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SUPRACHIASMATIC NUCLEUS PROJECTION TO THE MEDIAL PREFRONTAL CORTEX: A VIRAL TRANSNEURONAL TRACING STUDY

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Abstract—The viral transneuronal labeling method was used to examine whether the suprachiasmatic nucleus (SCN) is linked by multisynaptic connections to the medial prefrontal cortex of the rat. In separate experiments, pseudorabies virus (PRV) was injected into one of the three different cytoarchitectonic regions that comprise the medial prefrontal cortex: infralimbic (Brodmann area 25), prelimbic (Brodmann area 32), and cingulate (Brodmann area 24) cortical areas. After 4-days survival, extensive SCN transneuronal labeling was found following infralimbic cortex (ILC) injections, but almost none occurred when the PRV injections were centered in the prelimbic or cingulate areas. In the ILC cases, transneuronal labeling was localized mainly in the dorsomedial SCN, although a moderate number of labeled neurons were found in the ventrolateral SCN. About 13% of the infected neurons were vasopressin immunoreactive and 4% were vasoactive intestinal polypeptide-positive. Another set of experiments was performed in which the paraventricular thalamic nucleus (PVT) was destroyed 2 weeks prior to making PRV injections into the ILC. Almost no SCN transneuronal labeling occurred in these animals, suggesting that the SCN projection to the ILC is dependent on a relay in the PVT.

We propose that the SCN sends timing signals, via its relay in the PVT, to the ILC. This pathway may modulate higher-level brain functions, such as attention, mood, or working memory. Assuming that a homologous circuit exists in humans, we speculate that neurochemical changes affecting this pathway may account for some of the symptoms associated with clinical depression and attention-deficit/hyperactivity disorder.

Key words: attention, cerebral cortex, circadian rhythm, hypothalamus, mood, depression.

The medial prefrontal cortex (mPFC) has been implicated in a number of higher-brain functions, such as selective attention, behavioral flexibility, working memory and regulation of mood (Drevets et al., 1997; Delatour and Gisquet-Verrier, 2000; Dias and Aggleton, 2000; Ragozzino et al., 2002). Many of these functions are subject to diurnal rhythmicity. For example, attention (Kraemer et al., 2000) and mood (Owens et al., 2000; Adan and Sanchez-Turet, 2001) exhibit stereotypical circadian patterning. In addition, disruption of normal biological rhythms alters short-term memory and attention (Cho et al., 2000) and also affects mood (Florida-James et al., 1996). These findings suggest that a common underlying circadian mechanism may affect these higher-level brain functions.

The suprachiasmatic nucleus (SCN) is a probable source of these rhythms since it is the master circadian pacemaker in the brain (Reppert and Weaver, 2001). However, the SCN does not project directly to the mPFC (Watts et al., 1987), indicating that a multisynaptic pathway must be involved. The paraventricular thalamic nucleus (PVT) is potentially the main relay site where information is transmitted from the SCN to cortical sites. This midline thalamic nucleus receives a dense innervation from the SCN and projects to several cortical regions, including the mPFC (Watts et al., 1987; Berendse and Groenewegen, 1991; Moga et al., 1995; Kawano et al., 2001).

The purpose of this investigation was to establish whether the SCN is anatomically linked to the mPFC and thus could modulate higher-brain functions associated with this cortical area. To accomplish this, we used the viral transneuronal labeling method (Loewy, 1998; Aston-Jones and Card, 2000). Pseudorabies virus (PRV) injections were made into selective cytoarchitectonic fields of the mPFC and then, 4 days later, the pattern of SCN transneuronal labeling was studied. Our results indicate that extensive SCN transneuronal labeling occurred after viral injections were made into the infralimbic cortex (ILC), but not in other mPFC areas. A second experiment demonstrated that this pathway was dependent on a relay in the PVT. These data are discussed in terms of the circadian effects on higher-brain functions, such as attention and mood.

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Abbreviations: AVP, arginine vasopressin; CTb, cholera toxin β-subunit; ILC, infralimbic cortex; mPFC, medial prefontal cortex; PRV, pseudorabies virus; PVT, paraventricular thalamic nucleus; SCN, suprachiasmatic nucleus; VIP, vasoactive intestinal polypeptide.

EXPERIMENTAL PROCEDURES

Sprague–Dawley rats (female, wt = 240–325 g, Harlan Lab., Indianapolis, IN, USA) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), placed in a stereotaxic apparatus and skull leveled. After a craniotomy, a glass micropipette (tip = 25 μm) containing a mixture of the Bartha strain of PRV and cholera toxin β-subunit (CTb, product #103B, List Biologicals, Campbell, CA, USA) was advanced into a mPFC target based on coordinates from a rat brain atlas (Paxinos and Watson, 1998). All injections were made on the right side of the brain. The injectate was made as follows: 2 μl of 0.1% CTb solution was added to a 5-μl aliquot of Bartha PRV (viral titer = 1×10^8 plaque-forming U/ml; Dr. K. Platt, Iowa State University, Ames, IA, USA). The volume delivered was 40 nl; ≈2000 virions were injected. The CTb was used to localize the injection site (Chen et al., 1999).

