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SYMPATHETIC REINNERVATION OF THE PINEAL GLAND AFTER POSTGANGLIONIC NERVE LESION DOES NOT RESTORE NORMAL PINEAL FUNCTION[1]

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Abstract

The activity of the enzyme serotonin *N*-acetyltransferase (NAT) in the rat pineal gland exhibits a large circadian rhythm, with peak activity occurring at night. This rhythm is dependent on stimulation of the pineal gland by neurons whose cell bodies are in the superior cervical ganglia and whose axons reach the gland via the internal carotid nerves (ICNs). Two days after both ICN were cut, crushed, or frozen, nighttime NAT activity was decreased by 90%. The remaining low level of enzyme activity was not affected by decentralization of the superior cervical ganglia. Thus, this enzyme activity did not depend on the activity of neurons in these ganglia. Bilaterally lesioning the ICN also abolished the neuronal uptake of norepinephrine in the pineal, further indicating that the sympathetic innervation of the gland had been destroyed.

Three months after crushing both ICNs, nighttime NAT activity was only 20% of control values. However, in these animals, bilateral decentralization of the superior cervical ganglion reduced this low level of NAT activity by 90%. Thus, NAT activity, although low, was again dependent on sympathetic nerve stimulation. In contrast to this rather small recovery of nocturnal NAT activity, the norepinephrine uptake capacity of the gland recovered to 60% of control values. A similar discrepancy between the extent of recovery of NAT activity and of norepinephrine uptake was observed when the ICNs were frozen rather than crushed.

To determine to what extent the sympathetic nerves that had reinnervated the pineal gland in these lesioned animals were capable of regulating NAT activity, their cervical sympathetic trunks were stimulated electrically at 5 Hz for 3 hr during the daytime. NAT activity increased in these animals, as it did in sham-operated animals, from low daytime values to near peak nighttime values. Thus, the sympathetic nerves reinnervating the pineal gland are capable of increasing NAT activity to high levels when electrically stimulated, and yet these animals do not recover a normal NAT rhythm. We hypothesize that, following bilateral lesioning of the ICN, the pineal gland is reinnervated by different sympathetic neurons than those that had previously innervated this tissue and that these neurons do not receive the type of neural information from the central nervous system that is necessary for regulating a normal circadian rhythm in NAT activity.

When a peripheral nerve trunk is lesioned, the lesioned neurons form regenerative sprouts that often reinnervate the denervated target tissues (Guth, 1956). In the cervical sympathetic nervous system, regeneration of this type occurs after both preganglionic and postganglionic nerve lesion. Langley (1895, 1897) and later Nja and Purves (1977) and Purves and Thompson (1979) examined the anatomical specificity of the new connections that are formed following such lesions. For this purpose, they made use of the fact that the mammalian superior cervical ganglion is innervated by preganglionic neurons residing in several spinal roots and that these roots, when activated, elicit different clusters of autonomic responses. For example, stimulation of the first thoracic ventral root leads to pupillary dilation but produces little contraction of the arterioles in the ear (Langley, 1897; Nja and Purves, 1977). Stimulation of the fourth thoracic ventral root produces the opposite effects. These patterns of responses to nerve stimulation are re-established within 2 to 4 months after the preganglionic cervical sympathetic trunks are lesioned but not after the corresponding postganglionic trunks are lesioned (Langley, 1895, 1897; Nja and Purves, 1977; Purves and Thompson, 1979).

While these studies indicate a difference in the specificity of regeneration following the two types of lesions, they do not indicate whether physiological regulation of various autonomic functions will be normal after either lesion. For example, the population of fibers in each spinal root controls more than one

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autonomic function. Thus, stimulation of the first thoracic ventral root leads not only to dilation of the pupil but also to retraction of the nictitating membrane (Langley, 1897). Assuming that these sympathetic functions are controlled by different preganglionic neurons, it remains to be determined whether these functional subgroups of neurons re-established normal connections in the superior cervical ganglion after preganglionic nerve lesion. Furthermore, while the loss of specificity in the end organ responses to stimulation of spinal roots after postganglionic nerve lesion suggests that postganglionic neurons do not specifically reinnervate their original targets, the functional consequences of this anatomical reorganization are unclear. Presumably, these consequences will depend on whether the pattern of neuronal firing in the neurons innervating a particular tissue is or is not "functionally equivalent" to the pattern of firing in the original neurons. For example, Langley (1895) observed that pupil size and position of the nictitating membrane of operated cats appeared normal several months after either preganglionic or postganglionic nerve lesion.

