GABA and glutamate mediate rapid neurotransmission from suprachiasmatic nucleus to hypothalamic paraventricular nucleus in rat

Michael L. H. J. Hermes*†‡, Elaine M. Coderre*, Ruud M. Buijs† and Leo P. Renaud*

*Neurosciences, Loeb Research Institute, University of Ottawa and Ottawa Civic Hospital, 1053 Carling Avenue, Ottawa, Ontario, Canada K1Y 4E9 and †Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, The Netherlands

1. Intracellular sharp electrode and whole-cell patch-clamp recording from characterized paraventricular nucleus (PVN) neurones in rat hypothalamic slices were used to study the synaptic mechanisms and associated neurotransmitters that mediate their response to suprachiasmatic nucleus (SCN) stimulation.

2. Electrical stimulation restricted to SCN evoked short-latency inhibitory postsynaptic potentials (IPSPs) or combinations of IPSPs and excitatory postsynaptic potentials (EPSPs) in all ($n = 59$) PVN neurones tested. Type I neurones ($n = 18$) were magnocellular and a majority (13/18) demonstrated monosynaptic IPSPs that reversed polarity at the chloride equilibrium potential and were sensitive to bicuculline.

3. Type II ($n = 10$) and III parvocellular ($n = 13$), and unclassifiable neurones ($n = 18$) displayed combinations of IPSPs and EPSPs following similar stimuli applied to SCN. IPSP blockade with bicuculline uncovered SCN-evoked monosynaptic dual-component EPSPs that were sensitive to *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists. In addition, chemical microstimulation within SCN was associated with transient increases in spontaneous EPSPs recorded from these PVN neurones.

4. These data imply that the amino acids GABA and glutamate are important mediators of fast monosynaptic transmission from SCN to defined neurones in PVN, and are candidates for conveying circadian rhythmicity to PVN regulation of neuroendocrine and autonomic processes.

The mammalian suprachiasmatic nucleus (SCN), a chemically heterogeneous population of parvocellular neurones located in the rostroventral hypothalamus, is site of origin of circadian rhythms in various physiological and behavioural processes (Moore & Eichler, 1972; Stephan & Zucker, 1972; Inouye & Kawamura, 1979; Ralph, Foster, Davis & Menaker, 1990). SCN neurones, displaying circadian rhythmicity in electrical activity (Groos & Hendriks, 1982; Shibata, Liou, Ueki & Oomura, 1984; Welsh, Logothetis, Meister & Reppert, 1995), are believed to entrain their rhythm through the rhythmic release of transmitters at defined axon projection sites within the CNS (Earnest & Sladek, 1986; Schwartz, Gross & Morton, 1987; Watts, Swanson & Sanchez-Watts, 1987).

Despite considerable knowledge of the anatomy and neurochemical content of SCN efferents, there remains controversy as to the precise identity of either the cellular targets or the transmitters that participate in the expression of SCN rhythmicity. Conceivably an important target for SCN efferents is the hypothalamic paraventricular nucleus (PVN), since its neurones subserve important regulatory roles in neuroendocrine and autonomic functions that display circadian rhythmicity (Swanson & Sawchenko, 1983). Indeed, an electrophysiological analysis using *in vivo* extracellular recording techniques has recently provided evidence for the existence of functional SCN inputs to various types of PVN neurones, identified by antidromic activation as projecting to the posterior pituitary, median eminence, or dorsomedial medulla (Hermes & Renaud, 1993*a*). However, since several anatomical investigations suggest that SCN fibres innervate only a zone ventral to PVN (the subparaventricular zone: Watts *et al.* 1987; Buijs, Hou, Shinn & Renaud, 1994), the question remains as to whether these observed influences of SCN on PVN neurones

‡To whom correspondence should be addressed at the Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, The Netherlands.

are mediated directly, i.e. monosynaptically, or through interneurones located in the subparaventricular zone.

To investigate functional aspects of the SCN innervation of PVN in more detail, the present study utilized conventional intracellular sharp electrode and whole-cell patch-clamp recording techniques in an *in vitro* slice preparation of the hypothalamus. The objectives were to identify the properties of SCN-evoked postsynaptic potentials (PSPs), to determine a mono- or polysynaptic generation, and to characterize the neurotransmitters mediating the responses. In agreement with previous reports (Hoffman, Tasker & Dudek, 1991; Tasker & Dudek, 1991), our results reveal the presence of at least three different types of PVN neurones distinguished on the basis of their electrical and morphological properties. We have observed that SCN stimulation preferentially evokes monosynaptic inhibitory postsynaptic potentials (IPSPs) in type I magnocellular neurones, and combinations of monosynaptic IPSPs and excitatory postsynaptic potentials (EPSPs) in type II and III parvocellular, and unclassifiable neurones. The IPSPs are sensitive to the $GABA_A$ receptor antagonist bicuculline whereas EPSPs have components that are sensitive to *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists. These results have been reported briefly (Hermes & Renaud, 1993*b*; Hermes, Spanswick & Renaud, 1994).

METHODS

Slice preparation

Adult male Long–Evans or Wistar rats (Long–Evans rats obtained from Charles River, St Constant, Quebec, Canada; Wistar rats obtained from TNO, Delft, The Netherlands; both 175–350 g), housed in a temperature-controlled (21 ± 1 °C) environment on a 12 h light–12 h dark schedule (lights on at 07.00 h, circadian time 0), were used for this study. To minimize inadvertent effects of anaesthetics, animals were killed by decapitation using a guillotine (between 10.00 and 15.00 h), and the brains rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF). Following 1–2 min of cooling, a hypothalamic block was dissected and 400–500 μm-thick oblique transverse slices cut on a vibratome (model 1000, Vibratome, Pelco Int., Redding, CA, USA), so as to retain SCN and PVN in the same section. After incubation for at least 1 h at room temperature (20–25 °C) in ACSF equilibrated with 95% O_2 and 5% CO_2, slices were transferred to a recording chamber where they were submerged in gassed ACSF. Temperature of the ACSF was 33–35 °C for sharp electrode recordings and room temperature for whole-cell patch-clamp recordings. Flow rate of the gravity-fed ACSF ranged from 1·5 to 5·0 ml min^{-1}.