The stereotaxic coordinates for the ILC were: bregma = +2.6 mm, lateral = 1.0 mm; and depth = 4.5 mm ($n = 6$) (Paxinos and Watson, 1998). In other rats, additional injections were made into the prelimbic cortex ($n = 6$) and in the cingulate cortex ($n = 7$).

To determine whether the PVT was the key relay point for the SCN projection to the mPFC, another set of rats were prepared that had chronic midline thalamic lesions ($n = 15$). The animals were anesthetized as described above, placed in a stereotaxic apparatus and a midline craniotomy was performed 1.3–5.0 mm caudal to the bregma. The rostral and caudal limits of the superior sagittal sinus were ligated. Then, a 6-cm sterile stainless spatula with a blade that measured 4.0 mm × 0.9 mm was clamped into position on a micromanipulator and the blade was slowly advanced 7.0 mm into the brain from the dorsalmost surface of the cerebral cortex. As determined during the post-hoc analysis, this caused a 4.0-mm long plane of destruction through the PVT. Two weeks later, PRV injections were made into the ILC (as described above) and the rats processed as described below.

All of the rats used in this study received an intraventricular injection of colchicine 3 days after the PRV injection in order to increase neuropeptide levels for immunohistochemical visualization. The rats were anesthetized with sodium pentobarbital (as above), placed in a stereotaxic apparatus and colchicine (100 μg/10 μl of sterile saline; Sigma, St. Louis, MO, USA) was injected into the right lateral ventricle with a 50-μl microsyringe that was held with a micromanipulator. After 24 h, the rats were anesthetized, perfused through the heart with 0.9% saline, followed by 4% paraformaldehyde made in 0.1 M sodium phosphate buffer (pH = 7.4).

The brains were removed and stored in fixative for 3–7 days prior to processing for immunohistochemistry. Transverse sections of the brain were cut on freezing microtome. A complete 1-in-5 series of transverse sections (50 μm thick) through the forebrain was immunostained for CTb to localize the injection site. The sections were reacted with a goat anti-CTb antibody (1:40 000; List) followed by a biotinylated donkey anti-goat solution (1:200; Jackson ImmunoResearch, West Grove, PA, USA). The avidin–biotin complex method (ABC, Vectastain kit, Vector Laboratories, Burlingame, CA, USA) was used in conjunction with diaminobenzidine (DAB; SigmaFast; 1 tablet/15 ml distilled water; Sigma) as the chromagen (ABC/DAB method). After the sections were mounted on gelatinized glass slides, they were counter-stained with 0.6% Thionin, and coverslipped using DPX mountant (Poole, UK). The sections containing the injection sites were scanned with a Hewlett-Packard flat bed scanner, imported into the Adobe Photoshop 5.0 program, and traced using the CorelDraw 8.0 program (Corel, Ottawa, ON, Canada).

Sections through the SCN were immunostained by a double-color procedure. All solutions were made in a 0.1 M potassium phosphate buffer (pH = 7.4) containing 1.0% normal donkey serum (KPBS solution). The sections were first placed in the KPBS buffer solution containing pig anti-PRV (1:25 000, K. Platt) and guinea-pig anti-arginine vasopressin (AVP; 1:20 000, product no. GHC-8103; Peninsula Lab., Belmont, CA, USA). After 24 h, they were washed with KPBS solution containing donkey serum and transferred to a biotinylated rabbit anti-pig antibody solution (1:100; Sigma) for 3 h. The sections were washed in KPBS solution, and placed in the ABC solution for 1 h, washed, and transferred to KPBS solution containing Cy 3-strepavidin (1:75; Molecular Probes, Eugene, OR, USA) and Cy 2-donkey anti-guinea-pig (1:300; Jackson ImmunoResearch, West Grove, PA, USA) for 2 h. The sections were washed in KPBS solution, mounted on gelatinized slides, air-dried and coverslipped with a glycerol anti-fade solution containing 0.1% *n*-propyl gallate (Sigma) and 0.01% sodium azide (Sigma). A second set of sections were co-stained for PRV and vasoactive intestinal polypeptide using a rabbit antibody (VIP, 1:5000; product #982, Chemicon, Temecula, CA, USA). The same procedure was followed, except that the final staining solution contained Alexa 488-strepavidin (1:75; Molecular Probes) and Cy 3-donkey anti-rabbit (1:200; Jackson). Sections were examined using a conventional fluorescence microscope and the distribution of PRV and PRV+neuropeptide-containing (either AVP or VIP) neurons were mapped in three sections through the SCN that were spaced at 200-μm intervals. The data were recorded using an X–Y plotting system (AccuStage, Shorewood, MN, USA). Single PRV-positive neurons that also contained AVP or VIP were identified as double-labeled cells. These neurons had matched somatic and dendritic morphology and were identified at a single focal plane in order to avoid false positive labeling due to overlap of separate neurons.

In order to evaluate the extent of the thalamic lesions, sections from PVT-lesioned rats were reacted with a monoclonal antibody against glial fibrillary acidic protein (GFAP, clone G-A-5 ascites fluid, 1:500; product no. 3893; Sigma). Free-floating sections through the thalamus were incubated in anti-GFAP made in KPBS solution for 24 h. The sections were washed, transferred to biotinylated donkey anti-mouse (1:200, Jackson) for 3 h and processed by the ABC/DAB method (see above). The sections were dried and then counter-stained with Thionin (see above).