We have examined the question of functional recovery following postganglionic nerve lesion with respect to the sympathetic regulation of the activity of the enzyme serotonin *N*-acetyltransferase (NAT) in the pineal gland. NAT normally shows a large circadian rhythm in its activity, with peak activity occurring in the nighttime (Klein et al., 1971). This rhythm is almost totally abolished by lesioning the internal carotid nerves (ICNs), which contain the sympathetic axons that innervate the pineal gland (Zigmond et al., 1981). A great deal of evidence suggests that the rhythm in NAT activity is dependent on a rhythm in the activity of these sympathetic neurons (Klein, 1979; Bowers and Zigmond, 1980, 1982). In the present report we have examined whether normal neural regulation of this enzyme is re-established following bilateral lesioning of the ICNs. A preliminary report of these studies was presented to the Society for Neuroscience (Bowers et al., 1982).

Materials and Methods

Adult male Sprague-Dawley rats (100 to 125 gm at the time of purchase from Charles River Breeding Laboratories, Wilmington, MA) were housed in individual cages under controlled lighting (12-hr light:12-hr darkness) with *ad libitum* access to food and water for 10 days prior to the beginning of an experiment. All surgical procedures were performed during the last 2 hr of the light cycle. Animals were anesthetized with chloral hydrate prior to surgery (either 380 mg/kg, i.p., in experiments in which the acute effects of lesions were examined or 640 mg/kg, s.c., in experiments in which chronic effects were examined; Sigma Chemical Co., St. Louis, MO). The ICNs were exposed on both sides and either cut (removing a piece of nerve about 1 mm in length), crushed repeatedly with fine watchmaker forceps, or frozen repeatedly using a KRYMED MC-1000 cryosurgery unit (Cryomedics, Inc. Bridgeport, CT) (Fig. 1). In experiments in which the superior cervical ganglia were decentralized, a 2-mm piece of each cervical sympathetic trunk was removed about 5 mm proximal to the ganglion (Fig. 1). In certain animals both superior cervical ganglia were removed.

Nighttime NAT activity was measured between 7 and 8 hr into the dark cycle, by which time enzyme activity in unoperated animals in our laboratory has been found to reach peak nighttime levels (Bowers and Zigmond, 1980). Rats were individually removed from the animal room in a light-tight box and were killed in a second room under dim red light. Pineals were quickly removed, frozen on dry ice, and stored at −80°C prior to assay. Daytime NAT activity was determined 10 hr into the animal's light cycle. NAT activity was determined by measuring the rate of conversion of tryptamine to [^{14}C]-*N*-acetyltryptamine by the method of Deguchi and Axelrod (1972) with modifications described by Parfitt et al. (1975). The protein content of the pineal was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. The data are expressed as mean picomoles of *N*-acetyltryptamine formed per microgram of protein per 20 min ± SEM.

Norepinephrine uptake capacity was determined by a modification of the procedure described by Holz et al. (1974). Animals were killed during the light cycle and their pineals removed and stored in Krebs phosphate solution at 4°C prior to assay. Individual pineal glands were pre-incubated at 37°C in 35-mm Petri dishes containing 0.8 ml of BGJ_b medium (GIBCO, Grand Island, NY) supplemented with penicillin (100 units/ml, GIBCO) streptomycin (100 μg/ml, GIBCO), *l*-glutamine (2 mM, GIBCO), and *l*-ascorbic acid (0.1 mg/ml, J. T. Baker Chemical Co., Bricktown, NJ). The Petri dishes were pre-incubated without covers for 60 to 90 min in an organ culture chamber (Bellco Glass, Inc., Vineland, NJ) containing a humidified atmosphere of 95% O_2/5% CO_2. The pineals were then incubated for 15 min in a Dubnoff shaking water bath equilibrated with 95% O_2/5% CO_2 in beakers containing 0.65 ml of BGJ_b (as above) with 1 to 3 × 10^{-7} M [^{3}H]-*l*-norepinephrine (specific activity ~3 Ci/mmol, New England Nuclear Corp., Boston, MA) and 12.5 μM nialamide (Sigma). Uptake was terminated by transferring each pineal gland to a beaker containing 3 ml of Earle's balanced salt solution (GIBCO) at 4°C. The total radioactivity retained by the pineal glands was determined by sonicating them in 0.3 ml of distilled water and measuring the tritium content in a liquid scintillation counter. Non-neuronal uptake was determined by including pineals from animals whose superior cervical ganglia had been removed. Uptake data are expressed as mean picomoles of [^{3}H]norepinephrine per pineal per 15 min ± SEM.

