In Search of the Pathways for Light-Induced Pacemaker Resetting in the Suprachiasmatic Nucleus

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Abstract Within the suprachiasmatic nucleus (SCN) of the mammalian hypothalamus is a circadian pacemaker that functions as a clock. Its endogenous period is adjusted to the external 24-h light-dark cycle, primarily by light-induced phase shifts that reset the pacemaker's oscillation. Evidence using a wide variety of neurobiological and molecular genetic tools has elucidated key elements that comprise the visual input pathway for SCN photoentrainment in rodents. Important questions remain regarding the intracellular signals that reset the autoregulatory molecular loop within photoresponsive cells in the SCN's retino-recipient subdivision, as well as the intercellular coupling mechanisms that enable SCN tissue to generate phase shifts of overt behavioral and physiological circadian rhythms such as locomotion and SCN neuronal firing rate. Multiple neurotransmitters, protein kinases, and photoinducible genes add to system complexity, and we still do not fully understand how dawn and dusk light pulses ultimately produce bidirectional, advancing and delaying phase shifts for pacemaker entrainment.

Key words circadian, CREB, entrainment, Fos, glutamate, Per

Three decades ago, a novel retinofugal projection to the hypothalamus was demonstrated, innervating a relatively unknown structure, the suprachiasmatic nucleus (SCN) (Hendrickson et al., 1972; Moore and Lenn, 1972). Since then, the monosynaptic retinohypothalamic tract (RHT) has played a key role in studies identifying the SCN as a circadian pacemaker and in experiments seeking to trace the cascade of events that comprise the pacemaker's photic entrainment pathway. Remarkable advances have been made in understanding the "circadian visual system" (Morin, 1994) and its underlying substrates, and here we provide an overview of our knowledge (and ignorance) of mechanisms for light-induced pacemaker resetting in the rodent SCN.

THE STANDARD (LINEAR) MODEL FOR MAMMALIAN CIRCADIAN PHOTOENTRAINMENT

In its simplest form, the model posits that light stimulates a specialized group of retinal ganglion cells (see Rollag et al., 2003 [this issue]) whose unmyelinated axons form the RHT in the optic nerves and whose transmitter(s) synaptically affect SCN "clock" cells, leading to the activation of proteins that reset the circadian pacemaker's core autoregulatory transcription-translation loop. Some of the details of this idealized signaling pathway have now been clarified.

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The RHT Is a Necessary and Sufficient Visual Projection

The RHT appears to be a universal feature of mammals (even in the blind mole rat) (see Morin, 1994). It generally terminates within the ventral and lateral SCN but also extends beyond the nucleus. Species differences exist, and in the mouse, for example, the RHT projects more diffusely throughout the ventro-dorsal extent of the SCN (Abrahamson and Moore, 2001). SCN cells receive direct retinal input via conventional synapses, and the innervated population includes some neurons containing immunoreactive vasoactive intestinal polypeptide (VIP), gastrin-releasing peptide (GRP), and, in the hamster, calbindin (CalB) (Tanaka et al., 1993; Aïoun et al., 1998; Bryant et al., 2000).

RHT transection, sparing the optic nerves, prevents the photic entrainment of circadian locomotor rhythmicity but preserves the ability to make visual discriminations in the hamster (Johnson et al., 1988). When the SCN is isolated in a brain slice with the optic nerves still attached, the RHT can be electrically stimulated in the absence of other photic afferents. Such stimulation in the rat generates a light-like phase response curve (PRC), with phase delays and advances after stimulation during early and late subjective night, respectively (Shibata and Moore, 1993).

SCN Neurons Are Luminance Detectors

Retinal illumination elicits a change in membrane potential and discharge rate in a subpopulation of SCN neurons. Extracellular single-unit recordings show that 32% and 38% of SCN neurons are light-responsive in the rat and hamster, respectively (Groos and Mason, 1980; Meijer et al., 1986; Kim and Dudek, 1993; Cui and Dyball, 1996; Jiang et al., 1997; Aggelopoulos and Meissl, 2000). Histological confirmation of SCN recording location shows that light-responsive neurons are located in the ventral aspect in the rat and the ventrolateral aspect in the hamster, corresponding to the terminal field of the RHT in both species (Meijer et al., 1986, 1998). Outside the SCN, a much lower percentage of neurons are light responsive. Intracellular recordings show that retino-recipient SCN neurons are characterized by a high input resistance and a membrane potential of –60 mV (Kim and Dudek, 1993; Jiang et al., 1997). As shown by Jiang et al. (1997), these neurons exhibit increased conductance during day and decreased conductance during dusk, while membrane potential is high during day and low during night, as also observed in non-retino-recipient neurons (de Jeu et al., 1998; Schaap et al., 1999).

