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Lesions of the Paraventricular Nucleus Area of the Hypothalamus Disrupt the Suprachiasmatic→Spinal Cord Circuit in the Melatonin Rhythm Generating System

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KLEIN, D. C., R. SMOOT, J. L. WELLER, S. HIGA, S. P. MARKEY, G. J. CREED AND D. M. JACOBOWITZ. *Lesions of the paraventricular nucleus area of the hypothalamus disrupt the suprachiasmatic→spinal cord circuit in the melatonin rhythm generating system.* BRAIN RES BULL 10(5) 647–652, 1983.—The circadian rhythm in melatonin production in mammals is regulated by a suprachiasmatic (SCN)→spinal cord→pineal circuit. In the present investigation the possible participation of the paraventricular nucleus of the hypothalamus (PVN) in the SCN→spinal cord segment of this circuit was investigated in the rat. Bilateral lesions of the PVN area were produced and one to two weeks later melatonin production was evaluated by measuring the activities of the two pineal enzymes required for the formation of melatonin from serotonin, indoleamine N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT), and urinary 6-hydroxymelatonin, the major melatonin metabolite. In some cases pineal melatonin was also measured. Control animals received sham-PVN lesions. Histological examination of the lesions indicated that the PVN were bilaterally destroyed 100% in 12 animals. The nighttime pineal melatonin and urinary 6-hydroxymelatonin values in this group were reduced about 90%, nighttime pineal NAT activity was reduced about 98%, and HIOMT activity about 75%. The urinary 6-hydroxymelatonin values of PVN-lesioned animals and animals with denervated pineal glands were similar. In animals with hypothalamic lesions involving <30% of the PVN, nighttime values of NAT, HIOMT, and urinary 6-hydroxymelatonin were normal; in animals with 30 to 95% PVN damage these parameters were altered to a small degree. These studies, together with histochemical observations, indicate the SCN neurons responsible for pineal circadian rhythms project to the PVN area of the hypothalamus.

Pineal Suprachiasmatic nucleus Paraventricular nucleus Rat Circadian rhythms Melatonin

THE pineal gland and the suprachiasmatic nucleus (SCN) are major components of the melatonin rhythm generating system in mammals [6,17]. The SCN acts as a self-sustaining master oscillator controlling many circadian rhythms [13,14], including melatonin production. Each night it stimulates the pineal gland for a 10 to 14 hour period [7, 15, 18]. Light acts on this system via a retinohypothalamic projection terminating in the SCN. Light can both reset the SCN clock on a daily basis, insuring that it is synchronized with the environmental lighting cycle, and inhibit transmission of signals from the SCN to the pineal gland.

Stimulation of the pineal gland by the SCN at night increases pineal melatonin and melatonin production by increasing N-acetyltransferase (NAT) activity 30- to 70-fold [8]. This is the first enzyme in the conversion of serotonin to melatonin [6]. Stimulation also maintains the normal activity of hydroxyindole-O-methyltransferase (HIOMT), the second enzyme in the conversion of serotonin to melatonin [15]. When SCN→pineal stimulation is interrupted surgically, pineal NAT activity and melatonin do not increase each night, and HIOMT activity decreases slowly over the course of a week [7,15].

The SCN→pineal circuit is unusual because it passes through both central and peripheral neural structures [6,17]. The gland is innervated by postganglionic projections from cells in the superior cervical ganglia. These cells in turn are innervated by preganglionic cells originating in the intermediolateral cell column of the spinal cord. The precise

route of the SCN→spinal cord pathway has not been established, although lesion studies have been interpreted to indicate that it passes through the retrochiasmatic area of the hypothalamus and the medial forebrain bundle [7,15].

Histochemical studies have identified SCN projections in the paraventricular nucleus of the hypothalamus (PVN), which is considered here to include the periventricular section [21–23]. These SCN projections pass in close proximity to cells which project to the intermediolateral cell column of the spinal cord [3, 19, 21–23]. Based on these observations, it was proposed that an SCN→PVN→spinal cord circuit might be involved in circadian rhythms in general [24] and pineal regulation specifically [3].

In the study described in this report, the effect of bilateral lesions of the PVN area on rat pineal function was investigated by measuring pineal melatonin content, the activities of pineal NAT and HIOMT, and the amount of urinary 6-hydroxymelatonin, the major melatonin metabolite [6]. The latter serves as an approximation of melatonin made by the pineal gland. The effects of PVN lesions were compared to those of established procedures which alter melatonin production, including pinealectomy (PNX), bilateral superior cervical ganglionectomy (SCGX) and superior cervical ganglia decentralization (DEC).