In experiments in which the cervical sympathetic trunks were stimulated, animals were removed from the animal room no sooner than 4 hr into their light period and were anesthetized with chloral hydrate subcutaneously. Both cervical sympathetic trunks were exposed and cut, and the distal ends were placed in suction electrodes. The current intensity used was twice that required to produce maximal exophthalmus in the ipsilateral eye. The nerves were stimulated at 5 Hz for 3 hr, after which the rats were decapitated immediately and their pineals removed and frozen on dry ice. "Sham-stimulated" animals had their preganglionic trunks cut and were maintained under anesthesia for a period of time equivalent to that of the stimulated animals. Further details of the stimulation procedure can be found in Bowers and Zigmond (1982).

The significance of differences between groups was analyzed by the Student's *t* test for two means (two-tailed, except where noted).

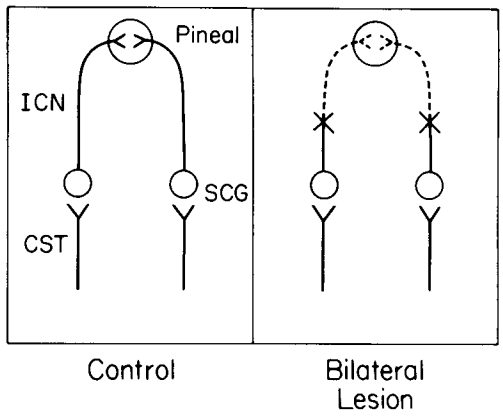

Figure 1. Diagram of the sympathetic innervation of the pineal gland and of the locations of the lesions made to denervate the gland. The cell bodies of sympathetic neurons innervating the pineal gland are located in the superior cervical ganglia (*SCG*), and they reach the pineal gland via the internal carotid nerves (*ICN*). To denervate the pineal gland, both ICNs were lesioned. In one group of animals, the cervical sympathetic trunks (*CST*) were also cut to determine whether the NAT activity remaining a few days after lesioning the ICNs depended on the activity of some neurons in the SCG whose axons had been inadvertently spared. In some of the animals, 3 months after the ICNs were lesioned, both SCG were removed or both CSTs were cut to determine whether the NAT activity or norepinephrine uptake which had recovered in these animals was dependent on stimulation via neurons in the SCG.

Results

Acute effects of ICN lesions. Anatomical studies indicate that an essentially complete sympathetic denervation of the pineal gland can be accomplished by bilaterally transecting the ICNs

(Bowers et al., 1984). This view is supported by the findings that cutting or crushing the ICNs decreased the uptake of norepinephrine in the pineal gland by 90% (Fig. 2*B*). Experiments with pineal glands from animals whose superior cervical ganglia had been removed or with pineal glands from intact animals incubated in the presence of desmethylimipramine (10^{-6} M), a specific inhibitor of the neuronal uptake of norepinephrine, suggest that the uptake remaining after bilaterally lesioning the ICNs is non-neuronal (data not shown). Lesioning the ICNs also resulted in a 90% decrease in nighttime NAT activity 2 days after the lesion (Fig. 2*A*). While these biochemical data indicate that the lesion produced an extensive denervation of the pineal gland, a small diurnal rhythm in NAT activity persisted after bilaterally crushing the ICNs (Table I). To determine whether the nocturnal level of NAT activity in lesioned animals is the result of the activity of sympathetic nerves that were inadvertently spared during surgery, animals whose ICNs were crushed 2 days earlier were lesioned again by transection of the preganglionic trunks of the superior cervical ganglia. The animals with both crushed ICNs and cut preganglionic trunks did not have significantly different nocturnal NAT activity than animals with only crushed ICNs (Fig. 3). Thus, the pineal NAT activity in animals with ICN lesions is not directly dependent on cervical sympathetic nerve activity. A small residual rhythm in pineal NAT activity after bilateral superior cervical ganglionectomy has been observed previously by other workers and is probably due to a rhythm in the concentration of circulating catecholamines (Klein et al., 1971; Bäckström et al., 1976).

