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# SUPRACHIASMATIC NUCLEUS COMMUNICATES WITH ANTERIOR THALAMIC PARAVENTRICULAR NUCLEUS NEURONS VIA RAPID GLUTAMATERGIC AND GABAERGIC NEUROTRANSMISSION: STATE-DEPENDENT RESPONSE PATTERNS OBSERVED *IN VITRO*

**L. ZHANG, M. KOLAJ AND L. P. RENAUD***

*Neurosciences, Ottawa Health Research Institute and University of Ottawa, 725 Parkdale Avenue, Ottawa, Ontario, Canada K1Y 4E9*

**Abstract—The hypothalamic suprachiasmatic nucleus uniquely projects to the midline thalamic paraventricular nucleus. To characterize this projection, patch clamp techniques applied in acute rat brain slice preparations examined responses of anterior thalamic paraventricular nucleus neurons to focal suprachiasmatic nucleus stimulation. Whole cell recordings from slices obtained during daytime ($n$=40) revealed neurons with a mean membrane potential of −66±1.2 mV, input conductance of 1.5±0.1 nS and state-dependent tonic or burst firing patterns. Electrical stimulation (one or four pulses) in suprachiasmatic nucleus elicited monosynaptic excitatory postsynaptic potentials (mean latency of 12.6±0.6 ms; $n$=12), featuring both AMPA and *N*-methyl-D-aspartate–glutamate receptor-mediated components, and monosynaptic bicuculline-sensitive inhibitory postsynaptic potentials (mean latency of 16.6±0.6 ms; $n$=7) reversing polarity at −72±2.6 mV, close to the chloride equilibrium potential. Glutamate microstimulation of suprachiasmatic nucleus also elicited transient increases in spontaneous excitatory or inhibitory postsynaptic currents in anterior thalamic paraventricular neurons. Recordings from rats under reverse light/dark conditions ($n$=22) yielded essentially similar responses to electrical stimulation. At depolarized membrane potentials, suprachiasmatic nucleus-evoked excitatory postsynaptic potentials triggered single action potentials, while evoked inhibitory postsynaptic potentials elicited a silent period in ongoing tonic firing. By contrast, after manual adjustment of membrane potentials to hyperpolarized levels, neuronal response to the same 'excitatory' stimulus was a low threshold spike and superimposed burst firing, while responses to 'inhibitory' stimuli paradoxically elicited excitatory rebound low threshold spikes and burst firing. These data support the existence of glutamatergic and GABAergic efferents from the suprachiasmatic nucleus to its target neurons. Additionally, in thalamic paraventricular nucleus neurons, responses to activation of their suprachiasmatic afferents may vary in accordance with their membrane potential–dependent intrinsic properties, a characteristic typical of thalamocortical neurons.** 

**Key words: SCN, PVT, circadian rhythms, evoked responses.**

*Corresponding author. Tel: +1-613-761-5070; fax: +1-613-761-5330.
E-mail address: lprenaud@ohri.ca (L. P. Renaud).

*Abbreviations:* ACSF, artificial cerebrospinal fluid; aPVT, anterior thalamic midline paraventricular nucleus; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; I–V, current–voltage; LTS, low threshold spike; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium; PVN, paraventricular nucleus; PVT, thalamic midline paraventricular nucleus; SCN, suprachiasmatic nucleus.

In mammals, the hypothalamic suprachiasmatic nucleus (SCN) is recognized as the site of the circadian pacemaker. SCN neurons display intrinsic circadian oscillations in their electrical and metabolic activity, orchestrated at the molecular level by translational and transcriptional loops in specific 'clock' genes (see Reppert and Weaver, 2002 for review). The activity of these genes is tuned to the solar day through direct and indirect photic inputs from the retina and intergeniculate leaflet respectively. Although it is clear from ablation studies that the rhythmicity in SCN is in turn conveyed to various endogenous neural systems governing physiological and behavioral functions (reviewed in Klein et al., 1991), our level of understanding as to how SCN neurons actually communicate with their CNS target neurons remains relatively meager. On the one hand, unidentified diffusible signals from encapsulated transplanted SCN neurons are sufficient to restore and maintain rhythmic locomotor and drinking behaviors following SCN ablation (Silver et al., 1996; Tousson and Meissl, 2004). However, it is also apparent that post-ablation restoration of endocrine (e.g. cortisol and melatonin release) and other rhythms depends on the re-establishment of neuronal communication with target cells via precise efferent pathways and neuronal connectivity that arises from neurons located in both the shell and the core regions of the SCN (Lehman et al., 1987; Meyer-Bernstein et al., 1999).