Photoimages were taken either with a DAGE-MIT SIT 68 digital camera (Michigan City, IN, USA) or a Canon digital camera (D30) and then transposed by Adobe Photoshop 5.5 program to create the photomicrographs used in this report. Except for brightness and contrast adjustments, no other changes were made of the data.

The research studies performed here were reviewed and approved by the Washington University School of Medicine Animal Care and Biological Safety Committees and conformed to NIH guidelines.

RESULTS

PRV was injected into three different cytoarchitectonic fields of the mPFC. Cases that had injections confined to cortical layers 5 and 6 in the ILC, prelimbic or cingulate

Abbreviations used in the figures

3V	third ventricle	PLC	prelimbic cortex
AM	anteromedial thalamic nucleus	PT	paratenial thalamic nucleus
CG	cingulate cortex	Re	nucleus reuniens
Ox	optic chiasm	Rt	reticular thalamic nucleus

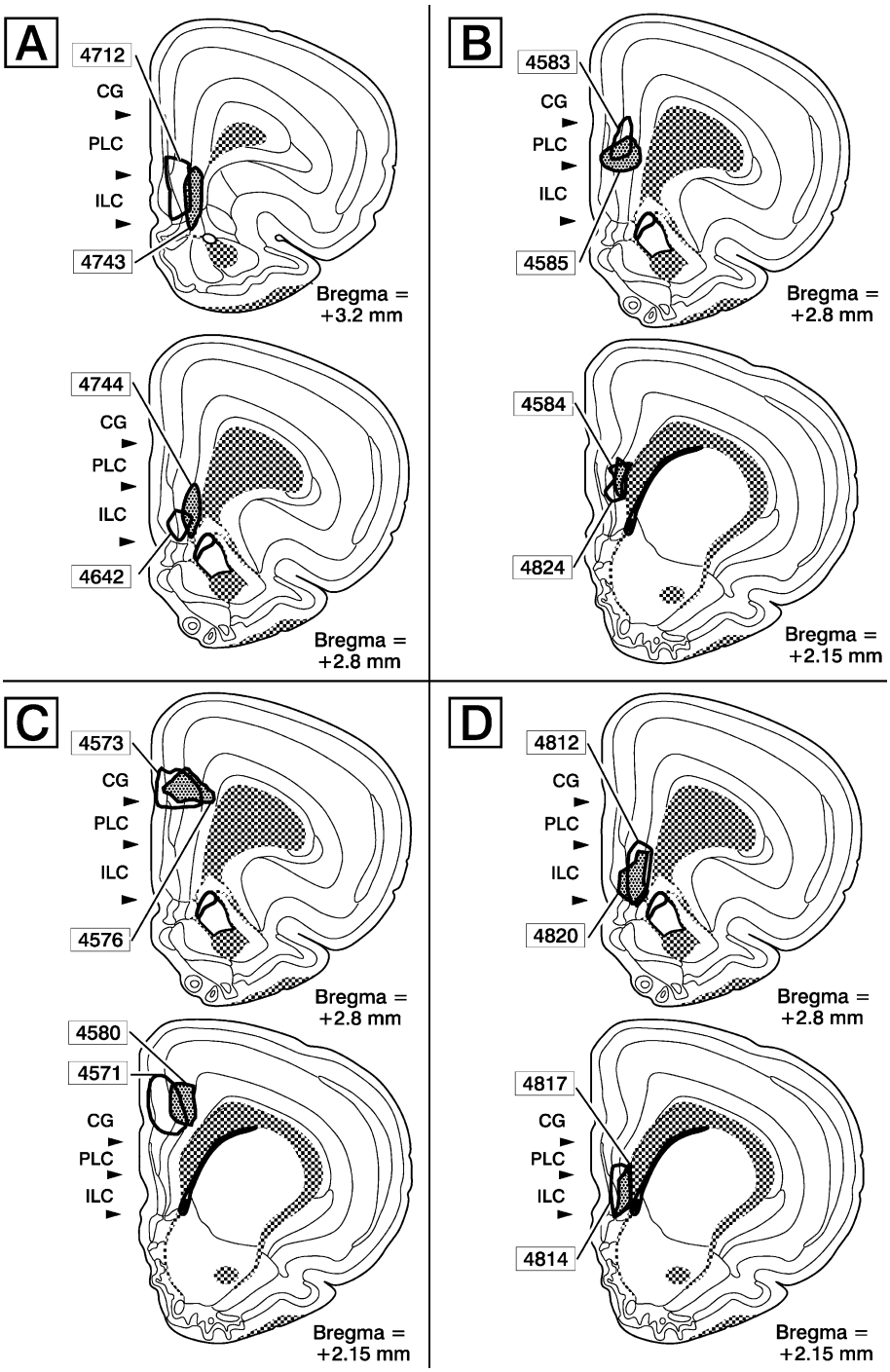

Fig. 1. A cocktail of PRV and CTb was injected into different cytoarchitectonic regions of the medial prefrontal cortex (mPFC): (A) infralimbic cortex (ILC), (B) prelimbic cortex (PLC), (C) cingulate cortex (CG), and (D) ILC of rats that had lesions of the paraventricular thalamic nucleus (PVT). Because PRV is rapidly cleared from the neuropil, the injection sites were determined on the basis of the extent of the CTb immunostaining in the mPFC. Four representative cases from each type of experiment are shown. Drawings were adapted from (Swanson, 1996).