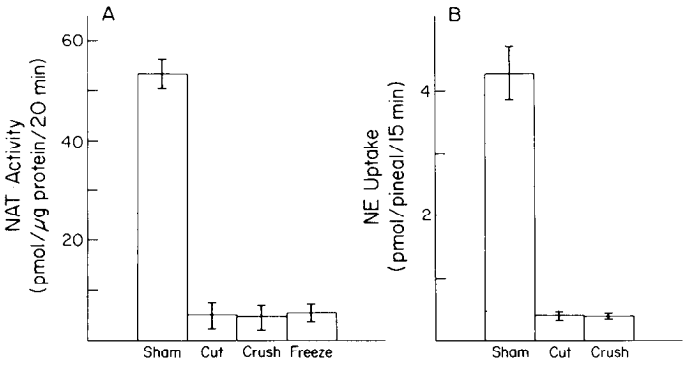

Figure 2. Acute effects of lesioning the ICNs on NAT activity and norepinephrine (*NE*) uptake in the pineal gland. Animals were operated on at the end of the light period. The data represent the means ± SEM of six to eight pineal glands. *A*, Pineal NAT activity 32 hr after the ICNs were cut, crushed, or frozen. Sham-operated animals served as controls. NAT activity was measured in pineal homogenates between 7 and 8 hr into the dark period. *B*, Norepinephrine uptake 48 hr after cutting or crushing the ICNs. Norepinephrine uptake was measured in intact pineal glands *in vitro*.

TABLE I

Acute effect on the rhythm in NAT activity of crushing the internal carotid nerves

Animals either were sham-operated or had their ICNs crushed. Thirty-two and 48 hr later, the activity of NAT was measured in the nighttime and in the daytime, respectively. The values are the mean NAT activities ± SEM for groups of nine pineals. A significant day-night difference in NAT activity was seen both in sham-operated and lesioned animals ($p < 0.05$ for the latter, by the Student's t test, one-tailed).

	NAT Activity
	pmol/μg of protein/20 min
Sham-operated	
Night	44.3 ± 3.5
Day	0.17 ± 0.03
Lesioned	
Night	3.5 ± 1.2
Day	0.9 ± 0.3

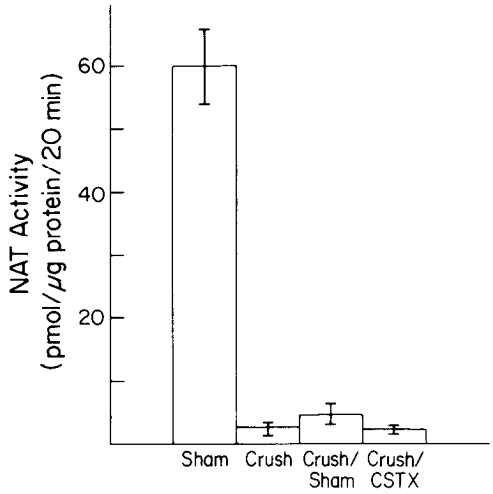

Figure 3. Acute effect of decentralization on the residual NAT activity in the pineal found after bilaterally crushing the ICNs. Animals were either subjected to a sham operation (*Sham*) or had their ICNs crushed (*Crush*). Two days later, some of the lesioned animals either had their cervical sympathetic trunks cut (*Crush/CSTX*) or had a sham operation (*Crush/Sham*). All operations were performed at the end of the light period. Nighttime NAT activity was measured 7 to 8 hr after the second operation. The data are expressed as the mean values ± SEM for eight to nine pineal glands. No significant difference was found between the Crush/Sham and the Crush/CSTX groups nor between either of these groups and the Crush group.

Reinnervation of the pineal gland. The time course of the reinnervation of the pineal gland was monitored by measuring the uptake of [^{3}H]norepinephrine 2 days and 1, 2, and 3 months after bilaterally crushing the ICNs (Fig. 4). The neuronal uptake of norepinephrine by the pineal gland recovered to 40% of normal 1 month after the nerve crush and increased significantly (to 55% of normal) during the subsequent month. No significant increase was seen during the third month (Fig. 4). The neuronal uptake of norepinephrine in the reinnervated pineal glands could be totally abolished by removing the superior cervical ganglia 1 week prior to the assay, suggesting that the recovery of norepinephrine uptake was due to reinnervating sympathetic neurons (Fig. 5*B*).