Light responses in the SCN are sustained for prolonged periods of retinal illumination (Groos and Mason, 1980; Meijer et al., 1986). This is in contrast to the transient responses in most visual areas. Responses in the SCN are either light activated or light suppressed, which means that neurons increase or decrease their sustained firing level in response to an increment in light intensity. For both light-activated and -suppressed neurons, long response latencies from 400 ms to several seconds are observed (Groos and Mason, 1980; Meijer et al., 1986). Glutamate antagonists block both types of responses to optic nerve stimulation in vitro, while bicuculline blocks only suppressed responses, suggesting that the latter are mediated by gamma-aminobutyric acid (GABA) neurons downstream from the activated response (Jiang et al., 1997).

The steady-state discharge rate of both light-activated and -suppressed neurons codes for light intensity within a small range, corresponding to intensities around dawn and dusk (Meijer et al., 1986). Response thresholds—about 0.1 lux in the rat and 1 lux in the hamster, measured against a dark background—are extremely high compared to that for vision. Above threshold levels, SCN light-responsive neurons code for intensities in a near linear way, with saturation at around 100 to 1000 lux. The shape of this intensity-response curve corresponds well to that for behavioral phase shifting (Takahashi et al., 1984; Meijer et al., 1989; Nelson and Takahashi, 1991b). Light-responsive SCN neurons have large receptive fields, often exceeding 20 degrees in diameter (Groos and Mason, 1980). As SCN-projecting ganglion cells have receptive fields of only 2 to 5 degrees (Pu, 2000), a convergence of these cells is necessary to account for the much larger receptive fields in the SCN. All of these features make SCN neurons especially suited to discriminate between day and night.

Importantly, many of these response characteristics have been confirmed in alert animals, demonstrating that the high threshold levels are not an artifact of anesthesia (Meijer et al., 1998). Recordings in alert animals also allow for the investigation of light responsiveness as a function of circadian time. Under saturating light conditions, responses in neuronal discharge

rate are found across the circadian cycle and are qualitatively similar in light-activated and -suppressed neurons. But with subsaturating light intensities, large differences are observed, with maximum responsiveness to light during the night.

Finally, in 2 diurnal species of animals, the 13-lined ground squirrel (*Spermophilus tridecemlineatus*) and degu (*Octodon degus*), extracellular single-unit recordings have revealed some striking differences from nocturnal species. The total proportion of light-responsive neurons is much lower in diurnal than in nocturnal animals (about 10%). A small majority of these neurons are suppressed by light, while a minority are activated by light (Meijer et al., 1989; Jiao et al., 1999). Threshold intensities of several hundred lux are required to stimulate light-responsive neurons in the diurnal species. Such intensities occur during early dusk or late dawn.

Glutamate Released from RHT Terminals Leads to an Influx of Postsynaptic Ca^{2+}

Multiple lines of evidence in rodents suggest that the excitatory amino acid glutamate is the primary RHT neurotransmitter responsible for mediating the circadian actions of light (for review and references, see Ebling, 1996). Glutamate is localized to RHT terminals innervating SCN neurons, and it is released by optic nerve stimulation of SCN slices in vitro; glutamate receptor blockers inhibit both the phase-shifting actions of light in vivo and the electrophysiological effects of optic nerve stimulation of slices in vitro. Glutamate application excites SCN neurons in vitro, with the resulting phase shifts mimicking a light-like PRC. Of note, excitatory amino acids are present not only in RHT terminals but also in afferent projections from other sources (Moga and Moore, 1996).