METHOD

The animals used in this study were 250 to 350 g male Sprague Dawley rats (Zivic Miller Co., Allison Park, PA). PNX, SCGX, and DEC (Experiment 1) were performed by the supplier. Animals were maintained in a windowless facility with automatically controlled lighting (LD 14:10); lights were turned on at 0500 hours. Food and water were provided ad lib.

Stereotaxic Lesions

Animals were anesthetized with halothane, and high frequency lesions were placed bilaterally in or near the PVN. The coordinates (in mm) were according to König and Klippel [10]. Coordinates and current for Experiment 2 were: AP 6.5, L 0.8, DV 6.4 from dura, 5 mA for 25 seconds. Coordinates and current for Experiment 3 were: AP 7.8, L 0.8, DV 7.2 from dura, 10 mA for 25 seconds. The lesioning electrode was 0.15 mm in diameter and 0.5 mm in length and was inserted to the appropriate site at a 4° angle. Current was delivered by a Grass LM4 lesion maker. Sham rats were treated identically except that the electrodes were placed 1.0 mm below dura and current was not delivered.

Sample Collection

Animals were placed into metal metabolic cages for urine collection; they were provided water ad lib and were deprived of food. Urine was collected in plastic tubes in solid CO_2 and stored at −30°C. The night and day collection periods were 1800 to 0600 and 0900 to 1700, respectively.

After urine collection had been completed in Experiment 1, animals were killed without further analyses. In Experiments 2 and 3 animals were returned to regular cages for a period of 2 to 3 days. Animals in these experiments were killed between 1100 and 1200 hours (day pineal glands) or under dim red light between 2300 and 2400 hours (night pineal glands). The pineal glands were rapidly removed, placed in plastic microtubes on solid CO_2 and stored at −30°C. Brains were carefully removed and frozen on dry ice.

TABLE 1

EFFECTS OF PINEALECTOMY, SCGX AND DEC ON URINARY 6-HYDROXYMELATONIN (EXPERIMENT 1)

Surgical treatment	Urinary 6-hydroxymelatonin (ng/collection) Day	Night	Total
None (N=6)	35.4 ± 6.8	264.2 ± 70.3†	299.5 ± 69.3†
SCGX (N=6)	18.3 ± 5.0	42.4 ± 17.6	60.8 ± 16.4
DEC (N=6)	6.4 ± 1.3*	17.1 ± 4.1	23.5 ± 4.5
Pinealectomy (N=6)	N.D.	2.7 + 1.3†	2.7 + 1.3†

For further details see the Method section and text.

*Statistically different than first two day values, $p>0.05$ (pinealectomy day value not included in analysis). N.D.—not detectable. †Statistically different from all other groups in column, based on analysis of log transformed data, $p>0.05$.

Biochemical Analyses

Individual pineal glands were homogenized in 120 μl of 0.05 M sodium phosphate buffer, pH 7.9, and 5 to 30 μl samples of this were used for each analysis. Established procedures were used to measure pineal NAT activity, HIOMT activity, and melatonin content and urinary 6-hydroxymelatonin [21, 25, 26].

Data are presented as the mean (±SEM). Statistical analysis was performed using Duncan's Multiple Range Test [5,11]. In certain cases, as indicated in the legends, log transformation of data was indicated because of heterogeneity of variance [1]. For urine samples in which 6-hydroxymelatonin was undetectable, the value was set at 1 ng/collection, the limit of detection.

Histological Analysis

Frozen brains were placed on specimen plates and sectioned in a cryostat. Transverse sections (50 μm) were cut through the lesion. The sections were stained with 0.1% thionen for verification of the lesion site. The extent of PVN damage was estimated by comparative microscopic examination of sections of lesioned and normal brains, and was expressed as a percentage.

RESULTS

Effects of PNX, SCGX and DEC on 6-Hydroxymelatonin Production (Table 1)

Urinary 6-hydroxymelatonin values in control and sham animals were about 30 ng during the day and 300 ng at night. SCGX and DEC markedly reduced night urinary 6-hydroxymelatonin values, to about 60 ng and 24 ng, respectively, consistent with known effects of these procedures [9]. PNX reduced day urinary 6-hydroxymelatonin to undetectable levels and night 6-hydroxymelatonin about 99%, consistent with earlier reports [11,12]. It should be added that 6-hydroxymelatonin was not detectable in the day urine collection from three of the six PNX animals.