Solutions and drugs

The ACSF contained (mM): NaCl, 123; KCl, 3; $CaCl_2$, 2·4; $MgSO_4$ or $MgCl_2$, 1·3; $NaHCO_3$, 26; NaH_2PO_4, 1·25; glucose 10; pH 7·35, osmolality 295–305 mosmol kg^{-1}. Sharp electrodes were filled with 3 M potassium acetate or 1·5 M potassium acetate containing 1·5% biocytin (Sigma). Patch pipettes contained (mM): potassium gluconate, 130; KCl, 10; Hepes (pH 7·4 with KOH), 10; EGTA, 0·5; Na_2ATP, 2; biocytin, 5; pH 7·3 with NaOH, osmolality 270–280 mosmol kg^{-1}. Drugs included tetrodotoxin (TTX; Sigma), monosodium glutamate (Sigma), bicuculline methiodide (BMI; Sigma), bicuculline methochloride (BMC; Tocris Cookson Ltd, Bristol, UK), D-2-amino-5-phosphonovaleric acid (D-APV), D-(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid (D-CPP), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; all from Research Biochemicals Int., Natick, MA, USA), and 6-nitro-7-sulphamobenzo(*f*)quinoxaline-2-3-dione (NBQX; Tocris Cookson Ltd).

Recording and stimulation

Intracellular current-clamp recordings were made from PVN neurones between circadian time 10 and 20, with sharp electrodes (resistance, 110–180 MΩ) or patch pipettes (resistance, 4–7 MΩ), using an Axoclamp-2A or Axopatch-1D amplifier, respectively (Axon Instruments, Foster City, CA, USA). Signals were filtered at 2 or 3 kHz. Whole-cell patch-clamp recordings (seal resistance > 5 GΩ) were obtained using the 'blind' whole-cell recording technique (Blanton, Loturco & Kriegstein, 1989). Only recordings with a series resistance (R_s) of < 30 MΩ were included, measured from the peak amplitude of the whole-cell capacitive current (I_c; after cancellation of the pipette capacitance), in voltage-clamp mode, as $R_s = E_t/I_c$, where E_t is the the test pulse of usually 10 mV (see O'Connor, Rowan & Anwyl, 1995). The input resistance (R_N) of the cell was repeatedly monitored and the recording terminated if R_N decreased by more than 20%.

Two separate monopolar nichrome stimulating electrodes (tip diameter, 70 μm), used together as a bipolar stimulating electrode, were placed in the medial and lateral aspects of the SCN. In order to assess the precise location that provided responses, the electrodes were occasionally placed in regions surrounding the SCN and the result of stimulation evaluated. Constant voltage or current electrical pulses (7–25 V or 10–300 μA, 0·2 ms, 0·3 Hz) were delivered by isolated stimulation units (model DS2A, Digitimer Ltd, Welwyn Garden City, UK) driven by a programmable clock (model D4030 or D100, Digitimer Ltd) or by pCLAMP software. For chemical stimulation of SCN, a picospritzer II (General Valve Corp., Fairfield, NJ, USA) was attached to single channels of triple-barrelled micropipettes (total tip diameter, 30–75 μm) that contained either 0, 100, 200 or 500 μM monosodium glutamate with 0·1% Pontamine Sky Blue dye in distilled water: NaCl and NaOH were added to adjust the osmolality to 295–305 mosmol kg^{-1} and the pH to 7·35, respectively. The Pontamine Sky Blue dye spot was visually monitored, and remained within SCN.

Data analysis

Specific events were captured on-line through a TL-1 DMA or Digidata 1200 interface (Axon Instruments) on a personal computer running pCLAMP software (Axon Instruments, version 5.5.1 or 5.7.1). For off-line data analysis, all analog signals were also digitized through an A/D converter (Neuro-corder, model DR-384, Neuro Data Instruments Corp., New York, NY, USA) and stored on videotape. PVN neurones were characterized for their spike waveform and their current–voltage relationship. The latter, defined from a series of positive and negative current pulses passed through the recording electrode, was used to determine R_N, to detect the presence of time-independent or -dependent inward rectification, a transient outward conductance, and low-threshold potentials (cf. Bourque, 1988; Tasker & Dudek, 1991; Erickson, Ronnekleiv & Kelly, 1993).

Histology

Only a single neurone was recorded from any individual slice. During recording cells were marked by intracellular perfusion of biocytin. Slices were fixed (1–2 h) in 4% paraformaldehyde in 0·1 M phosphate buffer (pH 7·4) and subsequently stored in 0·05 M Tris buffer containing 1% Triton X-100, pH 7·4 (12–36 h, at 5 °C). The

slices were rinsed in 100% methanol containing 0·5% H_2O_2 (30 min), followed by incubation with a streptavidin–horseradish peroxidase (HRP) complex (Amersham Canada, Oakville, Ontario, Canada) in Tris buffer containing 1% Triton X-100 and 1% fish gelatin, diluted 1:200 (for 2 h, at room temperature). The HRP was visualized by incubation with Tris buffer containing 0·05% 3,3′-diaminobenzidine, 2% ammonium nickel sulphate, and 0·05% H_2O_2. Following tissue staining with 2% Neutral Red dye, slices were dehydrated in a graded alcohol series and stored in methylsalicylate. Cell morphologies were reconstructed from camera lucida drawings.