cortical areas were selected. Preliminary studies indicated that injections centered on superficial layers (e.g. layer 3 of ILC), but that did not involve the deeper cortical layers, failed to cause robust transneuronal labeling in the SCN. In addition, cases that were centered in the deep layers of the ILC but that spread into the lateral septal nuclei, indusium griseum, or rostral part of the corpus callosum were excluded from the study. Thus, the present analysis is based on PRV experiments confined either to the ILC, prelimbic cortex, or cingulate cortex (Fig. 1). Figure 2 presents a photomicrograph of an ILC injection site.

To test the hypothesis that the PVT is the key relay site for the SCN → ILC circuit, another group of rats was

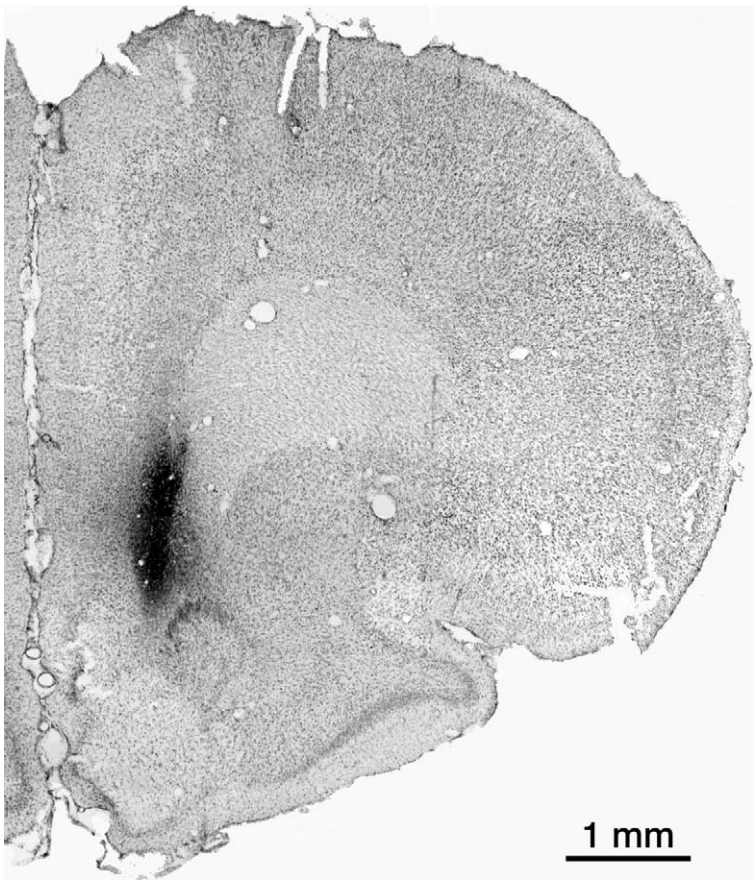

Fig. 2. Photomicrograph illustrating a CTb-PRV injection site in the ILC (case #4744).

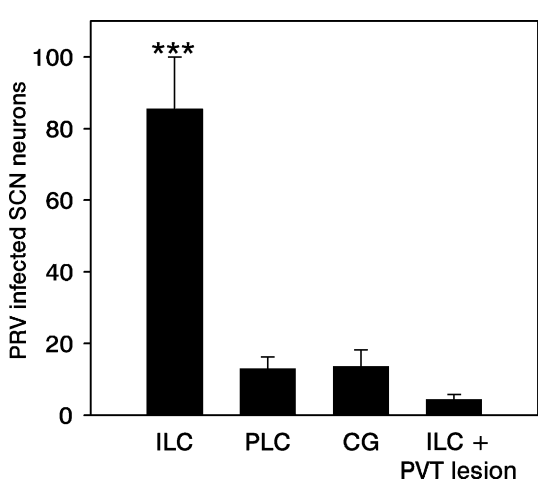

Fig. 3. Bar graph showing the mean number (±S.E.M.) of transneuronally labeled neurons in the ipsilateral suprachiasmatic nucleus (SCN) following PRV injections into the mPFC. Counts were based on three sections spaced at 200-μm intervals through the SCN of each case. The ILC injections resulted in statistically higher (***) SCN transneuronal labeling than that found after injections into either the prelimbic (PLC) or cingulate (CG) cortical regions ($P < 0.001$; ANOVA and Tukey's pairwise comparison test). In addition, the number of PRV labeled neurons in the SCN was significantly higher in the normal ILC cases versus ILC cases that had midline lesions that destroyed the PVT ($P < 0.001$).

used. These rats had chronic midline lesions that destroyed the PVT. After 2 weeks recovery time, PRV was injected into the ILC of these rats. The rats used in this analysis were selected on the basis of two criteria: PRV injections that were centered in the ILC (Fig. 1) and the near complete destruction of the PVT. Out of a total of 15 experiments, four were selected that met these criteria. In each case, however, a small amount of the lateralmost wing of the anterior PVT was left intact. The extent of the thalamic lesion was assessed by examination of the immunofluorescence sections through the PVT region and by adjacent bright-field histological sections stained with GFAP and Thionin. Two of the cases had no PRV retrograde labeling in the PVT, and the two other cases had two and 15 labeled neurons, respectively, in the two rostralmost sections through the PVT. However, this did not appear to correlate with the number of transneuronally labeled cells in the SCN. For example, in case #4820, 15 labeled neurons were found in the PVT, but only one PRV labeled neuron was found in the entire SCN.