Although the norepinephrine uptake capacity had recovered to 60% of normal 3 months after the lesions, the nocturnal NAT activity was only 20% of normal (Table II and Fig. 5*A*). While the absolute level of nocturnal NAT activity changed very little between 2 days (Fig. 2*A*) and 3 months (Fig. 5*A*) after lesioning the ICNs, the enzyme activity in the latter group was extremely sensitive to cutting the preganglionic cervical sympathetic trunks (Fig. 5*A*). Thus, 3 months after lesioning the ICNs, the pineal NAT activity was again under sympathetic neuronal control. When the ICNs were frozen rather than crushed, similar results were obtained concerning the extent of recovery of the norepinephrine uptake capacity and the nocturnal NAT activity (Fig. 6 *A* and *B*).

The decreased NAT rhythm in lesioned animals could result from a decrease in the number of sympathetic neurons innervating the pineal gland or from a decrease in the efficiency with which these reinnervating neurons stimulate the gland. To test these possibilities directly, we electrically stimulated both cervical sympathetic trunks in sham-operated and le-

sioned animals to determine whether the NAT response to nerve stimulation differed in the two groups. The preganglionic nerve trunks were stimulated for 3 hr during the daytime at a frequency of 5 Hz. NAT activity increased in both groups of animals, and the magnitudes of the increases were not significantly different (Table III). In the lesioned animals nerve stimulation increased NAT activity to a level 6- to 7-fold higher than that seen during the middle of the dark period. These data suggest that the nerves that have reinnervated the pineal gland are as capable as the original nerves of stimulating pineal NAT activity.

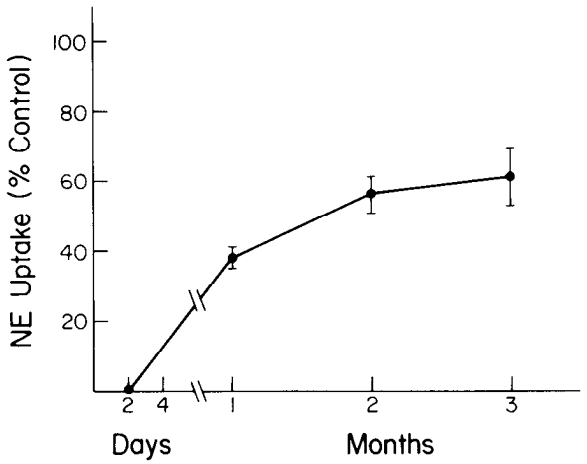

Figure 4. Time course of the return of neuronal uptake of norepinephrine (*NE*) in the pineal gland following crushing of the ICNs. The neuronal norepinephrine uptake is expressed as a percentage of the values found in pineal glands from control animals. To determine the amount of non-neuronal uptake, pineal glands were included from animals whose superior cervical ganglia had been removed 7 days previously. The uptake in these pineals was approximately 10% of that found in pineals taken from normal animals and was equal to that in pineals of normal animals which were incubated in the presence of 10^{-6} M desmethylimipramine. Thus, this level of uptake was considered to be non-neuronal and was subtracted from the total norepinephrine uptake to determine the specific neuronal uptake. Eight animals were included in each group.

