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Changes in Ingestive Behavior Following Interruption of a Noradrenergic Projection to the Paraventricular Nucleus: Histochemical and Neurochemical Analyses

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O'DONOHUE, T. L., W. R. CROWLEY AND D. M. JACOBOWITZ. *Changes in ingestive behavior following interruption of a noradrenergic projection to the paraventricular nucleus: Histochemical and neurochemical analysis.* PHARMAC. BIOCHEM. BEHAV. 9(1) 99–105, 1978.—Histochemical investigation of the paraventricular nucleus (PVN) identified the tractus filiformis lateralis (TF) as a major noradrenergic preterminal pathway to the PVN. Bilateral knife cuts transecting the TF resulted in a discrete deafferentation of 56% of the noradrenergic input to the PVN region. TF transection also resulted in hypophagia and hypodipsia during the first four days after surgery. A marked hyperdipsia developed between postoperative Days 11 and 13 and persisted until sacrifice on Day 17. The results suggest that the TF contains a noradrenergic system which innervates the PVN and is involved in mediation of ingestive behaviors.

Paraventricular nucleus Tractus filiformis lateralis Norepinephrine Feeding behavior Drinking behavior

FLUORESCENCE histochemical [8, 9, 17, 26] and microneurochemical studies [22] in rats have demonstrated that the hypothalamic paraventricular nucleus (PVN) receives a particularly dense noradrenergic innervation. The heavy noradrenergic input to this nucleus has been implicated in both neuroendocrine [19] and behavioral regulatory functions. A role in regulation of ingestive behavior has been suggested from studies in which knife cuts, in proximity to the PVN, produced disruption of feeding and drinking [6]. Furthermore, the PVN has been identified as the most responsive site for stimulation of feeding and drinking behaviors by intracerebral microinjection of norepinephrine (NE) or noradrenergic agonists [16].

A previous report [8] has described a preterminal noradrenergic fiber system which courses in the tractus filiformis lateralis (TF), passing dorsal to the fornix, to innervate the PVN. The present studies were undertaken to, first, describe more completely the noradrenergic input to the PVN via the TF; second, interrupt this pathway in an attempt to create a discrete denervation of the PVN; and, third, assess the role of this projection in feeding and drinking behavior.

EXPERIMENT 1

A fluorescence microscopic study was performed in order to determine major inputs of catecholaminergic axons to the PVN.

METHOD

Animals

Sprague-Dawley male albino rats (Zivic-Miller, Allison, Park, PA) weighing 250–350 g were housed six to a cage containing freely available Purina lab chow and tap water.

Catecholamine Histofluorescence Microscopy

Rats were sacrificed by decapitation. Brains were removed and a 3 mm thick transverse slice containing the PVN was made in the stereotaxic plane of König and Klippel [11]. The specimen was placed on a copper grid and frozen by immersion in isopentane cooled by liquid nitrogen. Tissue samples were dehydrated by freeze drying for 5–7 days and were subsequently processed for catecholamine fluorescence microscopy by the method of Falck and Hillarp [4,7]. Specimens were exposed to dry paraformaldehyde for 1.5 hr followed by a 0.5 hr exposure to humid (about 60% humidity) paraformaldehyde. The tissue was then embedded in paraffin. Sections were cut at 14 μm and studied in the fluorescence microscope containing a GB3 exciter filter and a Leitz 490 emission filter.

RESULTS AND DISCUSSION

Catecholamine histofluorescence analyses revealed an abundance of fine green fluorescent varicosities in the

paraventricular and periventricular nuclei. A slightly skewed section through the paraventricular region (Fig. 1) allowed visualization of a major catecholaminergic tract which innervates this nucleus as was originally reported by Jacobowitz [8]. Fluorescent axons emanate from both the ansa lenticularis and the medial forebrain bundle, pass dorsal to the fornix and enter the lateral margin of the PVN. This movement of catecholaminergic fibers flows within the tractus filiformis lateralis, a system originally described and named by Krieg [13]. Upon entering the PVN, some of the aminergic neurons of the tractus filiformis appear to terminate on the oxytocin and vasopressin-synthesizing magnocellular perikarya in the lateral aspect of this nucleus (Figs. 1 and 3), suggesting a possible role for this system in control of oxytocin and vasopressin secretion.