Both ionotropic (NMDA and AMPA/kainate) and metabotropic receptors are present in the SCN. In vivo microinjection of NMDA into the region of the SCN mimics the phase-shifting effects of light in nocturnal hamsters (Mintz et al., 1999) and diurnal grass rats (*Arvicanthis niloticus*) (Novak and Albers, 2002), and in situ hybridization studies of receptor mRNA expression suggest that the SCN is relatively enriched in the NMDA-R1 and NMDA-R2C subtype variants. Several excitatory amino acids have been tested for their direct stimulatory effects on rat SCN electrical activity (Kim and Dudek, 1991; Bos and Mirmiran, 1993; Meijer et al., 1993). Patch clamp recordings confirm that the membrane potential is depolarized by glutamate application (Meijer et al., 1993) and that depolarization is mediated by both NMDA and non-NMDA receptors (Jiang et al., 1997; Michel et al., 2002).

Activation of the NMDA-type glutamate receptor evokes Ca^{2+} transients within rat SCN cells (van den Pol et al., 1992; Tominaga et al., 1994; Colwell, 2001; Pennartz et al., 2001). Ca^{2+} influx is triggered by glutamate binding to the NMDA receptor, as well as by depolarization of the membrane. Depolarization is an important determinant for Ca^{2+} influx because at resting potential, the channel is blocked by extracellular Mg^{2+}. Upon depolarization, the Mg^{2+} block is removed, and Ca^{2+} influx through the NMDA receptor can occur. In the SCN of acutely-prepared slices, NMDA-induced excitatory postsynaptic currents and Ca^{2+} transients are larger (and the duration of Ca^{2+} transients is longer) during the night than during the day (Colwell, 2001; Pennartz et al., 2001). AMPA/kainate-induced currents do not show a circadian rhythm, but at night they lead to increased Ca^{2+} influx (Michel et al., 2002).

Some Genes in the SCN Exhibit a Phase-Dependent Photoinduction

Since light-induced pacemaker resetting occurs within a few hours after light onset (Best et al., 1999; Watanabe et al., 2001) and depends on protein synthesis (Zhang et al., 1996b), much work has focused on genes involved in transcriptional regulation as candidate elements in the SCN's photic entrainment pathway. The prototypical photoinducible protein of this type, and the one most extensively studied to date, is c-Fos. The principal features of SCN c-*fos* gene expression in rodents are by now well known (for review and references, see Schwartz et al., 1995). The levels of c-*fos* mRNA and immunoreactive c-Fos protein in the retino-recipient subdivision of the SCN are dramatically elevated after either (1) lights-on at dawn during a light-dark cycle or (2) a light pulse administered during the subjective night (but not during most of the subjective day). This response is rapid, with peak expression of mRNA and immunoreactive protein at about 30 min and 1 to 2 h after light onset, respectively, and transient, with mRNA and immunoreactive protein disappearing by about 2 h and 6 h after light onset. Light pulses as short as 5 min are effective. Stimulation

with saturating light pulses indicates an upper limit to the number of photoinducible c-Fos cells at about 20% of the total SCN cell population, at least in the mouse (Castel et al., 1997). These in situ hybridization and immunohistochemical data have been interpreted to suggest that photic stimulation increases actual c-*fos* gene transcription (rather than mRNA stability). This has not been rigorously demonstrated (e.g., by nuclear run-on assays), although light does induce histone modifications in the mouse SCN that lead to the chromatin remodeling associated with transcriptional regulation (Crosio et al., 2000).

There are strong correlations between the photic induction of c-*fos* and phase shifts of overt rhythmicity. The illumination threshold for gene expression in the hamster matches the threshold for behavioral phase shifts, even when the threshold is altered by age or prior history (Zhang et al., 1996a; Shimomura et al., 1998), and expression in the gerbil is directly proportional to the number of photons, rather than to irradiance or duration alone (Dkhissi-Benyahya et al., 2000). The degree of c-*fos* activation correlates with the magnitude of photic phase shifts in the rat (Trávnícková et al., 1996). The phase dependence of c-*fos* stimulation is similar to that for light-induced phase shifts of locomotor rhythmicity, and some of the pharmacological agents that block these behavioral phase shifts also block the photic stimulation of c-Fos protein in specific regions of the SCN.