Effects of Hypothalamic Lesions

The fifteen lesioned animals in Experiment 2 (Table 2) were divided into three groups. The first, Lesion A, was

TABLE 2

EFFECTS OF HYPOTHALAMIC LESIONS ON PINEAL NAT ACTIVITY AND HIOMT ACTIVITY, AND ON URINARY 6-HYDROXYMELATONIN (EXPERIMENT 2)

	NAT (nmol/gl/hr)		HIOMT (pmol/gl/hr)			Urinary 6-hydroxymelatonin (ng/collection)		
Group	Day	Night	Day	Night	Day and Night Pool[1]	Day	Night	Total
Sham	0.38±0.05(6)	31± 5.9(5)	244±29(6)	219± 21(5)	225± 18(11)	30± 7.5(11)	239±40(11)	270±43(11)
Lesion A[2]	0.17±0.07(3)	21± 4.3(5)	172±43(3)	194± 16(5)	186± 18 (8)	17± 2.7 (8)	242±32 (8)	259±32 (8)
Lesion B[3]	0.32±0.08(5)	14 (1)	151±15(5)*	77(1)†	139± 18 (6)*	17± 4.1 (6)	162±17 (6)	178±19 (6)
Lesion C[4]		0.4(1)‡		120(1)	120 (1)	25 (1)	68 (1)§	93 (1)

For further details see the Method section and the text. The numbers in parentheses indicate the size of the group. [1]The pool of HIOMT values are the means of the day and night HIOMT determinations in each group. [2]Animals in this group had partial PVN damage (<30%). [3]Animals in this group had partial PVN damage (30 to 95%). [4]This animal had complete PVN destruction.

*Significantly different from sham group, $p>0.05$.

†Significantly different from sham and lesion 2 groups, $p>0.05$.

‡Significantly different from sham and other groups, $p>0.05$, based on log transformed data.

§Significantly different from sham and lesion 2 groups, $p>0.05$, based on log transformed data.

TABLE 3

EFFECTS OF PVN LESIONS ON PINEAL NAT ACTIVITY, HIOMT ACTIVITY, AND MELATONIN CONTENT, AND ON URINARY HYDROXYMELATONIN (EXPERIMENT 3)

				Urinary 6-hydroxymelatonin (ng/collection)		
Group	Night NAT (nmol/gl/hr)	Night HIOMT (pmol/gl/hr)	Night melatonin (pg/gl)	Day	Night	Total
Sham (N=6)	16.4 ± 2.86	253 ± 23	1068 ± 255	22.6 ± 6.34	306.5 ± 35.8	329.1 ± 41.1
Lesion D* (N=11)	0.68 ± 0.42	54 ± 5.6	146 ± 18	6.2 ± 0.9	26.0 ± 8.1	32.2 ± 8.5
Lesion E† (N=1)	5.3	79	788	7.4	86	94

For further details see the Method section, and text. *Animals in this group had complete PVN destruction. †This animal had partial PVN damage (>95%), with a small number of cells surviving unilaterally dorsal to the fornix.

comprised of animals with <30% destruction of the PVN, typically in the rostral portion. These animals had normal NAT, HIOMT and 6-hydroxymelatonin values.

The second group in Experiment 2, Lesion B, was comprised of animals with approximately 30 to 95% PVN destruction; damage ranged from a complete unilateral PVN lesion to a bilateral rostral lesion which involved at least 50% of the nucleus. These animals exhibited a modest reduction in HIOMT activity, but did not have a significant reduction in urinary 6-hydroxymelatonin. Only a single animal in the Lesion B group had been killed at night, making judgement of the effects of this lesion on night NAT activity values difficult. Within the Lesion B group 5 animals had only 5 to 30% of the PVN remaining, but exhibited nearly normal biochemical indices. It should be added that lesions in the Lesion A and Lesion B groups produced partial destruction of the anterior hypothalamic nucleus and nucleus reunions, but did not involve the dorsomedial nucleus of the hypothalamus (DMH).

The third group in Experiment 2 was comprised of a single animal with a complete PVN lesion (Lesion C). This animal did not have a nocturnal increase in pineal NAT activity, and had low HIOMT activity and urinary 6-hydroxymelatonin values.