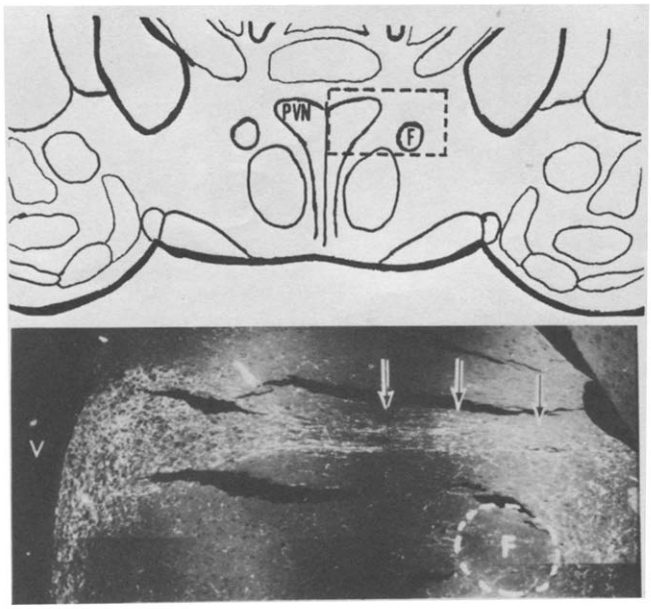

FIG. 1. Top: Schematic diagram of a coronal section (A 5660, [11]) at the level of the paraventricular nucleus (PVN). The dotted rectangle shows the location of the photomicrograph. Bottom: Catecholamine histofluorescence of the PVN region. Arrows show preterminal noradrenergic axons in the tractus filiformis, F—fornix, V—third ventricle.

EXPERIMENT 2

The results of Experiment 1 indicate that the TF contains a major noradrenergic afferent projection to the PVN. In Experiment 2, unilateral knife cuts of the TF were combined with histochemical and microneurochemical analyses of this region in order to determine the specificity and magnitude of the NE contribution to the PVN via this pathway. The effect of the knife cut on regional NE concentration over the course of time also was investigated to explore the possibility of plasticity of the noradrenergic innervation to the PVN.

METHOD

Animals

Rats, weighing between 275 and 400 g, were housed and maintained as described in Experiment 1.

Surgery

Stereotaxic surgery was performed under Halothane anesthesia. A stainless steel knife, 1.5 mm wide, was lowered in a parasagittal plane to transect the TF unilaterally (see Fig. 2). Coordinates for the knife placement were A: 6.5, L: 1.5, H: 8.4 (mm).

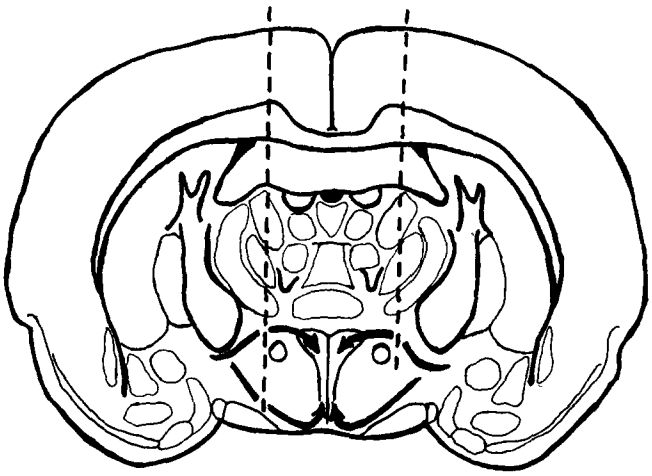

FIG. 2. Schematic representation of the deep knife cut (left) used in Experiment 2 and the more shallow transection employed in Experiment 3 (right). The arrows indicate probable routes of the noradrenergic innervation to the paraventricular and periventricular nuclei.