The *period* (*per*) genes, *per1* and *per2*, are also photoinducible in the rodent SCN with a phase dependence similar to that of behavioral rhythmicity (Albrecht et al., 1997; Shearman et al., 1997; Shigeyoshi et al., 1997; Takumi et al., 1998; Zylka et al., 1998; Moriya et al., 2000). The mRNA levels begin to rise 10 to 15 min after light onset during subjective night, peak at about 1 to 2 h (*per2* later than *per1*), and return to baseline after about 3 h. The protein immunoreactivities also rise, but they are most clearly resolved only after very long light pulses with the antibodies available (Field et al., 2000). At least for mouse *per1*, the photic threshold for gene expression matches that for behavioral phase shifts, and the intensity-response relationship shows reciprocity between light intensity and duration (Shigeyoshi et al., 1997). In the hamster, in vivo microinjection of NMDA into the region of the SCN during late subjective night increases both *per1* and *per2* mRNA levels, while injection of NMDA antagonists blocks their light-induced elevation (Moriya et al., 2000).

(Most) All Paths Lead to the Phosphorylation of CREB

A universal mediator of Ca^{2+}-dependent gene expression is the cAMP response element binding protein (CREB), which binds to a *cis*-regulatory element (the cAMP response element [CRE]) on the promoters of target genes (for review, see Lonze and Ginty, 2002). CREB is activated by phosphorylation of amino acid Ser-133; the interaction of phospho-CREB and its transcriptional coactivator, CREB binding protein (CBP), induces target gene expression. There are multiple kinases that mediate extracellular-, cAMP-, and Ca^{2+}-dependent CREB phosphorylation, including the extracellular signal-related kinase (ERK)-mitogen-activated protein kinase (MAPK) pathway, protein kinase A (PKA), and Ca^{2+}-calmodulin-dependent protein kinases (CaMK).

The c-*fos*, *per1*, and *per2* promoters all contain CREs that bind CREB from SCN tissue extracts (Ginty et al., 1993; Trávnícková-Bendova et al., 2002). SCN CREB is phosphorylated within minutes of photic or glutamatergic stimulation during the subjective night (but not during the subjective day) in rodents in vivo or in vitro (Ginty et al., 1993; Ding et al., 1997; McNulty et al., 1998; von Gall et al., 1998). Nighttime light pulses lead to CRE-mediated gene transcription in the SCN of a CRE-β-galactosidase reporter mouse strain (Obrietan et al., 1999), and CRE activation appears to be necessary for light- and glutamate-induced phase advances of the locomotor rhythm in the mouse in vivo and the SCN firing-rate rhythm in the rat slice in vitro, respectively (Tischkau et al., 2003). Recently, Gau et al. (2002) have shown a phase-dependent phosphorylation of SCN CREB at an additional amino acid site (Ser-142) by light in vivo and glutamate in vitro. These workers constructed a "knockin" mouse bearing a Ser-142Ala mutation; mutant mice exhibit reduced light-induced phase shifts of circadian locomotor activity and expression of immunoreactive c-Fos and *per1* mRNA levels (but Ser-133 phosphorylation and *per2* levels are spared).

BUT REAL PHOTOENTRAINMENT APPEARS MUCH MORE COMPLICATED THAN IN THE LINEAR MODEL

While our simple RHT ⇒ glutamate release ⇒ membrane depolarization ⇒ Ca^{2+} influx ⇒ CREB phosphorylation ⇒ gene transcription cascade pro-

vides a basic framework for a photoentrainment pathway, in reality there are complications that point to a considerably more complex mechanism.

SCN Neurons are Heterogeneous

Only a subset of all rodent SCN cells are light responsive, with estimates ranging from about one-fifth (by c-Fos immunoreactivity) to about one-third (by electrophysiology) located in the ventrolateral subdivision of the SCN. No more than about 20% of the cells in this subset can be attributed to any one identified peptidergic phenotype (Romijn et al., 1996; Castel et al., 1997). So, even though 80% of CalB cells in the hamster SCN express light-induced c-Fos, only 20% of the whole c-Fos population is $CalB^+$ (Silver et al., 1996); in the rat SCN, while 40% of GRP cells express light-induced c-Fos, less than 10% of the whole c-Fos population is GRP^+ (Earnest et al., 1993). SCN c-Fos cells are also heterogeneous in their connectivities, with some even identified as output neurons (de la Iglesia and Schwartz, 2002; Munch et al., 2002). Thus, the photo-responsive SCN subdivision includes cells distributed across a range of phenotypes, many of which remain unknown.