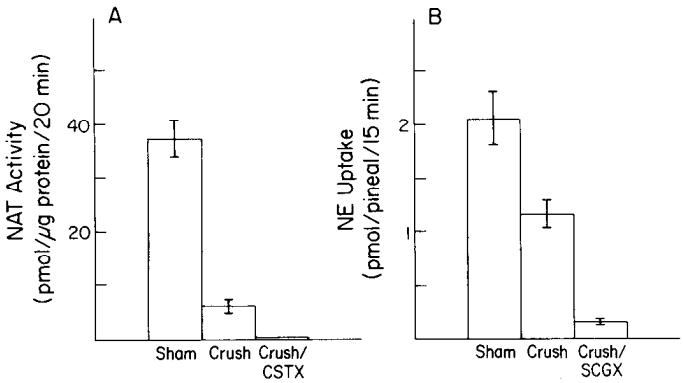

Figure 5. Chronic effects of crushing the ICNs on NAT activity and norepinephrine (*NE*) uptake in the pineal gland. *A*, NAT activity was measured in animals that had been sham-operated (*Sham*), had their ICNs crushed 3 months earlier (*Crush*), or had their ICNs crushed 3 months earlier and had their cervical sympathetic trunks cut 8 days earlier (*Crush/CSTX* group). Each group consisted of seven to eight animals. Enzyme activity was significantly lower in the Crush/CSTX group than in the Crush group ($p < 0.001$). *B*, Norepinephrine uptake was determined in pineals of animals that had been sham-operated (Sham), had their ICNs crushed 3 months earlier (Crush), or had their ICNs crushed 3 months earlier and had their superior cervical ganglia removed 8 days earlier (Crush/SCGX group). Each group consisted of eight animals except the Crush/SCGX group which contained only three animals. Norepinephrine uptake was significantly lower in the Crush/SCGX group than in the Crush group ($p < 0.005$).

TABLE II

Long-term effect on the rhythm in NAT activity of crushing the ICNs

Animals either were sham-operated or had their ICNs crushed. Three months later, daytime and nighttime NAT activities were determined. The number of animals in each group is shown in parentheses. A significant day-night difference in NAT activity was seen in both groups of animals.

	NAT Activity
	pmol/μg of protein/20 min
Sham-operated	
Night (7)	38.8 ± 3.3
Day (5)	0.06 ± 0.04
Lesioned	
Night (8)	8.6 ± 1.8
Day (9)	0.6 ± 0.2

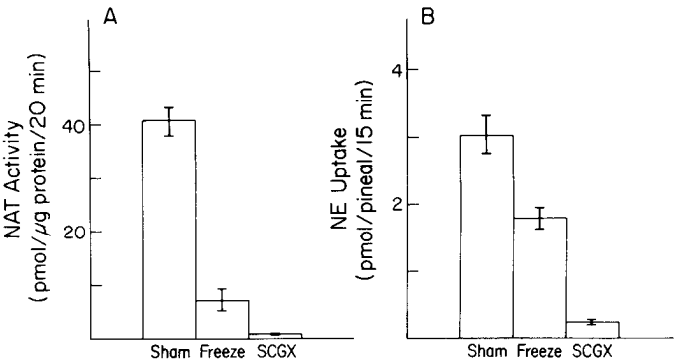

Figure 6. Long-term effects of freezing the ICNs on NAT activity and norepinephrine (*NE*) uptake in the pineal gland. NAT activity (*A*) and norepinephrine uptake (*B*) were determined in sham-operated animals, in animals whose ICNs had been frozen 3 months earlier, and in animals whose superior cervical ganglia had been removed 3 months earlier. Each group consisted of six to eight animals. Both NAT activity and norepinephrine uptake were lower in the animals whose ganglia were removed than in animals whose ICNs were frozen ($p < 0.01$ and $p < 0.001$, respectively).

Discussion

The pineal gland of the rat is innervated almost exclusively by sympathetic neurons whose cell bodies are in the superior cervical ganglia and whose axons are in the ICNs (Kappers, 1961; Owman, 1964; Bowers et al., 1984). The present results demonstrate that, after the pineal gland is denervated by bilaterally crushing the ICNs, sympathetic fibers begin reinnervating the gland within a month. The degree of reinnervation, as assayed by the uptake of norepinephrine, reached a plateau 2 months after the lesion, at which time the uptake of norepinephrine was about 60% of control.

In spite of this considerable reinnervation of the pineal gland, the neurally controlled rhythm in NAT activity remained greatly diminished. Three months after the lesion, peak nighttime NAT activity was only 20% of that found in sham-operated controls. The effects of freezing rather than crushing the ICNs were examined with the idea that regeneration might be more complete after the former. However, the extents of recovery of both norepinephrine uptake and NAT activity were similar after both types of lesions. This finding, together with

TABLE III

Effects on NAT activity of stimulating the cervical sympathetic trunks

Animals either were sham-operated or their ICNs were bilaterally crushed. About 3 months later NAT activity was determined in lesioned animals during the nighttime, during the daytime, after sham stimulation of the cervical sympathetic trunks, and after stimulation of the cervical sympathetic trunks at 5 Hz for 3 hr. NAT activity was also determined in sham-operated animals after stimulation of the preganglionic nerve at 5 Hz for 3 hr. The number of animals in each group is shown in parentheses.