The mean values ± S.E.M. of PRV labeled SCN neurons for the individual cases (Fig. 3) (ILC = 85.5 ± 14.4; prelimbic cortex = 13.0 ± 3.3; cingulate cortex = 13.7 ± 4.6; ILC with PVT lesion = 4.5 ± 1.3) were compared for possible differences using a single multifactorial analysis of variance (ANOVA). The ANOVA showed a significant difference between the groups, and a subsequent Tukey's pairwise comparison test revealed that the num-

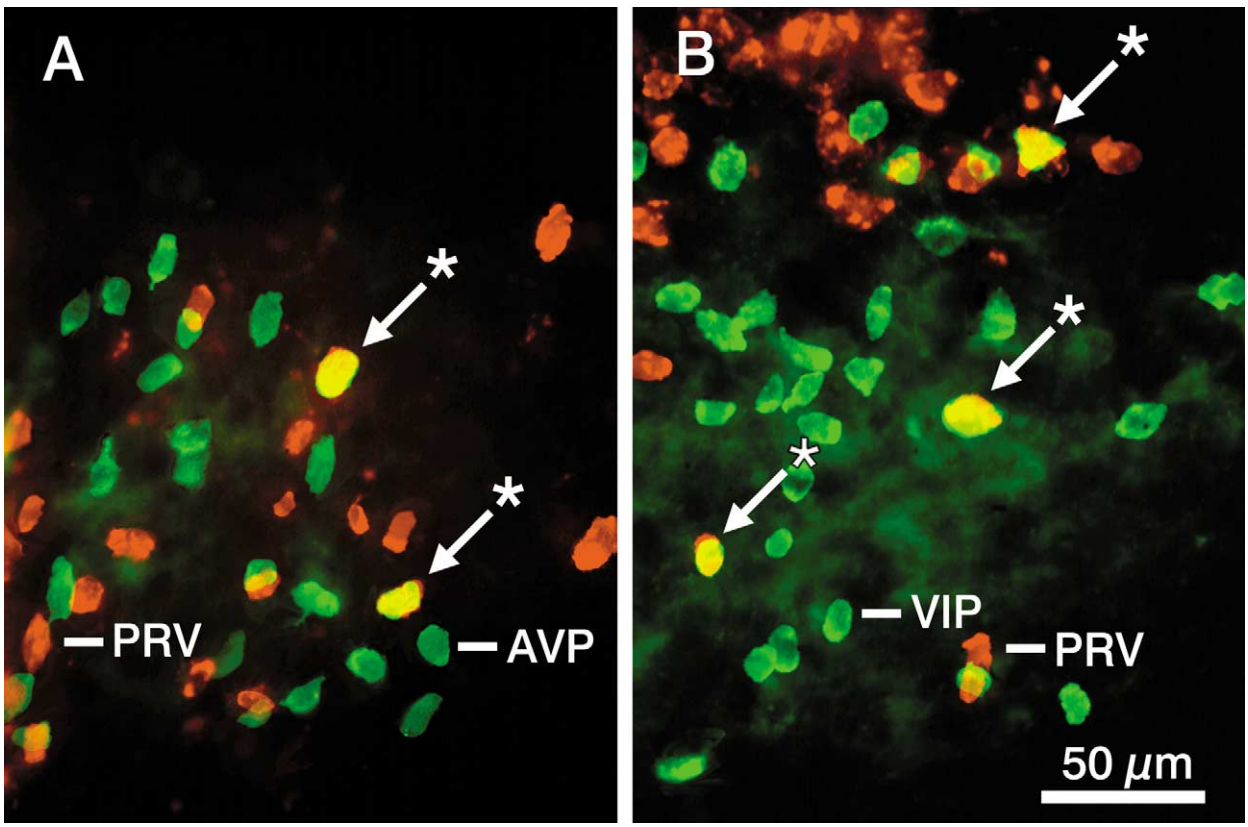

Fig. 4. (A) Photomicrograph of the dorsomedial SCN showing arginine vasopressin (AVP = green), PRV (red), and AVP+PRV (yellow; see arrows) immunoreactive neurons. The PRV injection was centered in the ipsilateral ILC (case #4744). (B) Photomicrograph of vasoactive intestinal polypeptide (VIP = green), PRV (red), and VIP+PRV (yellow; see arrows) immunoreactive neurons in the ventrolateral SCN from the same case.

ber of PRV labeled SCN neurons following injection into the ILC was significantly higher than following injections into the prelimbic cortex ($P < 0.001$), cingulate cortex ($P < 0.001$), or ILC with a PVT lesion ($P < 0.001$). No statistical difference was found in the PRV counts among the latter three groups.