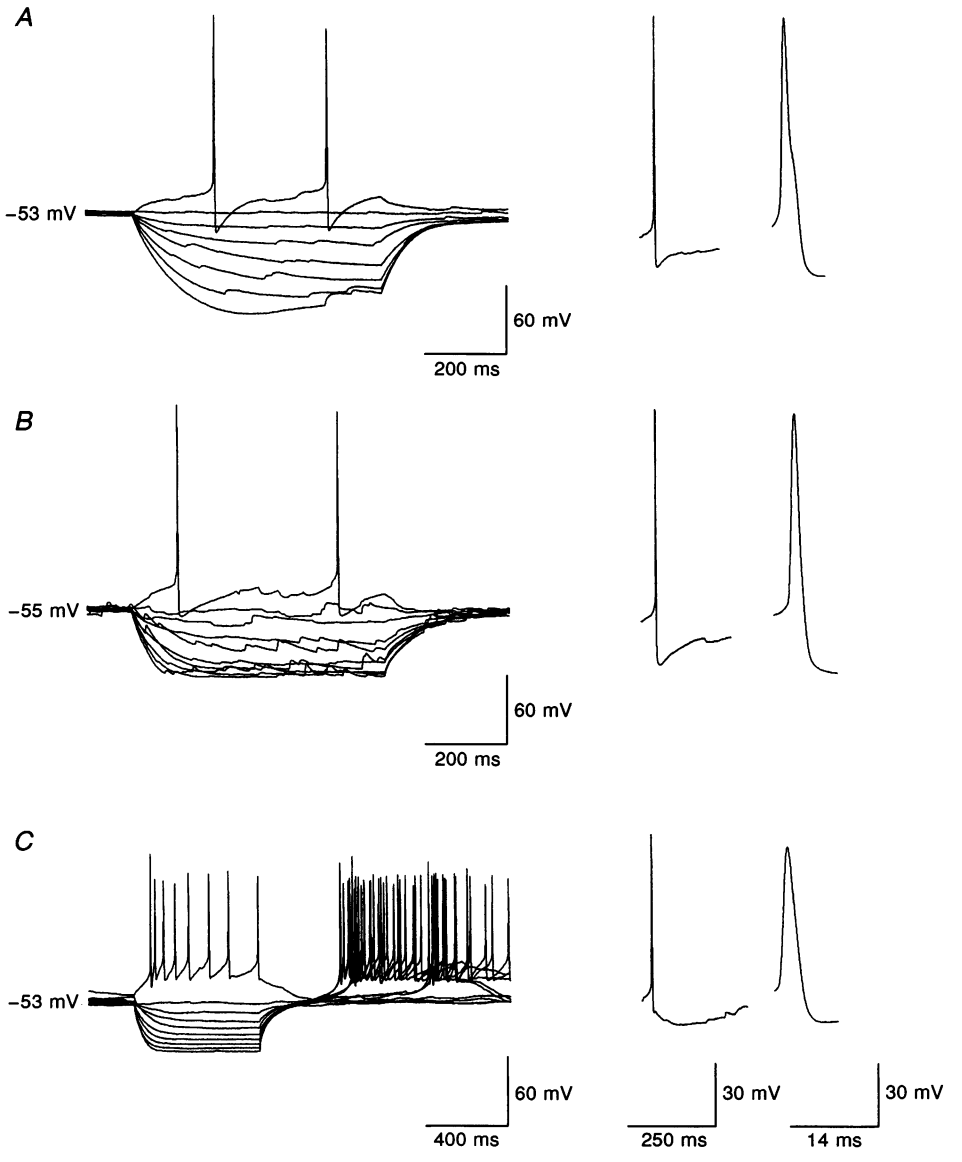

Figure 1. PVN neurones display distinct electrical properties

Left column shows voltage responses to depolarizing and hyperpolarizing current steps. Current pulse protocol was −30 to 0 pA, 5 pA increments and +10 pA for type I; −40 to 0 pA, 5 pA increments and +10 pA for type II and III. Duration of current pulse was 600 ms. Right column shows spontaneous action potentials at a slow and a fast time base. Both intracellular sharp electrode and whole-cell patch-clamp recordings (as shown here) revealed similar qualitative differences between PVN neurones. *A*, type I magnocellular neurones display a depolarizing sag (i.e. time-dependent inward rectification) and a delayed return to baseline membrane potential (caused by a transient outward conductance; Bourque, 1988) during and following membrane hyperpolarization, respectively. A shoulder is observed on the repolarizing phase of their action potentials. *B*, type II parvocellular neurones demonstrate pronounced instantaneous inward rectification and small low-threshold potentials (not shown). *C*, type III parvocellular neurones show large low-threshold potentials, observed here as depolarizing overshoots supporting bursts of action potentials following transient hyperpolarization. Their action potentials show a fast and a slow postspike hyperpolarization.

RESULTS

Neuronal classification

Based on intrinsic membrane properties and morphology, at least three cell types can be recognized in the medial parvocellular and posterior magnocellular subnucleus (nomenclature according to Swanson & Kuypers, 1980) of PVN (Fig. 1). Thus neurones were designated as type I if they displayed the electrical properties deemed typical of magnocellular neurones, i.e. a shoulder on the repolarizing phase of the action potential, time-dependent inward rectification, and a transient outward A-type conductance (Bourque, 1988; Erickson *et al.* 1993). Consistent with this was a morphology characteristic of magnocellular neurones, i.e. oval, round or triangular somata, 20–35 μm in length and two to four primary dendrites extending 75–200 μm, usually unbranched (Randle, Bourque & Renaud, 1986). In contrast, cells were classified as type II parvocellular neurones if they exhibited time-independent inward rectification, small-amplitude low-threshold potentials, and predominantly oval somata 10–20 μm in length, having two to four primary dendrites. Lastly, neurones were classified as type III if they showed large low-threshold potentials, either spontaneously or as a rebound following transient hyperpolarization. Type III cells had a similar morphology to type II neurones, suggesting that these cells also belong to the parvocellular population of PVN. The