Postoperative Analysis

Rats were allowed to survive for either 4, 7, 14, or 21 days after surgery. After decapitation, the brains were removed and either processed for catecholamine fluorescence microscopy, as described in Experiment 1, or prepared for microdissection and subsequent catecholamine analysis. In the latter case, brains were mounted on specimen plates and frozen rapidly with powdered dry ice. Alternate 300 μm thick slices for microdissection and 60 μm thin sections for histology were cut on a cryostat at -9°C. The thick sections then were microdissected by the technique of Palkovits [21]. The size and location of the microdissected regions are presented in Table 1. The periventricular nucleus was dissected into left and right of the ventricle using a microdissecting knife. Micropellets of the frozen thick sections were delivered into 100 μl of ice cold 0.1 N perchloric acid. Samples were homogenized by sonication and a 10 μl aliquot was removed for protein analysis [18]. The remaining acidified homogenate was stored at -70°C until catecholamines were assayed by a micromodification [22] of the method of Coyle and Henry [2].

RESULTS AND DISCUSSION

Figure 3 shows a montage of the TF transection and the ipsilateral and contralateral PVN 7 days after surgery. A substantial decrease in catecholamine fluorescence is evident ipsilateral to the transection, especially along the lateral margin of the nucleus which contains the magnocellular neurosecretory cells. The buildup of catecholamine lateral to