To determine the SCN neuropeptide phenotypes that contribute to the ILC projection system, SCN sections from the ILC cases were immunostained with both AVP and PRV; a second series of sections was reacted for VIP and PRV (Fig. 4). We found that $13.7 \pm 0.9\%$ (mean ± S.E.M.) of PRV labeled cells in the SCN were AVP-positive and $4.0 \pm 1.0\%$ were VIP immunoreactive.

When the normal ILC cases (i.e. without midline thalamic lesions) were compared to the ILC experiments from rats with chronic PVT lesions, some major differences were apparent. First, the number of SCN transneuronally labeled neurons between the two groups was statistically different at the $P < 0.001$ level (Fig. 3). Second, the pattern of cell body labeling in the thalamus was different as well. In the normal cases, large numbers of retrogradely labeled neurons were found in four thalamic nuclei: the PVT, and paratenial, anteromedial and reuniens thalamic nuclei. In the experimental group, the PVT was almost devoid of retrograde cell body labeling, and the three other thalamic nuclei showed a substantial reduction in the numbers of retrogradely labeled neurons (Fig. 5, middle panel). Third, there was prominent transneuronal labeling in the reticular thalamic nucleus, especially in its medialmost section, in the normal ILC experiments but in the experimental cases this was greatly reduced (Fig. 5, middle panel).

DISCUSSION

The present study demonstrates that the SCN is linked to a specific part of the mPFC, viz., the ILC, and that this pathway is dependent on a relay in the PVT. AVP- and VIP-containing SCN neurons contribute to this projection. However, since the VIP neurons may innervate vasopressin SCN neurons (Ibata et al., 1993; Romijn et al., 1997) as well as project to the PVT (Watts et al., 1987), it is not clear whether the labeled VIP neurons represent second- or third-order neurons in the SCN → PVT → ILC circuit.

Technical considerations regarding viral tracing experiments

The viral labeling method is an extremely useful technique for the analysis of multisynaptic central circuits (Kaufman et al., 1996; Jasmin et al., 1997; O'Donnell et al., 1997; Carr et al., 1999; Aston-Jones et al., 2001), but it has certain limitations (Chen et al., 1999; Aston-Jones and Card, 2000). First, it is difficult to localize