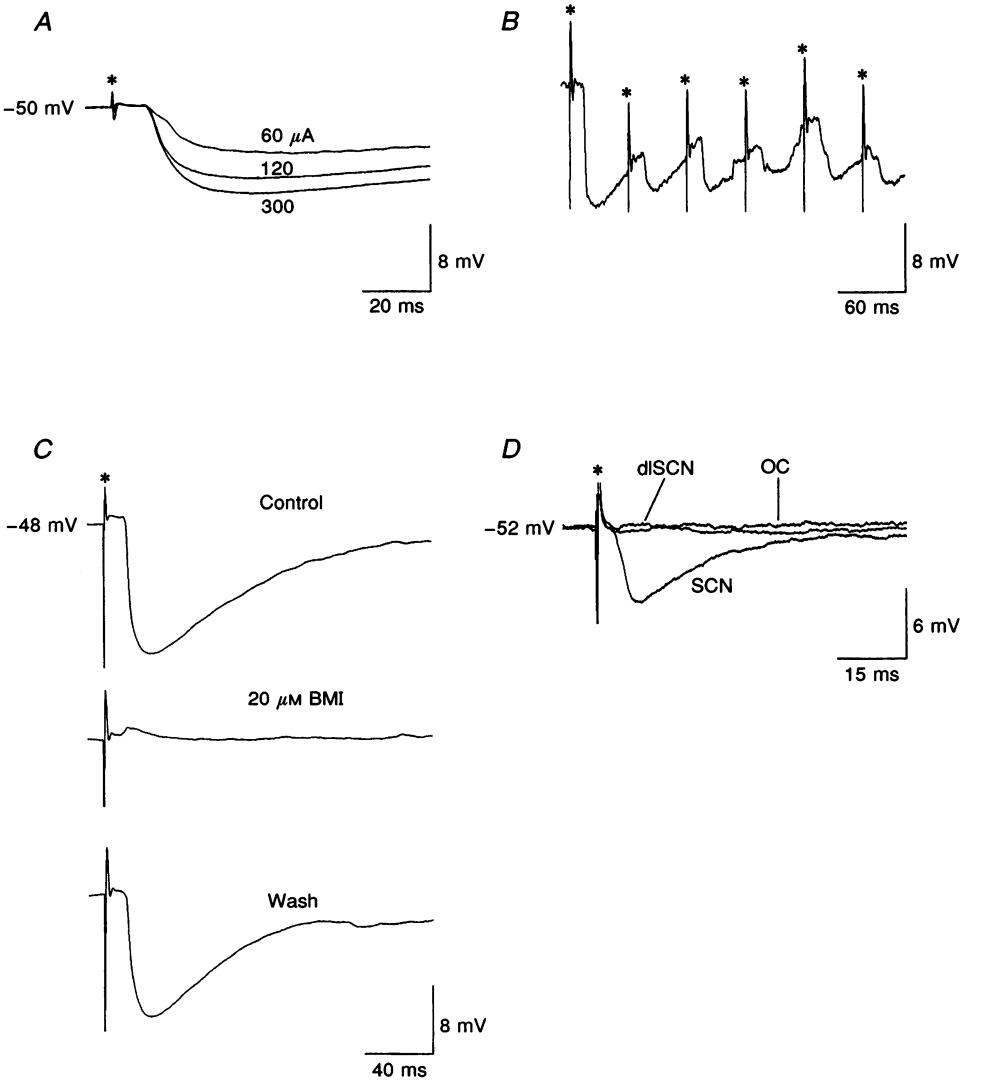

Figure 2. SCN stimulation evokes monosynaptic IPSPs in type I PVN neurones

A and *B*, SCN-evoked IPSPs in type I magnocellular neurones do not show shifts in latency with graded increases in stimulus intensities applied to SCN (*, stimulus; *A*, patch recording), or failures with high frequency stimulation of SCN (20 Hz: *B*, sharp electrode recording), suggesting mediation by monosynaptic pathways. Traces in *A* and following figures represent a mean of ten. *C*, evoked IPSPs in control media are reversibly abolished by the application of the $GABA_A$ receptor antagonist BMI (20 μM), leaving no residual effect (patch recording). *D*, superimposed records illustrate an IPSP evoked from stimulation in the ipsilateral SCN (SCN), whereas no response followed stimulation dorsolateral (dlSCN) or ventral from SCN in the optic chiasm (OC; sharp electrode recording).

classification of types I and II agrees with earlier observations (Hoffman *et al.* 1991; Tasker & Dudek, 1991); our type III resembles a class of non-paraventricular cells described by Tasker & Dudek (1991).

Postsynaptic responses to SCN stimulation

Observations were obtained from a total of fifty-nine neurones. All neurones displayed spontaneous PSPs and responded to SCN stimulation with short-latency (range, 2·95–7·50 ms at 33–35 °C: 7·20–14·60 ms at room temperature) IPSPs and/or EPSPs of varying amplitude. The amplitude of any PSP varied with the intensity of the stimulus delivered in SCN and may also reflect the number of SCN efferents to PVN retained in the slice. Therefore, no attempt was made to correlate amplitude of SCN-evoked responses with the time of recording in the circadian cycle. Moreover, only PSPs with an amplitude of 2 mV or higher when obtained with sharp electrodes, and 3 mV or higher when obtained with patch electrodes, were considered for analysis.

SCN-evoked PSPs were abolished by bath application of 500 nM TTX or replacement of Ca^{2+} by Mg^{2+} (5 mM $MgCl_2$ replaced $CaCl_2$ and $MgSO_4$ in the ACSF), indicating their synaptic mediation. Control responses (as observed in control media) were correlated to the type of neurone, and are described in the following sections.

Magnocellular (type I) neurones

At membrane potentials ranging from −50 to −62 mV (−45 to −55 mV with patch electrodes), short latency IPSPs to SCN stimulation were observed in a majority (13/18) of type I neurones, the remaining cells showing EPSPs or combinations of EPSPs and IPSPs. The 'pure' IPSPs did not display changes in latency with increasing intensities of stimulation (5/5 cases; Fig. 2*A*) or failures when stimuli were applied at frequencies up to 50 Hz ($n = 6$: Fig. 2*B*), characteristics that are consistent with a mediation by monosynaptic pathways (Berry & Pentreath, 1976). IPSPs evoked in type I neurones reversed polarity at −68·0 ± 6·4 mV ($n = 8$; sharp electrode recordings) and