Based on the results of Experiment 2, the lesion coordinates were modified, and in Experiment 3 (Table 3) complete bilateral PVN destruction (Lesion D) was produced in eleven of twelve lesioned animals (Fig. 1). Most of these eleven animals also had damage to the dorsal portion of the anterior hypothalamic nucleus, and the dorsorostral portion of the ventromedial nucleus. The rostral or mid DMH, medial portion of the zona incerta, fornix and nucleus reunions were also included within the lesion. However, the SCN and the retrochiasmatic area of the hypothalamus were not damaged.

Animals in the Lesion D group had night urinary 6-hydroxymelatonin values that were 10% of those in the Sham group, lower than that in SCGX animals and similar to those in DEC animals (Table 1). Night pineal melatonin content was reduced about 90%, NAT activity about 98%, and HIOMT activity about 75%. This indicates that a complete PVN lesion blocks neural stimulation of the pineal gland by the SCN. One animal was found to have surviving PVN cells (Lesion E). It had partially reduced indices of melatonin synthesis, suggesting that the lesion did not completely block neural stimulation of the pineal gland.

DISCUSSION

These observations, together with prior histochemical

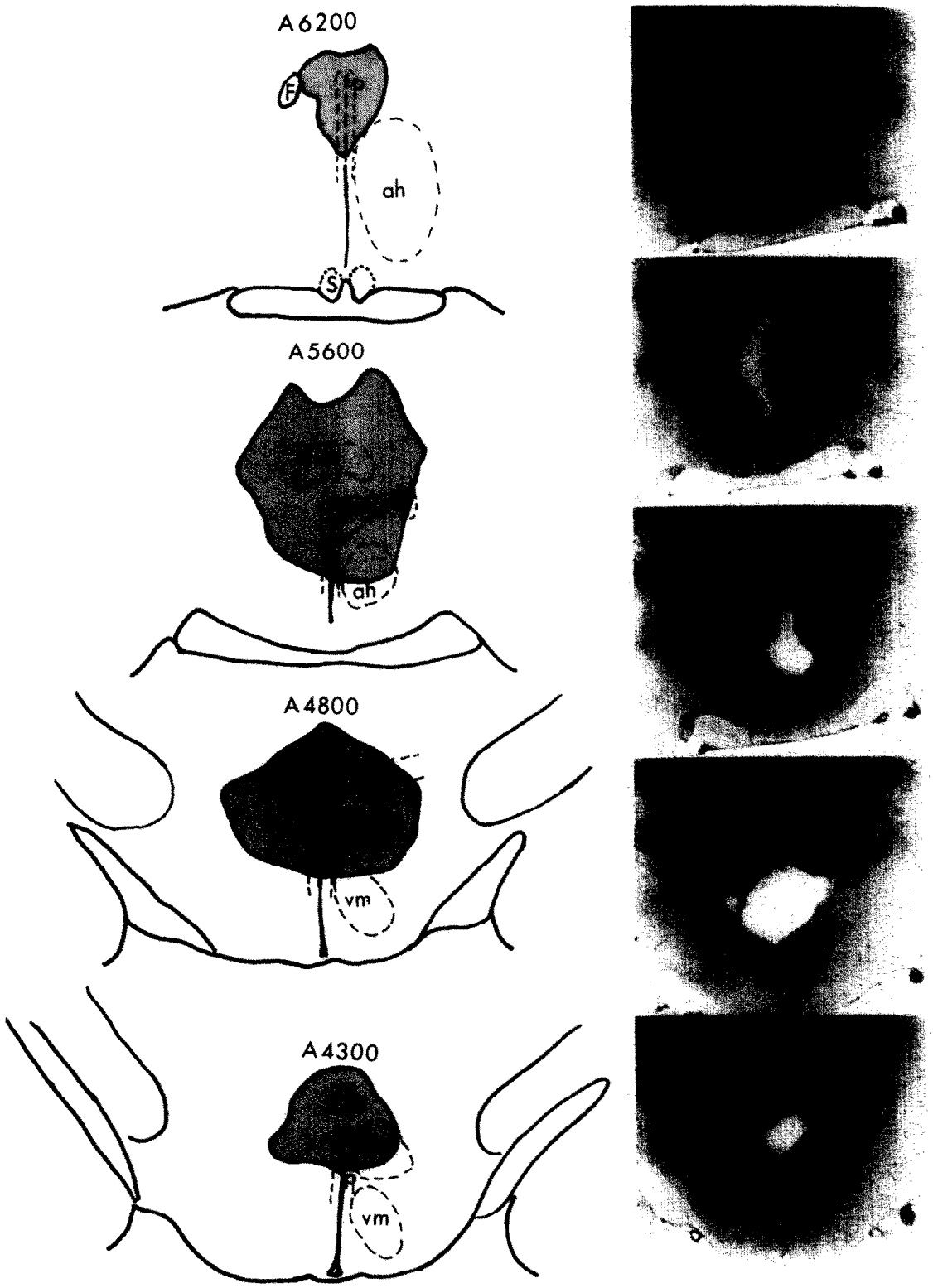

FIG. 1. Diagram and photomicrograph of typical PVN lesions. Left: Projection drawing of the rostral-caudal extent of a PVN-area lesion of one rat. The lesion included the PVN, part of the anterior hypothalamic nucleus (ah), dorsomedial nucleus (dm), periventricular nucleus (p), nucleus reunions (re), ventromedial nucleus (vmn) and the zona incerta (zi). Other abbreviations: F=fornix; S=suprachiasmatic nucleus. Coordinates taken from the atlas of König and Klippel [10]. Right: Coronal sections of the PVN-lesioned area of another rat, thionen stain (20 μM). ($\times$10)

studies identifying SCN→PVN [3] and PVN→spinal cord projections [3, 19, 21–23] support the hypothesis that the PVN is a synaptic relay station in the SCN→spinal cord circuit regulating the pineal gland in the rat and other mammals. However, there are two bodies of evidence that are not totally in concert with this position, both of which deserve discussion. One indicates the pathway passes through the retrochiasmatic hypothalamus, and the second raises the possibility that hypothalamic nuclei near the PVN, rather than the PVN itself, could be the relay station in the SCN→spinal cord circuit.