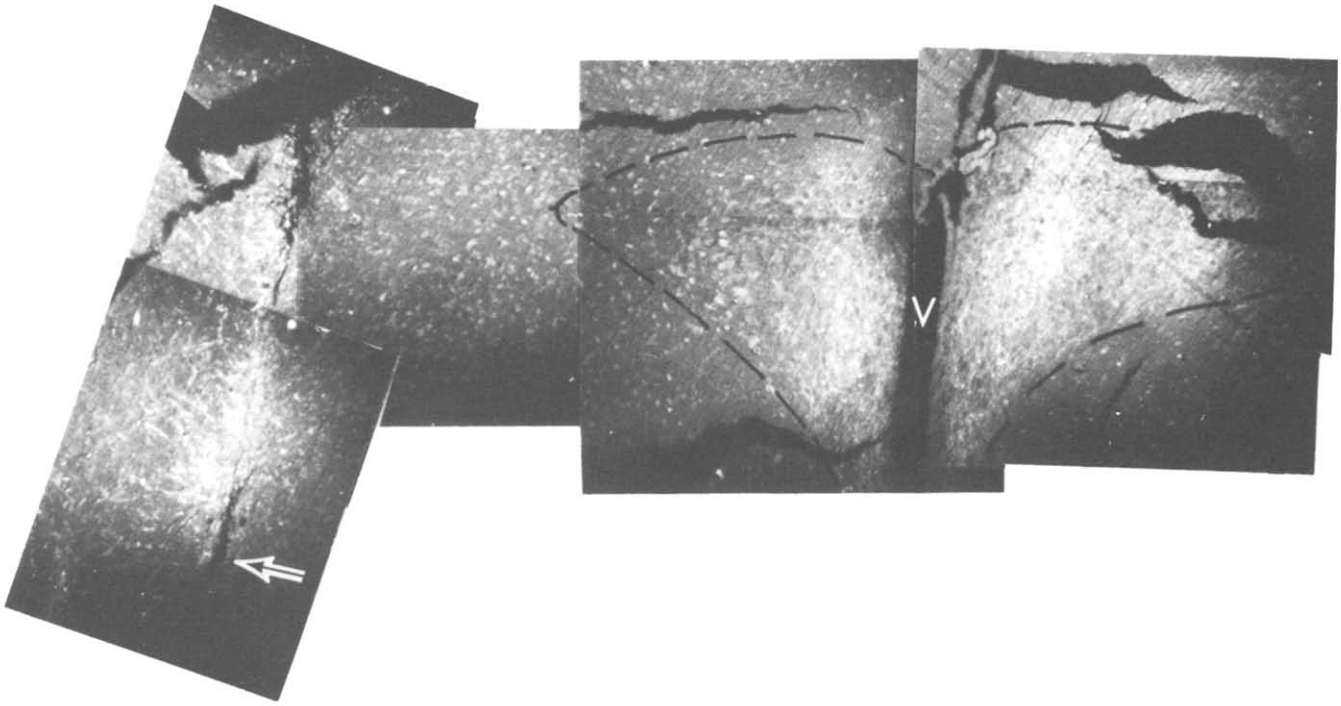

FIG. 3. Montage through the paraventricular region (PVN borders marked with dottted black line) 7 days after tractus filiformis knife cut. Note decreased catecholamine fluorescence ipsilateral to the transection (arrows) compared to the contralateral side. Also note the buildup of catecholamine lateral to the knife cut. V—third ventricle.

the knife cut confirms the medially directed flow of adrenergic fibers in the TF.

The magnitude of the TF noradrenergic contribution to the PVN was quantified further by assay of NE and dopamine ipsilateral and contralateral to a deep transection (Fig. 2). Tables 2 and 3 present norepinephrine and dopamine concentrations in several discrete brain nuclei 4, 7, 14 and 21 days after TF transection. These data were subjected to two factor (surgery × time) analysis of variance and subsequent Duncan's Multiple Range Tests [28]. Consistent with the histochemical results, NE was reduced significantly, between 26% and 36%, in the ipsilateral compared to contralateral PVN on Days 4, 7 and 14 (see also Fig. 4). A 27% decrease, which was not statistically significant, was observed in the ipsilateral PVN 21 days after the lesion.

The ipsilateral periventricular nucleus also showed a substantial decrease in NE, between 30% and 53%, up to 21 days after a deep knife cut (Table 2). Dopamine also was significantly reduced ipsilaterally in the periventricular nucleus four days post lesion, but recovered by Day 7 (Table 3). An unexpected finding was the marked elevation of NE concentrations in the PVN, both ipsilateral and contralateral to the transection on postoperative Days 7 and 14, compared to Days 4 or 21, even though NE levels ipsilateral to the

TABLE 1

MICRODISSECTION OF DISCRETE BRAIN REGIONS

Region	No. of Punches/Brain		Cannula Size (mm)		Approximate Coordinates*
	Exp. 2	Exp. 3	Exp. 2	Exp. 3	(μm)
Caudate-Putamen	1	2	1.0	1.0	A 6570
Anterior Hypothalamic Nucleus	1	2	0.75	0.75	A 5660
Supraoptic Nucleus	1	2	0.5	0.5	A 6060
Periventricular Nucleus	dissected (see text)	2	dissected (see text)	0.5	A 5660, 5340
Paraventricular Nucleus	2	4	0.5	0.5	A 5660, 5340
Retrochiasmatic Area	1	2	0.5	0.5	A 5660
Ventromedial Nucleus	1	2	0.5	0.5	A 4890

*Based on [9, 11].

TABLE 2

NOREPINEPHRINE (NE) LEVELS (MEAN ± SEM IN PG/μG PROTEIN) AFTER UNILATERAL TRACTUS FILIFORMIS TRANSECTION

Region	Days After Surgery 4	7	14	21
Paraventricular N				
Contralateral	51.4 ± 6.2	86.6 ± 6.4§	71.6 ± 4.90†	54.5 ± 6.4
Ipsilateral	32.6 ± 2.4*	55.0 ± 1.8‡§	53.0 ± 6.0*†	39.8 ± 3.4
Periventricular N				
Contralateral	23.1 ± 5.0	34.6 ± 2.5	37.9 ± 2.7§	40.2 ± 4.4§
Ipsilateral	10.8 ± 1.6‡	18.7 ± 2.8‡	26.4 ± 2.0*§	27.9 ± 2.2*§
Retrochiasmatic Area				
Contralateral	—	28.4 ± 7.3	14.1 ± 4.9	19.5 ± 4.0
Ipsilateral	—	26.1 ± 7.9	20.5 ± 3.7	20.2 ± 4.8
Caudate-Putamen				
Contralateral	3.1 ± 1.8	2.7 ± 0.3	2.1 ± 0.1	1.7 ± 0.1
Ipsilateral	4.4 ± 1.7	2.2 ± 0.2	2.7 ± 0.4	2.1 ± 0.2