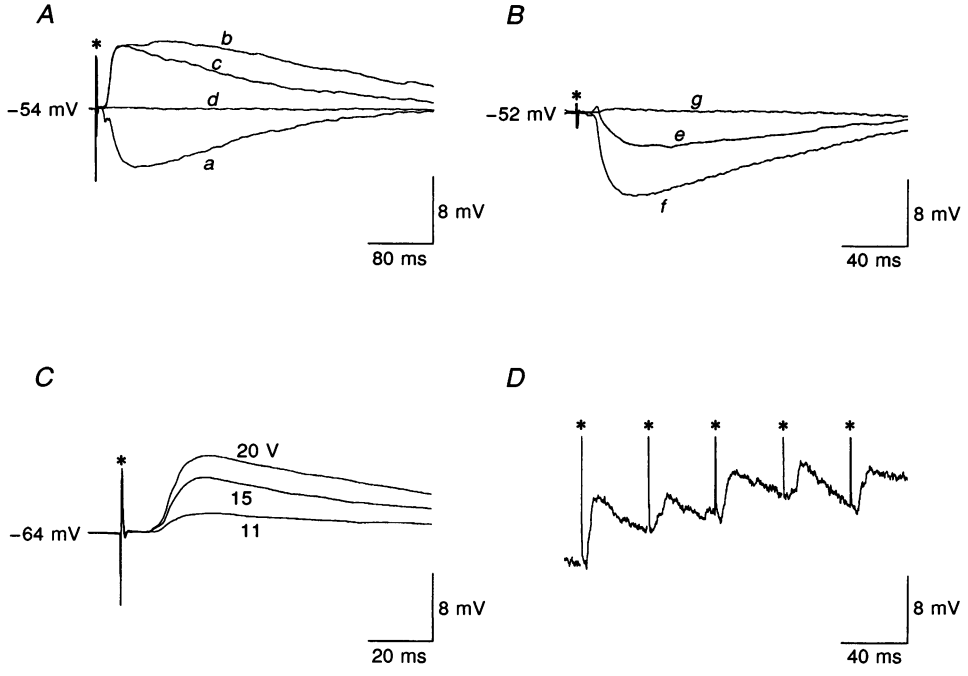

Figure 3. Type III and unclassifiable PVN neurones display hyperpolarizing mixed EPSP–IPSP responses to SCN stimulation

A and *B*, in normal ACSF, SCN stimulation (*) evoked IPSPs that were either interrupted (*a*), preceded (*e*), or followed by membrane depolarizations. Bath application of 20 μM BMI uncovered dual component EPSPs (*b*): the second, later, component was reduced in amplitude with hyperpolarization of the membrane potential (not shown) and by application of 10 μM D-CPP (*c*). The remaining fast EPSP was abolished by 2 μM NBQX (*d*). Alternatively, perfusion with a cocktail of NBQX and D-CPP increased the amplitude of the IPSPs (*f*), which were abolished by further addition of BMC (*g*) (patch recordings). Traces are a mean of 10. *C*, in a majority (10/19) of neurones SCN-evoked IPSPs or EPSPs did not shift latencies with increasing stimulus intensities, similar to the IPSPs evoked in type I magnocellular neurones. However, in a minority higher intensies of stimulation evoked additonal responses that occurred either at an earlier or a later latency, but remained constant with further increases of stimulation, as shown here for an EPSP recorded in 20 μM BMI plus 10 μM D-CPP. This is consistent with the recruitment of additional monosynaptic fibre pathways (patch recording). Traces are a mean of 10. *D*, EPSPs consistently follow repetitive stimuli in SCN (shown here at 25 Hz in the presence of BMI), characteristic of a mediation by monosynaptic pathways (sharp electrode recording).

were reversibly abolished by bath application of 20 μM BMI or BMC ($n = 8$), leaving no residual effect (Fig. 2*C*). There was no obvious reduction in the amplitude or a change in the latency of these IPSPs with perfusion of a cocktail containing 10 μM CNQX or 2–5 μM NBQX plus 20 μM D-APV or 10 μM D-CPP ($n = 5$; not shown). This latter result indicates that (parts of) the IPSPs were not generated disynaptically, for example through SCN glutamatergic input to local GABAergic interneurones, and provides further evidence for monosynaptic connections between SCN and type I neurones.

The responses reported above appear to be a feature of stimulation within SCN rather than in the adjacent tissue, since electrical stimulation in the areas lateral, dorsolateral, and ventral (in the optic chiasm) to SCN resulted in a reduction or a complete disappearance of the evoked responses in 2/2 cells (Fig. 2*D*).

Parvocellular (type II and III) neurones

The response of a majority (7/10) of type II neurones, at membrane potentials ranging from −53 to −68 mV, consisted of a fast EPSP. In all cases investigated, the EPSP was shunted by a concurrent $GABA_A$ receptor-mediated IPSP, as revealed by an increase in EPSP duration and/or amplitude following bath application of 20 μM BMI ($n = 6$). The EPSP displayed both fast and slow components. The amplitude of the 'slow' EPSP, measured 60–200 ms after the stimulus artefact, was reduced by membrane hyperpolarization, and by 20 μM D-APV ($n = 4$), features that are characteristic of an NMDA receptor-mediated response (Mayer, Westbrook & Guthrie, 1984; Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984). The remaining fast EPSP was abolished by 10 μM CNQX.

Type III neurones and a majority of unclassifiable PVN neurones exhibited combined EPSP–IPSP responses following SCN stimulation with membrane hyperpolarization as the dominant feature. Combining these cell types, 25/31 neurones (at membrane potentials of −52 to −64 mV (−46 to −53 mV with patch electrodes)) displayed predominant IPSPs that were either preceded, interrupted, or followed by small depolarizations indicative of underlying EPSPs (Fig. 3*A* and *B*). Initial bath application of BMI or BMC ($n = 7$) resulted in the unmasking of a prolonged EPSP which displayed characteristics in waveform and pharmacology that were similar to the EPSP evoked in type II neurones (Fig. 3*A*). In contrast, initial application of NBQX (CNQX) and D-CPP (D-APV) was associated with an increase in amplitude of the bicuculline-sensitive IPSP (Fig. 3*B*).

As with IPSPs in type I magnocellular neurones, both pharmacologically isolated IPSPs (not shown) and EPSPs evoked in type II, III and unclassifiable PVN cells demonstrated constant latencies with increasing stimulus intensities ($n = 19$) and faithful following of 20–50 Hz stimuli ($n = 11$), features consistent with monosynaptically generated responses (Fig. 3*C* and *D*). Similarly, these responses were specific to stimulation within SCN ($n = 3$; not shown).

Microinjections of glutamate (100–500 μM) into SCN to selectively activate neurones rather than fibres of passage, in the presence of BMI (intended to block activation of SCN