An objective of our research has been to define the characteristics and nature of synaptic transmission from SCN to its target neurons. Within the hypothalamus, we initially evaluated neurotransmission from SCN to the paraventricular nucleus (PVN) using both *in vivo* and *in vitro* approaches. These investigations revealed that PVN neurons responded to focal electrical and chemical (glutamate) stimulation in SCN with rapid inhibitory (GABA-mediated) and excitatory (glutamate-mediated) responses (see Hermes and Renaud, 1993; Hermes et al., 1996; Cui et al., 2000, 2001), indicating that rapid amino acid–mediated neurotransmission participated in this process. We now extend this analysis to a uniquely extrahypothalamic target of SCN efferents, the thalamic midline paraventricular nucleus (PVT). PVT, a member of the midline–intralaminar complex, is distinct from other 'specific' thalamocortical relay nuclei in terms of its putative role in stress, psychostimulants and reward-motivated behaviors (e.g. Brown et al., 1992; Cullinan et al., 1995; Young and Deutch, 1998; Sewards and Sewards, 2003) and linkage to

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cortical regions engaged in motivation and attention (Su and Bentivoglio, 1990; Cardinal et al., 2002; Christakou et al., 2004). SCN projections to PVT were initially reported in studies that presented an overview of the CNS distribution of arginine vasopressin-immunoreactive fibers (Buijs et al., 1978; Sofroniew and Weindl, 1978), a projection that has been subsequently verified with various anatomical tracers (e.g. Watts and Swanson, 1987; Watts et al., 1987; Peng and Bentivoglio, 2004). In addition to vasopressin, immunocytochemistry has revealed that other potential transmitter molecules synthesized in SCN neurons can contribute to the identity of this projection to PVT, notably vasoactive intestinal polypeptide (VIP; Watts and Swanson, 1987) and prokineticin 2 (PK2; Cheng et al., 2002). Using a combination of retrograde and anterograde tracers, Peng and Bentivoglio (2004) have demonstrated that SCN efferents to anterior thalamic midline paraventricular nucleus (aPVT) synapse with neurons that send their axons to the amygdala, supporting the notion that this connection may relay circadian-related information to the limbic system. Additionally, the detection of a modest reciprocal innervation with SCN (Moga et al., 1995) implies possible feedback of information from PVT back to SCN.

Whereas the morphological observations mentioned above imply a robust innervation of aPVT by SCN neurons, there is as yet no functional information on the nature of this connection. Taking advantage of a brain slice preparation that retains the connection from SCN to aPVT, we used cell attached and whole cell patch clamp techniques to evaluate the response of aPVT neurons to focal electrical and chemical stimulation in SCN. We now report that stimulation in SCN monosynaptically evokes rapid excitatory and inhibitory postsynaptic responses in aPVT neurons, mediated by glutamate and $GABA_A$ receptors, respectively. Responses were not significantly different in slice preparations obtained during the subjective day or night, implying that the connections are hard wired. In addition, we provide initial observations on the state-dependent properties of aPVT neurons and suggest how these may dictate the patterns (single spikes versus bursts) of response to synchronized inputs from SCN. A preliminary account of these observations was recently presented (Zhang et al., 2005).