In the non-retino-recipient, usually dorsomedial, subdivision of the rodent SCN, c-*fos* and *per* gene expression exhibit endogenous circadian rhythmicity (Sumová et al., 1998; Guido et al., 1999a, 1999b; Yan et al., 1999; Schwartz et al., 2000; Hamada et al., 2001; Yan and Okamura, 2002). Thus, any model of SCN photoentrainment must eventually include not only the synaptic signal(s) and *intracellular* transduction pathway(s) for resetting the autoregulatory molecular loop within photoresponsive cells but also the *intercellular* mechanisms for coupling to and then resetting others. Presumably, such coupling is necessary for generating photic phase shifts of overt behavioral and physiological circadian rhythms such as locomotion and SCN firing rate. The responsible coupling mechanisms within SCN tissue are not understood.

GABA has been considered a possible intercellular synchronizing agent. GABA applied to cultured mouse SCN cells causes phase-dependent phase shifts of the circadian rhythm of firing rate (Liu and Reppert, 2000), but studies of GABAergic agonists and antagonists in vivo have been difficult to perform and interpret (see Gillespie et al., 1999). Additional plausible candidates include the ventrolateral peptides VIP and/or GRP, as their microinjection into the hamster SCN in vivo (Piggins et al., 1995) and application onto the rat slice in vitro (McArthur et al., 2000; Reed et al., 2001) mimic light-like phase shifts of circadian locomotor and SCN firing rate rhythms, respectively. VIP (Nielsen et al., 2002) and GRP (Aida et al., 2002) elevate *per1* and *per2* mRNA levels during the night. In particular, Aida et al. (2002) have reported that intracerebroventricular injection of GRP during early night increases *per* mRNA and c-Fos immunoreactivity but primarily in the *dorsal* mouse SCN; the photic induction of *per* and c-Fos is reduced in GRP-receptor-deficient mutant mice, an effect also occurring primarily in the *dorsal* rather than in the ventral part of the nucleus.

The release of a diffusible gas, such as nitric oxide (NO), might also link cells in the ventrolateral and dorsomedial subdivisions. At least in the rat SCN, NO synthase immunoreactive cells are located ventrolaterally (Reuss et al., 1995; Wang and Morris, 1996). Generation of NO in slices in vitro mimics light-like phase shifts of the SCN firing rate rhythm (Ding et al., 1994). Inhibition of the NO synthase blocks glutamate-induced electrophysiological phase shifts in the rat in vitro (Ding et al., 1994; Watanabe et al., 1994) and light-induced behavioral phase shifts in the rat and hamster in vivo (Ding et al., 1994; Watanabe et al., 1995), but light-induced c-Fos expression in hamster ventrolateral SCN cells remains unaltered (Weber et al., 1995).

Multiple Transmitters and Multiple Tracts Impinge on SCN cells

Costored with glutamate in RHT terminals is pituitary adenylate cyclase activating polypeptide (PACAP, a member of the VIP/secretin/glucagon family) (Hannibal et al., 2000). Nanomolar PACAP microinjected into the SCN in vivo and applied onto slices in vitro mimics light-like phase shifts of hamster locomotor and SCN firing rate rhythms, respectively (Harrington et al., 1999). PACAP also elevates *per* mRNA levels during the night (Nielsen et al., 2001; Minami et al., 2002), an effect blocked by NMDA receptor antagonism (at least in mice in vivo; Minami et al., 2002). These data have raised the possibility that PACAP acts as a modulator of photic (glutamate)-induced Ca^{2+} signaling, but the mechanism and physiological function of this hypothesized modulatory role remains confusing and not yet clearly resolved

(Chen et al., 1999; Hannibal et al., 2001; Kopp et al., 2001; Dziema and Obrietan, 2002).