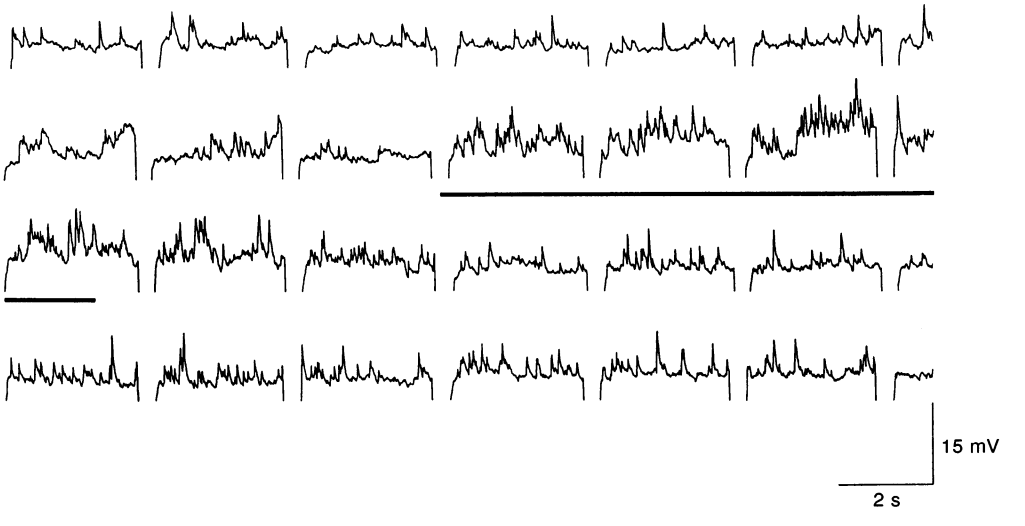

Figure 4. SCN-evoked EPSPs in type II and III parvocellular neurones are generated by activation of SCN neurones

Continuous record of the membrane potential of a type II neurone in the presence of BMI, showing the presence of spontaneous, CNQX-sensitive (not shown) EPSPs. Hyperpolarizing voltage deflections during negative current injections are truncated. Pressure application to SCN of 100 μM glutamate (horizontal bar) induced a reversible visible increase in the frequency of these EPSPs and a concomitant small membrane depolarization. Vehicle application was without effect.

GABAergic activity), were followed by transient increases in the frequency of EPSPs in 3/5 PVN cells, as judged by eye (Fig. 4). Since the glutamate microinjections were visibly confined to SCN, these features support the proposal that SCN-evoked EPSPs observed in PVN neurones arise specifically from SCN neurones, rather than from fibres of passage.

DISCUSSION

In mammals, circulating levels of vasopressin and oxytocin are regulated by hypothalamic magnocellular neurosecretory neurones, with enhanced hormone release from their neurohypophyseal axon terminals occurring in proportion to increases in the level of excitability recorded at their somata (Poulain & Wakerley, 1982). The observation that pituitary and plasma levels of neurohypophyseal hormones exhibit circadian variations (König & Meyer, 1967; König & Martin, 1968; Windle, Forsling & Guzek, 1992) implies an influence of the source of circadian rhythmicity, i.e. the SCN, on the excitability of magnocellular neurones. Indeed, an earlier *in vivo* investigation revealed that electrical stimulation in SCN depressed the excitability of a majority of PVN magnocellular neurosecretory neurones tested, including both putative vasopressin- and oxytocin-secreting neurones (Hermes & Renaud, 1993*a*). Although response latencies in the *in vivo* study were consistent with direct projections from SCN to PVN, limitations of extracellular recording methods precluded determination of their mono- or polysynaptic generation, or an analysis of the neurotransmitter mediating the observed changes in excitability.

The current observations of monosynaptic, $GABA_A$ receptor-mediated IPSPs in type I magnocellular neurones following electrical stimulation in SCN are in agreement with our earlier extracellular *in vivo* observations of SCN-evoked decreases in their excitability. Also, the consistent implication of $GABA_A$ receptors in the SCN influence on type I PVN neurones corresponds with recent proposals that GABA is an important transmitter in SCN efferents (cf. Moore & Speh, 1993; Buijs *et al.* 1994). In contrast, the monosynaptic nature of these IPSPs seems contradictory to anatomical tracer data indicating that most SCN efferents to PVN terminate in the subparaventricular zone and the medial periventricular subnucleus of PVN, rather than part of the PVN that contains the somata of magnocellular neurones (Watts *et al.* 1987; Buijs *et al.* 1994). While it is plausible that the anatomical tracers may not reveal all of the fine terminal arborizations of SCN efferents, an

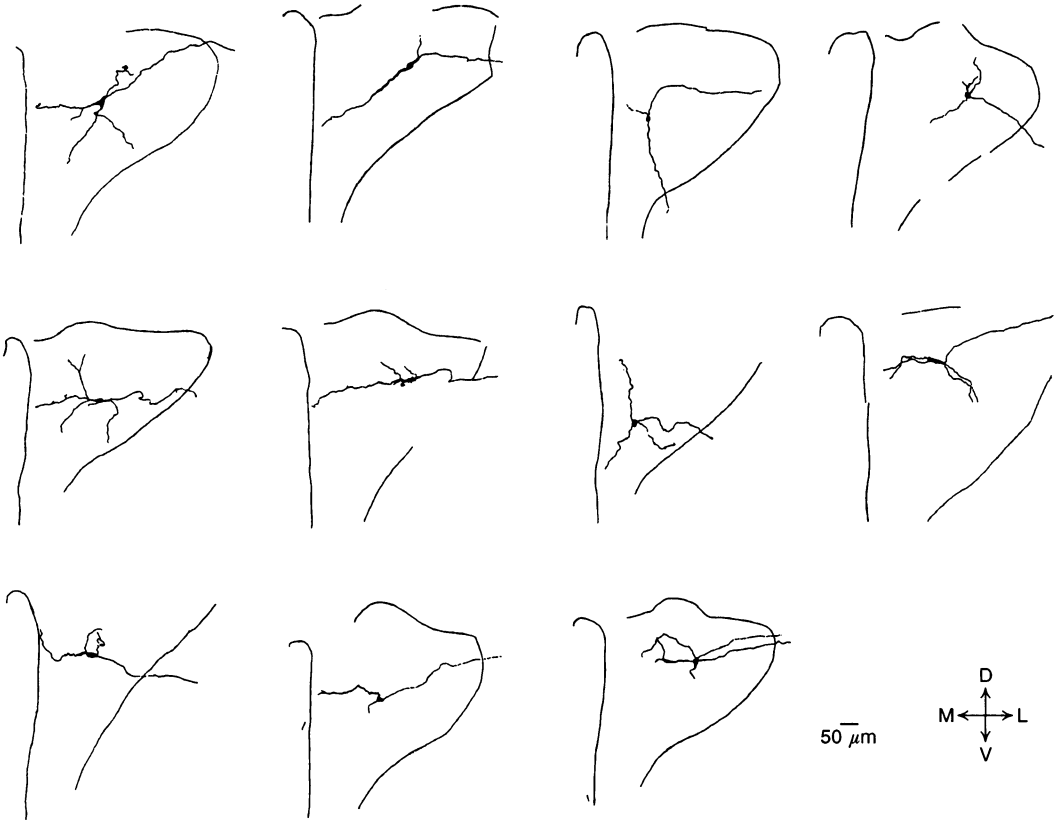

Figure 5. Most type I PVN neurones have a dendrite that extends into the periventricular subnucleus
Camera lucida drawings of the morphology of 11 type I neurones labelled by intracellular injection of biocytin. Axons (shown at a thickness similar to the dendrites) exit the PVN in a lateral direction. D, dorsal; V, ventral; M, medial; L, lateral.