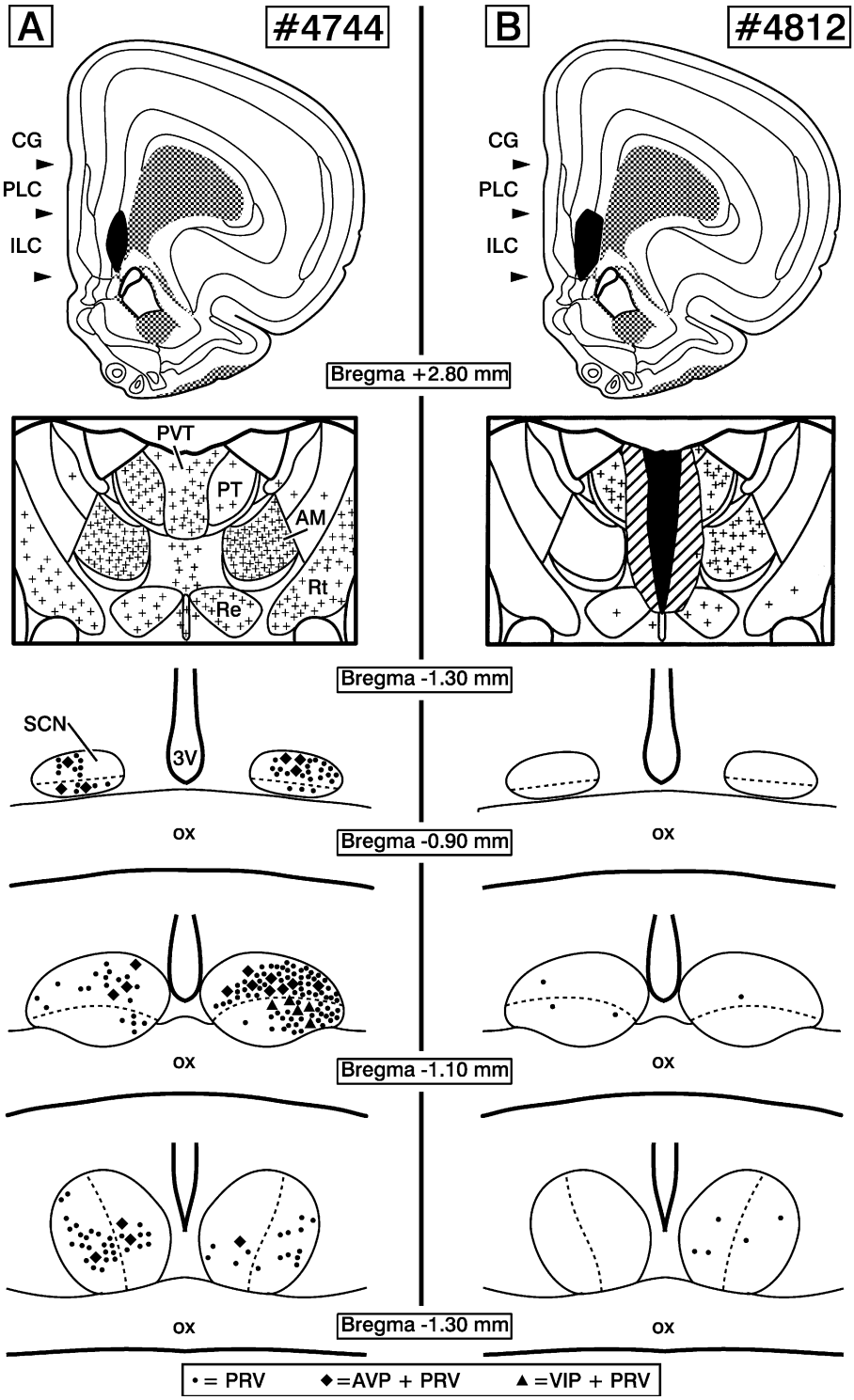

Fig. 5. Diagram showing the pattern of viral labeling following a PRV injection into the ILC in (A) a normal rat and (B) a PVT-lesioned rat. Top panel: Black areas indicate the PRV injection site in the ILC. Middle panel: Distribution of PRV cell body labeling ($^{+}=10$ virally labeled neurons) in the thalamus. The black area indicates the midline lesion and the diagonal shading illustrates the adjacent area that had a severe gliosis as determined by GFAP staining. Lower panels: Three sections through the SCN are presented showing the distribution of PRV transneuronal labeling. Drawings were adapted from (Swanson, 1996; Paxinos and Watson, 1998).

PRV injection sites using only the immunohistochemical staining of PRV because viruses are rapidly taken up by neurons and glial cells in the first 6 h after an injection is made (Card et al., 1999). When animals are allowed to survive for periods of several days, as was done in the present experiments, some other type of marker has to be used to approximate the PRV injection sites. Following the approach used by Chen et al. (1999), a cocktail of

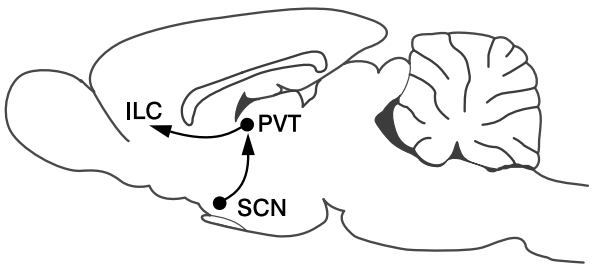

Fig. 6. A schematic diagram showing the proposed ascending neural pathway from the SCN to the ILC. Drawing modified from (Paxinos and Watson, 1998).

CTb and PRV was injected into different mPFC sites. Since CTb remains present in the neuropil for several days, immunohistochemical staining of CTb was used to approximate the viral injection sites. While CTb injection sites are approximately twice as large as PRV injection sites (Chen et al., 1999; Aston-Jones and Card, 2000) and thus overestimate the size of the PRV injection sites, this approach allowed us to select a series of cases in which the injections were confined to specific regions of the mPFC.

Second, the viral tracing method cannot be used with a high level of accuracy to determine the number of neurons that make up a neural circuit because several factors complicate the interpretation of the data generated in these types of experiments. These factors include the difficulty of determining whether local interneurons are interposed within the SCN → PVT → ILC circuit. In addition, there may be multiple pathways by which a viral infection may spread in the CNS to reach the same afferent target. Previous neuroanatomical studies have demonstrated that the ILC receives direct inputs from several sources. These include the thalamus (viz., PVT, anteromedial, mediodorsal, paratenial, reuniens and rhomboid thalamic nuclei), hippocampus (viz., ventral subiculum and ventral CA1 field), amgydala (viz., amygdalohippocampal area, amygdalopiriform transition area and basolateral amygdaloid nucleus), lateral hypothalamic area and ventral tegmental area (Swanson, 1981, 1982; McDonald, 1987, 1991; Conde et al., 1990, 1995; Berendse and Groenewegen, 1991;). Although this hypothesis was not tested, it is possible that longer survival periods (i.e. 5 or more days) in PVT-lesioned material could result in SCN labeling from one of these alternative pathways. Nevertheless, the simplest explanation of the present data is that the SCN modulates the mPFC by a two-neuron circuit, viz., the SCN → PVT → ILC pathway (Fig. 6). This model assumes that the PVT is composed of only projection neurons and that this nucleus has no local interneurons (Frassoni et al., 1997).

A third limitation of the viral tracing technique is that PRV may be taken up by fibers-of-passage. This issue is particularly important when the viral tracing method is used to analyze central circuits. In order to avoid this problem, we carefully selected cases on the basis that these injection sites were centered in the deep layers of the mPFC and did not spread laterally into the underlying white matter or caudally into the lateral septum or nucleus accumbens. The latter two areas receive dense inputs from the PVT (Berendse and Groenewegen, 1991; Moga et al., 1995) and conceivably could also be influenced by the SCN.

PRV injections into the prelimbic cortex or the cingulate cortex resulted in very low amounts of SCN transneuronal labeling (Fig. 3). The former result is consistent with earlier studies which suggest that the prelimbic area receives a very weak input from the PVT (see figure 2 of Moga et al., 1995 and figure 3 of Berendse and Groenewegen, 1991). PRV injections into the cingulate cortex resulted in sparse SCN transneuronal labeling. While the PVT does not innervate this region, it is important to note that the cingulate, prelimbic and infralimbic regions are reciprocally connected (Fisk and Wyss, 1999). The low amount of SCN labeling following cingulate cortex injections could be the result of viral spread within cortico-cortical circuits, followed by subsequent retrograde viral transport to the SCN via the PVT. However, additional experiments would be required to examine this possibility.