The observations indicating that the pathway passes through the retrochiasmatic hypothalamus are that SCN neurons project through this area [3, 19, 21], that there is a monosynaptic projection from the retrochiasmatic hypothalamus to the spinal cord [23], and that lesions of the retrochiasmatic hypothalamus block the rhythm in pineal NAT activity and photic stimulation of HIOMT activity [7,15]. However, a reexamination of the lesion which implicated the retrochiasmatic hypothalamus in pineal control indicates it probably would interrupt an SCN→PVN→spinal cord circuit [7,15]. This lesion was produced with a 180° knife cut (Halasz knife: radius=2.4 mm, depth=2.4 mm) at the base of the hypothalamus, extending in the horizontal plane from the fornix on one side, forward to a point just caudal to the optic chiasm and SCN, to the fornix on the other side. The more rostral and medial portions of this lesion would probably destroy an SCN→PVN→spinal cord connection, by either damaging the PVN or by cutting SCN→PVN or PVN→spinal cord connections, or by a combination of these effects. Hence, damage in the retrochiasmatic area may not have been the critical effect responsible for blocking neural stimulation of the pineal gland. Rather, damage to the PVN area may have been the critical event. Consistent with this position is our finding in the present report that it was possible to block neural stimulation of the pineal gland with complete PVN lesions, without damaging the retrochiasmatic hypothalamus.

Thus, if the only possible SCN→spinal cord routes were either via the PVN or the retrochiasmatic hypothalamus, then the former would appear to be the more likely choice. However, it is obvious that we cannot rule out the possibility that the circuit involves both. For example, PVN→spinal cord projections could course to the base of the hypothalamus through the retrochiasmatic hypothalamus. An alternative route, passing first through the retrochiasmatic hypothalamus and then to the PVN seems less likely because this is a less direct route and also because of the prominent periventricular SCN→PVN projections which exist [3, 19, 21].