alternative explanation based on the biocytin-stained profiles is that GABAergic efferents from SCN may actually terminate selectively on dendrites of magnocellular PVN neurones that extend medially into the periventricular subnucleus (Fig. 5). By providing evidence for monosynaptic transmission between SCN and PVN, the present study by no means excludes the possible presence of polysynaptic communication between these two hypothalamic nuclei, as suggested by the anatomy of SCN efferents. However, thus far, electrophysiological evidence for the existence of such communication between SCN and PVN has not been obtained.

The PVN is a hypothalamic centre implicated in regulation of various neuroendocrine and autonomic processes, many of which demonstrate circadian rhythmicity (i.e. adrenal corticosterone secretion, pineal melatonin release; Swanson & Sawchenko, 1983). Indeed, an influence of the circadian pacemaker was noted in the earlier *in vivo* study showing SCN-evoked excitatory or inhibitory influences on PVN neurones whose axons project to the median eminence or dorsomedial medulla (Hermes & Renaud, 1993*a*). The parvocellular type II and III, as well as the non-classifiable, neurones recorded in this study are presumed to be components of the neuroendocrine and autonomic roles of PVN, although an electrophysiological signature of their identity comparable to that of type I magnocellular neurosecretory neurones remains to be defined. The present results provide evidence that these PVN neurones are influenced by SCN through monosynaptic connections, and that the influence comprises both $GABA_A$ receptor-mediated inhibition and glutamate receptor-mediated excitation. Given recent findings on a predominant presence of GABA in SCN (Okamura *et al.* 1989; Francois-Bellan, Kachidian, Dusticier, Tonon, Vaudry & Bosler, 1991; Moore & Speh, 1993; Buijs *et al.* 1994) a possible role for glutamate in SCN efferent transmission, as indicated by our electrical stimulations of SCN, seems unexpected (but see also Meister, Nicholas & Hökfelt, 1992). However, control experiments demonstrating loss of the EPSPs when the stimulating electrodes are moved to sites outside the SCN and showing transient increases in EPSPs in PVN neurones after glutamate microstimulation in SCN, support the suggestion of a neurotransmitter role for glutamate in SCN efferent pathways. The mixed EPSPs–IPSPs evoked by electrical SCN stimulation are the consequence of a simultaneous activation of the GABAergic and glutamatergic systems of SCN. In normal conditions they may show an independent activation, perhaps at different times of the circadian cycle.

In conclusion, we have demonstrated that the amino acids GABA and glutamate are important transmitters in fast monosynaptic transmission from SCN to PVN. This adds to already strong indications for several peptides implicated in transfer of SCN information to the brain (Card, Brecha, Karten & Moore, 1981; Earnest & Sladek, 1986; Albers *et al.* 1990; Kalsbeek, Buijs, van Heerikhuize, Arts & van der Woude, 1992), although thus far electrophysiological evidence for a role of these peptides in SCN synaptic transmission is lacking. This will be a matter for future investigation.

Albers, H. E., Stopa, E. G., Zoeller, R. T., Kauer, J. S., King, J. C., Fink, J. S., Mobtaker, H. & Wolfe, H. (1990). Day–night variation in prepro vasoactive intestinal peptide/peptide histidine isoleucine mRNA within the rat suprachiasmatic nucleus. *Molecular Brain Research* **7**, 85–89.

Berry, M. S. & Pentreath, V. W. (1976). Criteria for distinguishing between monosynaptic and polysynaptic transmission. *Brain Research* **105**, 1–20.

Blanton, M. G., Loturco, J. J. & Kriegstein, A. R. (1989). Whole-cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *Journal of Neuroscience Methods* **30**, 203–210.

Bourque, C. W. (1988). Transient calcium-dependent potassium current in magnocellular neurosecretory cells of the rat supraoptic nucleus. *Journal of Physiology* **397**, 331–347.

Buijs, R. M., Hou, Y. X., Shinn, S. & Renaud, L. P. (1994). Ultrastructural evidence for intra- and extranuclear projections of GABAergic neurons of the suprachiasmatic nucleus. *Journal of Comparative Neurology* **340**, 381–391.

Card, J. P., Brecha, N., Karten, H. J. & Moore, R. Y. (1981). Immunocytochemical localization of vasoactive intestinal polypeptide-containing cells and processes in the suprachiasmatic nucleus of the rat: Light and electron microscopic analysis. *Journal of Neuroscience* **1**, 1289–1303.

Earnest, D. J. & Sladek, C. D. (1986). Circadian rhythms of vasopressin release from individual rat suprachiasmatic explants *in vitro*. *Brain Research* **382**, 129–133.

Erickson, K. R., Ronnekleiv, O. K. & Kelly, M. J. (1993). Electrophysiology of guinea-pig supraoptic neurones: role of a hyperpolarization-activated cation current in phasic firing. *Journal of Physiology* **460**, 407–425.

Francois-Bellan, A. M., Kachidian, P., Dusticier, G., Tonon, M. C., Vaudry, H. & Bosler, O. (1991). GABA neurons in the rat suprachiasmatic nucleus: Involvement in chemospecific synaptic circuitry and evidence for GAD-peptide colocalization. *Journal of Neurocytology* **19**, 937–947.

Groos, G. & Hendriks, J. (1982). Circadian rhythms in electrical discharge of rat suprachiasmatic neurones recorded *in vitro*. *Neuroscience Letters* **34**, 283–288.

Hermes, M. L. H. J. & Renaud, L. P. (1993*a*). Differential responses of identified rat hypothalamic paraventricular neurons to suprachiasmatic nucleus stimulation. *Neuroscience* **56**, 823–832.