* $p<0.05$ vs. contralateral
† $p<0.05$ vs. 4 days
‡ $p<0.01$ vs. contralateral
§ $p<0.01$ vs. 4 days

TABLE 3

DOPAMINE (DA) LEVELS (MEAN ± SEM IN PG/μG OF PROTEIN) AFTER UNILATERAL TRACTUS FILIFORMIS TRANSECTION

Region	Days After Surgery 4	7	14	21
Paraventricular N				
Contralateral	7.5 ± 1.7	10.3 ± 1.1	8.8 ± 0.7	6.5 ± 1.2
Ipsilateral	6.4 ± 1.2	7.7 ± 0.8	5.6 ± 0.4	5.0 ± 0.5
Periventricular N				
Contralateral	11.4 ± 1.9	6.0 ± 0.8†	7.8 ± 0.7	8.0 ± 1.6
Ipsilateral	4.9 ± 0.8*	4.0 ± 0.5	5.0 ± 0.3	5.2 ± 0.9
Retrochiasmatic Area				
Contralateral	—	28.4 ± 7.3	14.7 ± 5.0	19.5 ± 4.0
Ipsilateral	—	26.1 ± 7.9	20.5 ± 3.7	20.2 ± 4.8
Caudate-Putamen				
Contralateral	42.7 ± 7.2	63.0 ± 6.1	56.7 ± 3.9	47.2 ± 3.1
Ipsilateral	60.6 ± 9.6	60.5 ± 4.6	65.7 ± 14.7	53.3 ± 4.2

* $p<0.05$ vs. contralateral
† $p<0.05$ vs. 4 days
N=5-8

lesion always remained lower than on the contralateral side (Fig. 4). A similar pattern was observed in the periventricular nucleus (Table 2).

In summary, both the biochemical and fluorescence histochemical data indicate that the TF is a major source of noradrenergic fibers to the PVN.

EXPERIMENT 3

Having established the TF as a major noradrenergic afferent to the PVN, an investigation of the role of this system in ingestive behavior was undertaken.

METHOD

Animals

Sprague-Dawley male albino rats, weighing approximately 200 g at the start of the experiment, were housed individually in a colony room on a 12 hr light/dark cycle. They were maintained on a freely available diet of Purina lab chow and tap water. Animals were allowed two weeks to adapt to housing and laboratory conditions before behavioral observations commenced. Food and water intake was monitored for seven days to establish a proper baseline of consumption.

Surgery

Stereotaxic surgery was performed under halothane anesthesia. A 1.5 mm long stainless steel knife was lowered to transect the TF bilaterally. The knife was positioned just lateral and ventral to the fornix, as illustrated in Fig. 2 (right), to interrupt the TF noradrenergic preterminal path-

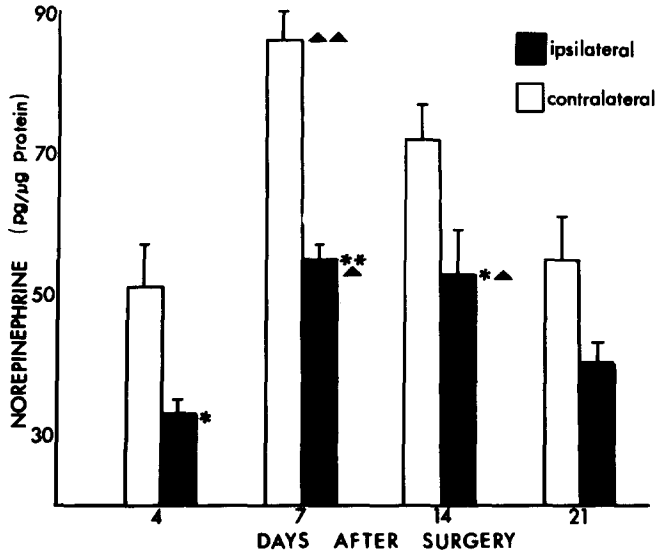

FIG. 4. Norepinephrine concentrations (mean ± SEM) in the paraventricular nucleus, ipsilateral and contralateral to tractus filiformis transection, *—$p<0.05$; **$p<0.01$ vs. contralateral on each day. ▲—$p<0.05$; ▲▲—$p<0.01$ vs. respective Day 4 value.