The second body of evidence which is inconsistent with the SCN→PVN→spinal cord hypothesis raises the possibility that other hypothalamic nuclei are involved; there are two central points. First, lesions in this study which produced total destruction of the PVN also damaged adjacent nuclei, including the DMN and the zona incerta. Second, SCN→DMN projections have been identified [3, 19, 21] and evidence of both DMN→spinal cord and zona incerta→spinal cord projections has been presented [22,23]. However, we feel it is unreasonable to favor the interpretation that either the DMN or zona incerta are synaptic relay stations in the SCN→spinal cord pathway because of several reasons. First, evidence of SCN→zona incerta projections does not exist; second, DMH→spinal cord projections are sparse [22,23], compared to the density of PVN→spinal cord

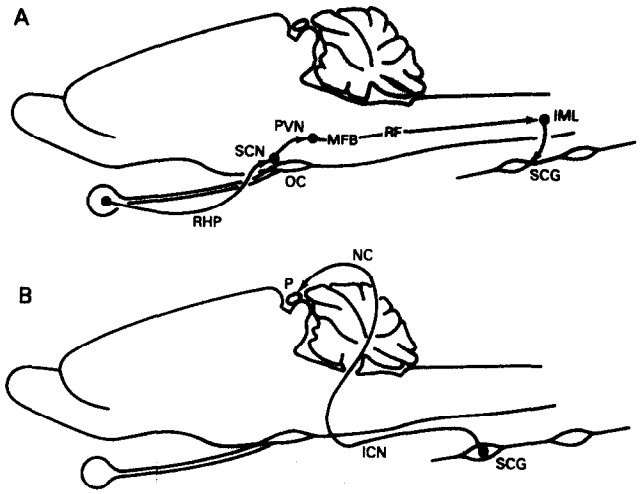

FIG. 2. Proposed circuits in the melatonin rhythm generating system. (A) Cells in the suprachiasmatic nucleus (SCN) receive photic stimulus via a monosynaptic retinal hypothalamic projection (RHP) which courses in the optic nerve and exits at the optic chiasm (OC) [13]. SCN neurons project to the paraventricular nucleus (PVN), and innervate cells which project through the medial forebrain bundle (MFB) and reticular formation (RF) to the intermediolateral cell column (IML) in the spinal cord [7,15]. These projections innervate preganglionic cells which innervate the superior cervical ganglion (SCG) [9]. (B) Postganglionic noradrenergic cells in the SCG course to the pineal gland via the inferior carotid nerve (ICN) and nervii coronarii (NC), which traverses in the tentorium cerebelli [6].

projections; and third, the lesions produced in the current study left a substantial amount of both the zona incerta and DVN intact, while producing complete PVN destruction. It seems probable that remaining cells in either the zona incerta or DMN would have compensated for the loss of neurons due to the lesion, and maintain stimulation of the pineal gland, if they were involved in pineal control.

Based on the above discussion, the results of our studies, and the available histological evidence [3, 19, 21–23], we strongly suspect that the SCN→spinal cord pathway regulating pineal function projects through the PVN (Fig. 2). We also suspect that synaptic contact might be made within the PVN because neither retrograde or anterograde labelling studies have provided evidence of a monosynaptic SCN→spinal cord circuit [3, 19, 21–24], and SCN→PVN projections in the PVN are close to the cells which project to the spinal cord [23].

An interesting point to consider is that our studies indicate complete or nearly complete PVN destruction is required to block stimulation of the pineal gland by the SCN; in specific animals as little as about 5 to 30% of the PVN was sufficient for apparently normal stimulation of the pineal gland as estimated by the parameters measured. Similarly, complete destruction of the SCN in the rat and monkey is necessary to block circadian rhythms in the pineal gland [7, 15, 18]; small surviving SCN areas maintain normal pineal rhythms. This may reflect substantial redundancy within this pathway, or that there is an impressive amount of anatomical or biochemical compensation for neural function lost due to lesioning, or that both redundancy and compensation are involved. Alternatively, it is possible that the specific neurons involved are not limited in location to the apparent boundaries of either the SCN or PVN.

An attractive extension of this line of investigation would

be to electrically or iontophoretically stimulate the PVN and monitor pineal function. An extremely useful endpoint would be urinary 6-hydroxymelatonin, as evidenced by these and previous studies [12,26]. This approach would provide a more detailed description of the role the PVN plays in the neural control of the pineal gland, including the transmitters involved. Regarding this issue, it should be noted parenthetically that the rhythm in pineal NAT appears unaltered in Brattelboro rats [16], indicating that vasopressin is probably not involved as a transmitter in the melatonin rhythm generating system.

The apparent participation of the PVN area in the melatonin rhythm generating system in the rat supports the speculation that SCN→PVN→spinal cord circuits control circadian rhythms in tissues under sympathetic control [24]. However, it will be necessary to examine this hypothesis further with other systems to determine the extent to which this view is correct.

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