Hermes, M. L. H. J. & Renaud, L. P. (1993*b*). Amino-acids-mediated inhibition and excitation of characterized rat hypothalamic paraventricular neurons following suprachiasmatic nucleus stimulation. *Society for Neuroscience Abstracts* **19**, 1613.

Hermes, M. L. H. J., Spanswick, D. & Renaud, L. P. (1994). Cell type specific amino-acids-mediated responses of rat hypothalamic paraventricular neurons following suprachiasmatic nucleus stimulation. *Society for Neuroscience Abstracts* **20**, 161.

Hoffman, N. W., Tasker, J. G. & Dudek, F. E. (1991). Immunohistochemical differentiation of electrophysiologically defined neuronal populations in the region of the rat hypothalamic paraventricular nucleus. *Journal of Comparative Neurology* **307**, 405–416.

INOUYE, S. I. & KAWAMURA, H. (1979). Persistence of circadian rhythmicity in a mammalian hypothalamic 'island' containing the suprachiasmatic nucleus. *Proceedings of the National Academy of Sciences of the USA* **11**, 5962–5966.

KALSBEEK, A., BUIJS, R. M., VAN HEERIKHUIZE, J. J., ARTS, M. & VAN DER WOUDE, T. P. (1992). Vasopressin-containing neurons of the suprachiasmatic nuclei inhibit corticosterone release. *Brain Research* **580**, 62–67.

KÖNIG, A. & MARTIN, B. (1968). Tagesperiodische schwankungen des neurohypophysären oxytocingehaltes bei männlichen wistar-ratten. *Acta Endocrinologica* **58**, 98–100.

KÖNIG, A. & MEYER, A. (1967). Tagesperiodische schwankungen der urinausscheidung und des adiuretingehaltes im hypophysen-hinterlappen männlicher wistar-ratten. *Acta Endocrinologica* **54**, 275–281.

MAYER, M. L., WESTBROOK, G. L. & GUTHRIE, P. B. (1984). Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* **309**, 261–263.

MEISTER, B., NICHOLAS, A. P. & HÖKFELT, T. (1992). Glutamate- and aspartate-like immunoreactivities in chemically identified hypothalamic neurons. *Society for Neuroscience Abstracts* **18**, 1416.

MOORE, R. Y. & EICHLER, V. B. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Research* **42**, 201–206.

MOORE, R. Y. & SPEH, J. C. (1993). GABA is the principal neurotransmitter of the circadian system. *Neuroscience Letters* **150**, 112–116.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., HERBET, A. & PROCHIANTZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurons. *Nature* **307**, 462–465.

O'CONNOR, J. J., ROWAN, M. J. & ANWYL, R. (1995). Tetanically induced LTP involves a similar increase in the AMPA and NMDA receptor components of the excitatory postsynaptic current: Investigations of the involvement of mGlu receptors. *Journal of Neuroscience* **15**, 2013–2020.

OKAMURA, H., BÉROD, A., JULIEN, J.-F., GEFFARD, M., KITAHAMA, K., MALLET, J. & BOBILLIER, P. (1989). Demonstration of GABAergic cell bodies in the suprachiasmatic nucleus: in situ hybridization of glutamic acid decarboxylase (GAD) mRNA and immunocytochemistry of GAD and GABA. *Neuroscience Letters* **102**, 131–136.

POULAIN, D. A. & WAKERLEY, J. B. (1982). Electrophysiology of hypothalamic magnocellular neurones secreting oxytocin and vasopressin. *Neuroscience* **7**, 773–808.

RALPH, M. R., FOSTER, R. G., DAVIS, F. C. & MENAKER, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science* **247**, 975–978.

RANDLE, J. C. R., BOURQUE, C. W. & RENAUD, L. P. (1986). Serial reconstruction of Lucifer Yellow-labeled supraoptic nucleus neurons in perfused rat hypothalamic explants. *Neuroscience* **17**, 453–467.

SCHWARTZ, W. J., GROSS, R. A. & MORTON, M. T. (1987). The suprachiasmatic nuclei contain a tetrodotoxin-resistant pacemaker. *Proceedings of the National Academy of Sciences of the USA* **84**, 1694–1698.

SHIBATA, S., LIOU, S. Y., UEKI, S. & OOMURA, Y. (1984). Influence of environmental light–dark cycle and enucleation on activity of suprachiasmatic neurons in slice preparations. *Brain Research* **302**, 75–81.

STEPHAN, F. K. & ZUCKER, I. (1972). Circadian rhythms in drinking behavior and locomotor activity are eliminated by hypothalamic lesions. *Proceedings of the National Academy Sciences of the USA* **69**, 1583–1586.

SWANSON, L. W. & KUYPERS, H. G. J. M. (1980). The paraventricular nucleus of the hypothalamus: Cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagus complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. *Journal of Comparative Neurology* **194**, 555–570.

SWANSON, L. W. & SAWCHENKO, P. E. (1983). Hypothalamic integration: Organization of the paraventricular and supraoptic nuclei. *Annual Review of Neuroscience* **6**, 269–324.

TASKER, J. G. & DUDEK, F. E. (1991). Electrophysiological properties of neurones in the region of the paraventricular nucleus in slices of the rat hypothalamus. *Journal of Physiology* **434**, 271–293.

WATTS, A. G., SWANSON, L. W. & SANCHEZ-WATTS, G. (1987). Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport of *Phaseolus vulgaris* leucoagglutinin in the rat. *Journal of Comparative Neurology* **258**, 204–229.

WELSH, D. K., LOGOTHETIS, D. E., MEISTER, M. & REPPERT, S. M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* **14**, 697–706.

WINDLE, R. J., FORSLING, M. L. & GUZEK, J. W. (1992). Daily rhythms in the hormone content of the neurohypophyseal system and release of oxytocin and vasopressin in the male rat: Effect of constant light. *Journal of Neuroendocrinology* **133**, 283–290.

Acknowledgements

The authors thank Dr D. Spanswick for his technical advice and comments on an earlier version of this manuscript. This work was supported by postdoctoral fellowships of the International Human Frontier Science Program Organization and the Heart and Stroke Foundation of Canada, and a grant from Institut de Recherches Internationales Servier (no. PHA-614-NLD).

Author's email address

M. L. H. J. Hermes: m.hermes@nih.knaw.nl

Received 16 April 1996; accepted 15 July 1996.