Fourth, AVP- and VIP-containing SCN neurons were found to be components of the SCN → PVT → ILC circuit. This finding is consistent with earlier work that indicated that both of these SCN neuronal phenotypes innervate the PVT (Watts and Swanson, 1987). However, since VIP-containing axon terminals are found on the AVP-containing SCN neurons (Ibata et al., 1993; Romijn et al., 1997) and have been assumed to originate from the VIP neurons lying in the ventrolateral SCN, this would suggest that the transneuronal labeling seen in this phenotype could possibly represent third-order transneuronal labeling. In any event, the neurochemical organization of this pathway is still unclear and further complicated because almost all SCN output neurons contain GABA (Okamura et al., 1989; Francois-Bellan et al., 1990; Moore and Speh, 1993). This raises the possibility that SCN outflow to the PVT may be an inhibitory system. In other studies, histochemical data indicate that the PVT projection neurons contain aspartate or glutamate (Frassoni et al., 1997) and this suggests that this limb of this circuit may provide an excitatory drive to the ILC.

Potential function of the SCN–mPFC pathway

Although the function of the SCN → PVT → ILC pathway is unknown, there is sufficient circumstantial evidence to suggest that it may be involved in modulating some higher-level brain functions which exhibit circadian

rhythms, such as mood and attention. Since these data come principally from human studies, the assumption of the following discussion is that there is a circuit in the human brain that is homologous to that which has been demonstrated here for the rat.

The evidence that the mPFC is involved in the regulation of mood comes from positron emission tomography and functional magnetic resonance imaging studies in humans (Drevets et al., 1997). Blood flow and size of the mPFC is reduced in individuals with unipolar depression while patients with bipolar disorder exhibit alternative bouts of decreased and increased blood flow to the mPFC that correlate with periods of depression and mania, respectively (Drevets et al., 1997). Additional work has shown that disruption of normal biological rhythms affects mood. For example, individuals working nocturnal shifts consistently report worse moods than daytime workers, a condition referred to as shift work maladaptation syndrome (Smith-Coggins et al., 1994; Florida-James et al., 1996). The mood of the night workers can be dramatically improved by employing strategies that reset the circadian pacemaker to nighttime activity via phase shifting (Eastman et al., 1994). Finally, Zhou and colleagues reported that the mRNA levels for vasopressin are reduced in the SCN from autopsy material from individuals with a clinical history of depression (Zhou et al., 2001). When all of these reports are considered along with the present findings, it is possible that the SCN → PVT → ILC circuit may provide timing cues that modulate mood.

The mPFC also plays an important role in attention in rats (Granon et al., 1998; Broersen and Uylings, 1999; Delatour and Gisquet-Verrier, 2000; Gill et al., 2000; Granon et al., 2000) and in humans (Carretie et al., 2001; Gusnard et al., 2001). For example, the frontal midline theta rhythm of humans, which reflects focused attention, is thought to be generated by the mPFC (Ishii et al., 1999). Because attention exhibits clear diurnal variation (Gillooly et al., 1990; Mathur and Bhattacharya, 1991; Carrier and Monk, 2000; Kraemer et al., 2000), it is reasonable to hypothesize that the SCN affects levels of attention. Direct evidence that the SCN may modulate attention comes from behavioral studies of a patient with a midline hypothalamic lesion that destroyed the SCN (Cohen and Albers, 1991; Cohen et al., 1997). This patient's scores on repetitions of the same cognitive tests revealed inconsistent performance; she was quite successful at certain times but failed the same tests at other times during the day. Cohen and coworkers suggested that her ability to properly regulate the timing of attentional focus had been disrupted (Cohen et al., 1997).

Children with attention-deficit/hyperactivity disorder (ADHD) show disruptions in functions modulated by the SCN, such as circadian rhythms of cortisol levels and sleep–wake cycle (Kaneko et al., 1993; Gruber et al., 2000). It is possible that dysfunction of the SCN may be responsible for many of the symptoms associated with the disorder. For example, the somatomotor hyperactivity could be viewed as analogous to wheel-running in rodents, a behavior shown to be modulated by the SCN (Stephan and Zucker, 1972). While it has not yet been possible to detect whether there are functional changes in SCN of ADHD patients, magnetic resonance imaging studies suggest that there is some pathological involvement of the frontal lobes (Baumeister and Hawkins, 2001; Giedd et al., 2001). When these clinical observations are considered in light of the present anatomical data, it is reasonable to hypothesize that abnormal timing signals generated from the SCN may contribute to the cognitive, sleep–wakefulness and neuroendocrine changes seen in this disorder.

CONCLUSION

In summary, the present study has demonstrated the existence of a SCN → PVT → ILC circuit (Fig. 6). Based on the neuroanatomical evidence presented here, we postulate that the SCN may send timing cues to the mPFC and thus could affect a number of higher-level functions associated with this region, including attention, mood and working memory.

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