	NAT Activity
	pmol/μg of protein/20 min
Lesioned	
Night (7)	6.6 ± 1.3
Day (6)	0.3 ± 0.1
Sham-stimulated (6)	1.5 ± 0.4[a]
Stimulated (7)	42.7 ± 4.6[b]
Sham-operated	
Stimulated (5)	28.0 ± 7.8

[a] Significantly different from daytime controls ($p < 0.025$).

[b] Not significantly different from stimulated sham-operated animals.

the finding that the recovery of norepinephrine uptake by the pineal gland appeared to reach a plateau between 2 and 3 months after crushing the ICNs, suggests that the degree of reinnervation of the gland at 3 months is near maximal for this system.

There are a number of possible reasons for the relatively small NAT rhythm in the reinnervated pineal glands. For example, one possibility is that the reduced rhythm may simply reflect a reduced number of sympathetic neurons innervating the pineal gland. However, it is interesting to note that we have previously observed an NAT rhythm of normal amplitude in pineal glands which have been denervated by 50% (i.e., following removal of one superior cervical ganglion) (Zigmond et al., 1981). A second possibility is that the lack of recovery of pineal function in the animals with ICN lesions results from regenerating axons establishing less efficient synaptic interactions with pineal cells than those found in normal animals. Such a loss in efficiency could be caused by a variety of factors operating at either a pre- or postsynaptic level. For example, in the reinnervated pineal glands there may be a decrease in quantal content of evoked norepinephrine release, a failure of conduction of action potentials in the sympathetic axons, an abnormal anatomical pattern of sympathetic innervation, or a subsensitivity of pinealocytes to adrenergic stimulation—although, in fact, from previous studies (Deguchi and Axelrod, 1973) one might expect a supersensitive response. Any of these and other related mechanisms in various combinations might result in the observed lack of recovery of the NAT rhythm. The stimulation experiment we performed was designed to test whether the sympathetic innervation of the pineal gland in the reinnervated animals was adequate (both in terms of numbers of synapses formed and in terms of synaptic efficacy) to restore normal neural regulation of NAT activity. Since bilateral stimulation of the cervical sympathetic trunks at 5 Hz produced as large an increase in enzyme activity in the reinnervated glands as in normal glands, we conclude that the regenerated axons are not less efficient than the original axons in increasing NAT activity and that sufficient neurons have reinnervated the pineal gland to produce a normal NAT rhythm.

Five hertz was chosen for these experiments because this frequency of stimulation appears to be just maximal for elevating NAT activity in animals at night following exposure to light to reduce their NAT activity to approximately daytime levels (Bowers and Zigmond, 1980). As a test of the adequacy of the sympathetic innervation of the pineal gland in lesioned animals, stimulation at 5 Hz was considered preferable to stimulation at higher frequencies to decrease the likelihood that superphysiological stimulation might obscure differences in responsiveness between lesioned and control animals. In fact, we have recently obtained evidence that during the daytime 5 Hz may produce a slightly submaximal elevation of NAT activity, further indicating that it is unlikely to be a superphysiological frequency (J. R. Lingappa and R. E. Zigmond, unpublished data). Nevertheless, it should be stressed that the normal frequency and pattern of firing of the sympathetic fibers innervating the rat pineal gland are not known at present. In addition, it is possible that stimulation of the cervical sympathetic trunk might indirectly increase pineal NAT activity in the lesioned animals via an elevation in circulating catecholamines (e.g., see Cannon and Rosenblueth, 1949). However, even if an increase in plasma catcholamines was produced during stimulation, it is not clear whether the reinnervated pineal glands would be especially sensitive to such a change, as they have recovered much of their capacity to take up catecholamines (Parfitt and Klein, 1976; Zigmond et al., 1981).

In spite of these possible limitations, the stimulation experiment suggests that the efficiency of transmission between sympathetic axons and pineal cells is not altered in the regenerated animals. Thus, the decreased NAT rhythm in these animals is likely to be due to a difference in the frequency and/or pattern of neural input to the pineal gland. One way such an alteration in neural information reaching the pineal gland could arise is if the neurons that reinnervate the gland are not identical to the neurons that originally innervated it and if the pattern of nerve firing of the reinnervating neurons is inappropriate for maintaining normal pineal function. Recent anatomical studies in our laboratory have indicated that in unlesioned rats only 250 to 450 neurons innervate the rat pineal gland from each superior cervical ganglion (Bowers et al., 1984). This represents approximately 3% of the neurons that project into the ICNs. Thus, in the extreme case, if the reinnervation of denervated tissues by fibers in the ICNs were random, one would expect very few of the original "pineal neurons" to reinnervate the gland.