TABLE 4

NOREPINEPHRINE (NE) AND DOPAMINE (DA) CONCENTRATIONS (MEAN ± SEM IN PG/µG OF PROTEIN) 17 DAYS AFTER BILATERAL TRACTUS FILIFORMIS TRANSECTION

	Region	Control Lesion	TF Lesion
A. NE	Paraventricular N.	67.6 ± 5.6	29.7 ± 9.1*
	Periventricular N.	22.8 ± 3.5	23.6 ± 4.2
	Anterior N.	19.4 ± 2.0	15.9 ± 1.1
	Supraoptic N.	23.9 ± 4.4	19.2 ± 3.6
	Ventromedial N.	7.8 ± 0.9	9.1 ± 1.4
	Retrochiasmatic Area	24.8 ± 4.2	29.5 ± 4.9
	Caudate-Putamen	2.2 ± 0.1	1.9 ± 0.1
B. DA	Paraventricular N.	5.8 ± 0.3	4.3 ± 0.5
	Periventricular N.	4.4 ± 0.9	5.8 ± 1.2
	Anterior N.	2.6 ± 0.5	2.8 ± 0.4
	Supraoptic N.	6.6 ± 1.1	7.0 ± 0.8
	Ventromedial N.	1.9 ± 0.5	2.0 ± 0.3
	Retrochiasmatic Area	3.0 ± 0.7	3.3 ± 0.6
	Caudate-Putamen	97.9 ± 5.7	87.2 ± 5.9

N=6-8
* $p<0.01$

way to the PVN. Lesion coordinates for the approximately 250 g rat were A: 6.5, L: 1.5, H: 8.2 (mm). Control animals were treated identically, but the knife was only lowered to H: 5.5, penetrating into the thalamus. Animals were assigned to control or experimental groups so that their mean preoperative food and water intakes and body weights were virtually identical.

Postoperative Analyses

Food and water intake was monitored daily until 17 days after surgery. On Day 17, animals were decapitated and their brains frozen. Discrete nuclei were microdissected and catecholamines assayed as described in Experiment 2.

RESULTS AND DISCUSSION

Catecholamine Levels

Placement of the knife just ventro-lateral to the fornix (Fig. 2) produced a significant decrease of NE only in the PVN (Table 4). The lesion coordinates used in this study did not decrease the NE content of the periventricular nucleus. The fact that a deep knife cut (Experiment 2) results in a depletion of NE in the periventricular nucleus, suggests a more ventral pathway innervates this nucleus while the TF innervates the PVN (Fig. 2).

Feeding and Drinking

Figures 5 and 6 present food and water intake for the control and TF-transected animals. These data were subjected to two factor (surgery × time) analysis of variance and Duncan's Multiple Range Tests [28] after transformation to natural logarithms. Food and water ingestion was reduced on the first postoperative day for both groups. Control lesioned animals returned to their preoperative rate of food consumption by the second day after surgery while both feeding and drinking in lesioned animals remained depressed until be-