## EXPERIMENTAL PROCEDURES

### Slice preparation

Experimental protocols conformed to the Canadian Council for Animal Care guidelines and were approved by the Ottawa Health Research Institute Animal Care and Use Committee. Care was taken to minimize the number of animals used and to reduce their suffering. Wistar rats weighing 50–120 g (21–35 days old) were housed in pairs in a temperature controlled environment under 12-h light/dark conditions. Recordings were obtained from aPVT neurons in acutely prepared brain slice preparations that were obtained from two circadian times: 4–6 h after lights on (subjective quiet period), or 4–6 h after lights off (subjective active period). Briefly, animals were killed by guillotine, the brain was quickly removed from the skull and immersed in oxygenated ($95\%O_2$–$5\%CO_2$) cooled (<4 °C) artificial cerebrospinal fluid (ACSF) of the following composition (mM): 127 NaCl, 3.1 KCl, 1.3 $MgCl_2$, 2.4 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, pH 7.3, osmolality 300–310 mOsm/kg. Using a vibrating blade microtome (Leica VT1000S; Nussloch, Germany), a 400–450 μm slice was cut in a coronal plane that contained both the SCN and the anterior PVT (Fig. 1A), incubated in gassed ACSF for >1 h at room temperature, then transferred to a submerged chamber and superfused (2–4 ml/min) with oxygenated ACSF at 22–24 °C.

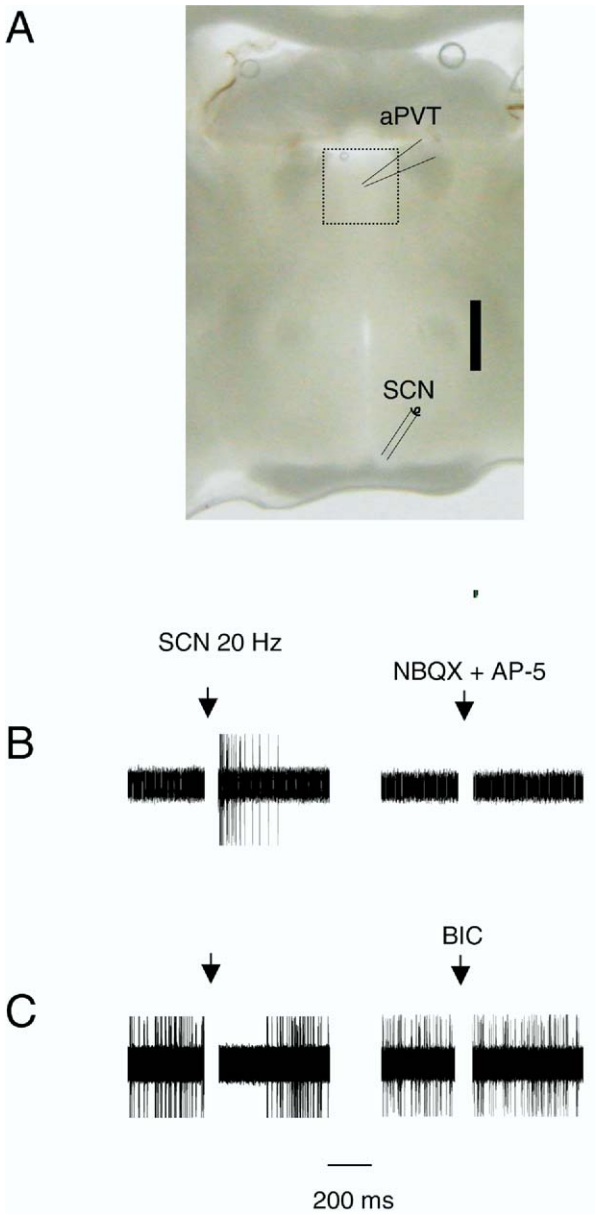


**Fig. 1.** (A) Photo of a brain slice preparation, sectioned to contain both the SCN stimulation site and the aPVT recording site in the same plane. Scale bar=1 mm. (B) Superimposed traces of cell attached recordings from a silent aPVT neuron display a transient increase in activity evoked by a train of SCN stimuli (left, four pulses at 20 Hz) and loss of this response after addition of NBQX (5 μM) and AP5 (20 μM) to the media (right). (C) In ACSF containing glutamate (350 μM) to enhance cell firing, superimposed traces of cell attached recordings from another PVT neuron reveal a transient reduction in excitability follows similar SCN stimuli (left) and loss of this response after addition of bicuculline (BIC; 20 μM).