A second possible mechanism that could account for a change in the neural information reaching the pineal gland is if the original neurons did specifically reinnervate the pineal gland, but the pattern of electrical activity of these neurons was altered because of neural rearrangements proximal to the lesions. Such rearrangements could conceivably occur in the CNS, but a more likely place might be in the superior cervical ganglia. For example, it is known that after axotomy the synaptic connections between preganglionic nerve terminals and neurons in the superior cervical ganglia of both the rat and guinea pig are temporarily lost, resulting in a failure of synaptic transmission (Matthews and Nelson, 1975; Purves, 1975). In addition, Purves (1975) has shown that approximately 50% of the neurons in the guinea pig superior cervical ganglia degenerate after crushing the postganglionic trunks, and we have found that extensive cell loss occurs in the rostral portion of the rat superior cervical ganglion after crushing the ICNs (C. W. Bowers, L. M. Dahm, and R. E. Zigmond, unpublished observations). It is possible that either of these two processes could result in preganglionic rearrangements that cause alterations in the neural information received by the surviving neurons. Nevertheless, Purves and Thompson (1979) found no evidence for rearrangement of connections in the guinea pig superior cervical ganglion following postganglionic axotomy. These authors found that the normal pattern of reinnervation of individual ganglion cells by various preganglionic roots was re-established after such lesions.

While our data suggest that a significant fraction of the

sympathetic fibers which reinnervate the pineal gland have electrical activity inappropriate for maintaining normal pineal function (either due to mistakes in reinnervation by regenerating sympathetic neurons or to synaptic rearrangements proximal to the lesion), it is impossible to determine from these data exactly what proportion of the fibers have abnormal activity. How the formation of incorrect circuits would affect pineal function presumably depends on the frequency and pattern of impulse activity in these circuits. For example, an extreme case would be one in which the formation of incorrect circuits leads to a completely inactive sympathetic input at night, resulting in no stimulation of the pineal gland. Previous studies have suggested that electrophysiologically inactive sympathetic neurons (i.e., decentralized neurons) can actually inhibit the ability of normal sympathetic neurons to stimulate pineal function. This inhibition appears to be mediated by the norepinephrine uptake system of the "silent" neurons (Zigmond et al., 1981). Presumably a similar inhibitory interaction could occur between neurons firing at very low frequencies and neurons firing at higher frequencies. On the other hand, if the "nonpineal neurons" exhibited a diurnal rhythmicity in their electrical activity, they might have the ability to mimic the effects of pineal neurons. Indirect biochemical evidence that neurons from the superior cervical ganglion other than pineal neurons show a diurnal rhythm in their electrical activity comes from studies on the norepinephrine content of sympathetically innervated tissues. Norepinephrine content was found to be higher at night than in the daytime in both the pineal gland and the submandibular gland, and it has been suggested that both rhythms are caused by a rhythm in the activity of the sympathetic innervation to these tissues (Wurtman and Axelrod, 1966; Moore and Smith, 1971). Furthermore, it has been found that, if the pineal gland is transplanted to the anterior chamber of the eye and is reinnervated (presumably by collateral sprouting of neurons innervating the iris), a small but distinct rhythm in NAT activity is established (Bäckström et al., 1976; Moore, 1975). These data suggest that neurons innervating the iris may also have a rhythm in their electrical activity. It is interesting to note that, although the NAT rhythm in animals whose ICNs were lesioned 3 months earlier is considerably reduced compared to that of normal animals (Table II), a distinct rhythm in enzyme activity is present in these animals. Thus, this rhythm could result from stimulation of the pineal gland by a small population of "pineal neurons" and by a population of other superior cervical ganglion neurons that shows some diurnal rhythmicity in their electrical activity.

Our finding of a lack of a rhythm in NAT activity of normal amplitude in lesioned animals that have a normal NAT response to electrical stimulation of their cervical sympathetic trunks suggests that a significant alteration in the frequency and/or pattern of electrical activity in the sympathetic innervation to the pineal gland has occurred. We suggest that it is most likely that this change results from the pineal gland being reinnervated by postganglionic neurons that are different from the original pineal neurons and that have different preganglionic inputs.

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