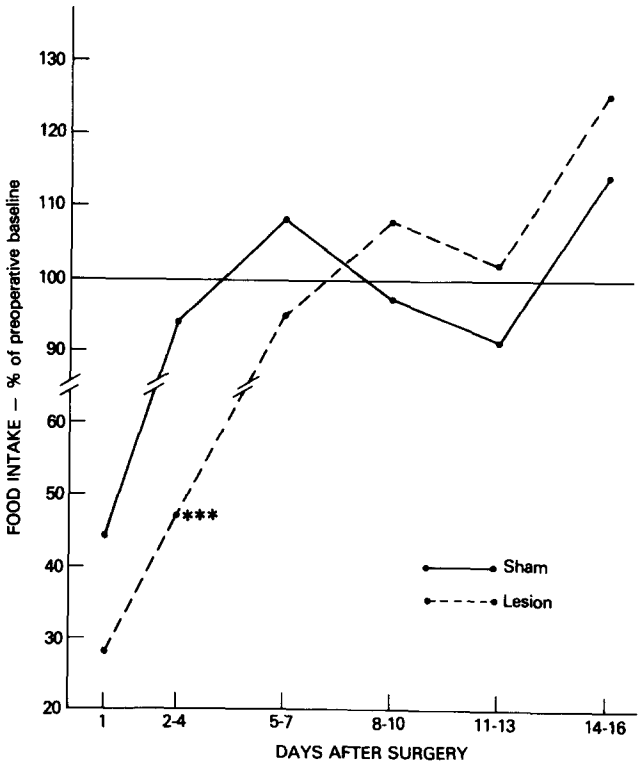

FIG. 5. Food intake, expressed as percentage of preoperative baseline, after sham lesion or tractus filiformis transection (***—$p<0.001$ vs. sham).

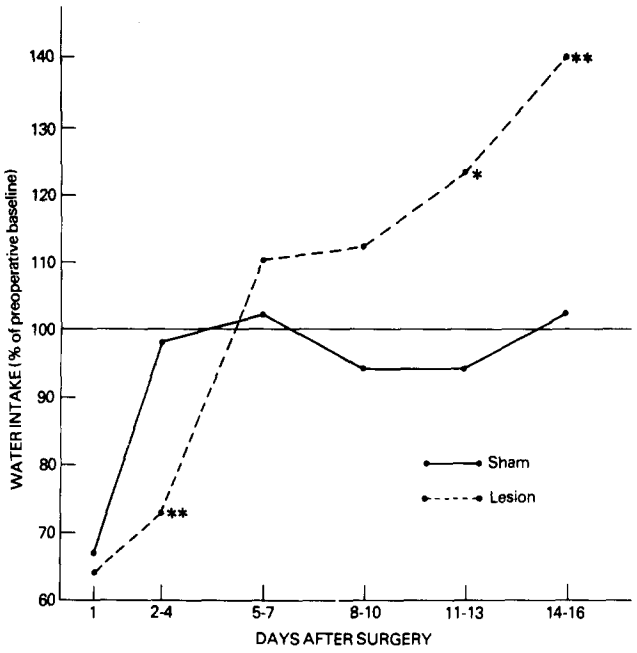

FIG. 6. Water intake, expressed as percentage of preoperative baseline, after sham lesion or tractus filiformis transection (*—$p<0.05$, **—$p<0.01$ vs. sham).

tween postoperative Days 5 and 7. TF-transected animals developed a hyperdipsia between 11 and 13 days that persisted until the animals were killed on Day 17. No other behavioral changes were obvious in the TF-transected animals.

GENERAL DISCUSSION

Through histochemical, neurochemical and lesion techniques, we have demonstrated that the TF contains a major noradrenergic projection to the PVN. The TF most likely contains noradrenergic axons originating in the A1 and/or A2 cell groups (O'Donohue, Crowley and Jacobwitz, in preparation) as well as a contribution from the locus coeruleus [10]. Our TF transection studies suggest that approximately half of the norepinephrine in the PVN is delivered by the TF. The remaining NE after TF lesion is possibly derived from the ventral periventricular system which enters the PVN caudally [17]. While it is likely that other non-catecholaminergic afferents to the PVN are present within the TF, no other transmitter system has yet been identified. To our knowledge, the TF transection represents the first successful interruption of a preterminal monoaminergic pathway to a discrete nucleus of the brain. Furthermore, the present study demonstrates that such a discrete denervation can also produce behavioral changes.

Although interruption of non-noradrenergic axons may have contributed to the changes in ingestive behavior seen after TF transection, the present results are consistent with previous work implicating a noradrenergic mediation of feeding and drinking within the PVN. For example, the transient hypophagia following bilateral TF transection is consistent with previous work showing temporary hypophagia after lesions of the locus coeruleus, a probable source of NE neurons in the TF [10] and also with findings that feeding may be elicited by microinjection of NE into the PVN [14, 15, 16, 24].

A noradrenergic influence in the PVN on ingestive behavior is also suggested by decreased NE content in the PVN of genetically obese rats [3]. The perifornical region also has been implicated in noradrenergic mediation of feeding [24,27] and denervation of this region, located just lateral to the PVN, may have contributed to the resulting feeding deficit. Gold *et al.* [6] also implicate the PVN in feeding behavior but find that deep transections, which penetrate the ventral surface of the brain, result in hyperphagia and obesity. These data strongly suggest that more than one satiety regulating system may exist in the PVN region.

The present results also suggest a relationship between PVN norepinephrine and drinking. Our findings demonstrating an initial decrease in drinking after deafferentation of a noradrenergic input to the PVN are consistent with reports of drinking elicited by NE injection into the PVN [14, 15, 16]. Interestingly, the initial hypodipsia reverses to a marked hyperdipsia between postoperative Days 11 and 16. These drinking changes may be due to a disruption of the PVN vasopressin system, either by removal of a noradrenergic influence on vasopressin-secreting neurons [19], or by damage incurred by vasopressin-containing axons that pass from the PVN to the neurohypophysis [25]. However, the striking similarity between behavioral results of TF transection and locus coeruleus lesions, i.e., initial hypophagia accompanied by a delayed hyperdipsia ([20] but see also [12]), raises the possibility that the noradrenergic cells of the locus coeruleus may influence ingestive behavior through their innervation of the PVN via the TF.

The transient feeding and drinking changes after TF transection are similar to those observed after other disruptions of catecholaminergic neurotransmission. After intraventricular administration of the catecholaminergic neurotoxins, 6-hydroxydopa or 6-hydroxydopamine, rats initially eat and drink less but recover preoperative intake rates quickly [1, 5, 23]. These and the present results suggest rapid neural mechanisms for the recovery of function after damage to a central catecholaminergic system.

The changes in NE concentration in the PVN and periventricular nucleus following unilateral TF transection may reflect compensatory processes that promote recovery of noradrenergically mediated function. The ipsilateral increase in NE concentration in both of these nuclei on postoperative Days 7 and 14 as compared to Day 4 (Table 2 and Fig. 4) may represent increased NE synthesis in the surviving noradrenergic neurons innervating these areas. This finding is consistent with physiological and pharmacological data suggesting that undamaged neurons in a partially denervated area may compensate by increasing catecholamine synthesis and release [29]. Interestingly, NE concentrations on the intact side of the periventricular and paraventricular nuclei also increased between postoperative Days 4 and 7. This finding raises the possibility that both an intact region and a partially denervated region may compensate to enable recovery of noradrenergically mediated function. More gradually developing mechanisms of recovery of function, such as receptor supersensitivity [29], could account for the subsequent decrease in ipsilateral and contralateral PVN NE concentrations between postoperative Days 14 and 21. Further work is required to determine whether the neurochemical changes observed after unilateral TF transection are involved in the recovery of ingestive behavior seen after bilateral TF transection.

In this regard, it is interesting to note that the PVN projects axons that descend and innervate various brain stem areas, including the locus coeruleus, the A1, and the A2 noradrenergic cell groups [25] which, in turn, give rise to ascending NE axons that innervate the PVN (O'Donohue, Crowley and Jacobowitz, in preparation). Thus it is possible that the PVN may modulate its own noradrenergic input by a long feedback loop. At present, we are investigating this potential relationship in our laboratory.

In summary, these studies demonstrate the possibility of investigating the neurochemical control of behavior in a discrete brain region by combining surgical intervention with histochemical, microdissection and microneurochemical techniques. Further work is required to elucidate the specific nature of the regulatory changes and the physiological mechanisms underlying recovery of function after transection of the TF.

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