An additional SCN peptide attributed to RHT terminals is substance P. Micromolar concentrations applied onto rat slices in vitro mimic light-like phase shifts of the SCN firing rate rhythm (Shibata et al., 1992), whereas application of a tachykinin (sp. NK_1) receptor antagonist blocks light-like shifts following optic nerve stimulation (Kim et al., 2001). Substance P's phase-shifting action appears to be mediated by changes in glutamate release (Hamada et al., 1999; Kim et al., 2001). However, a role for the peptide in the SCN of the hamster is not so obvious (Piggins and Rusak, 1997; Challet et al., 2001). Substance P terminals are not a feature of the hamster SCN's retinorecipient subdivision (Piggins et al., 2001), and, while the tachykinin receptor antagonist spantide can block light-induced c-Fos expression in the hamster SCN in vivo, the distribution of this effect is different from glutamate receptor blockade (Abe et al., 1996). Even in the rat, substance P may not actually be within RHT terminals (Hannibal and Fahrenkrug, 2002).

Besides the RHT, photic input can reach the SCN via indirect pathways. The best studied of these is from the intergeniculate leaflet (IGL) (for review, see Harrington, 1997), but the SCN also receives afferents via other structures that receive retinal innervation, such as the pretectum (Mikkelsen and Vrang, 1994) and possibly also the median and dorsal raphe. Neuropeptide Y (NPY) from the IGL and serotonin (5-HT) from the raphe have been implicated in nonphotic phase resetting (a subject beyond the scope of this review), but they also act as extra-SCN modulators of light-induced phase shifts during the night (for reviews and references, see Rea and Pickard, 2000; Yannielli and Harrington, 2001a). Their actions might account for the larger phase shifts observed in vitro than in vivo. NPY applied onto hamster slices in vitro can block glutamate- or NMDA-induced phase shifts of the SCN firing-rate rhythm, an effect attributed to the Y5 receptor (Yannielli and Harrington, 2001b). 5-$HT_{7/1A}$ and 5-HT_{1B} receptor agonists block light-induced behavioral phase shifts and SCN c-Fos immunoreactivity in the hamster in vivo, effects believed to be postsynaptic (7/1A) and presynaptic (1B) to RHT terminals (Belenky and Pickard, 2001; Smith et al., 2001).

There are even more neurochemicals that affect the amplitude of photic phase shifts, putatively by modulating glutamatergic signaling presynaptically or postsynaptically, including histamine (Meyer et al., 1998), brain-derived neurotrophic factor (Liang et al., 2000), adenosine (Hallworth et al., 2002), and opioids (Tierno et al., 2002).

Multiple Kinases and Multiple Genes Are Active within SCN Cells

The intracellular pathways leading from membrane excitability to gene expression involve a network of protein kinases that act as a "phospho-relay" system; their complexity is staggering, with the human "kinome" now estimated as a nonredundant set of 518 genes (Manning et al., 2002). Elevation of intracellular Ca^{2+} activates several pathways, which, depending on context, exhibit various combinatorial, conditional, and/or convergent interactions (West et al., 2001). CREB phosphorylation and transcriptional regulation depend on the spatio-temporal dynamics of Ca^{2+} influx and route of entry (primarily via NMDA receptors but also through L-type voltage-gated Ca^{2+} channels and release from intracellular stores). In neurons, the phosphorylation of CREB by both a fast CaMKIV and a slow MAPK pathway may be responsible for encoding the timing and amplitude, respectively, of incoming stimuli (Dolmetsch et al., 2001; Wu et al., 2001).

In the rodent SCN, PKA (Tischkau et al., 2000), protein kinase G (PKG) (Mathur et al., 1996), CaMK (Golombek and Ralph, 1994; Schurov et al., 1999), and MAPK (Obrietan et al., 1998) have all been implicated in light-induced resetting. Their relative roles are debated, with discrepancies (Yokota et al., 2001; Butcher et al., 2002) probably due to experimental details (e.g., species and irradiance levels). There are data to suggest that phase delays and advances might involve different substrates (Gillette and Mitchell, 2002), for example, the ryanodine receptor/intracellular Ca^{2+} stores in the former and cGMP/PKG in the latter (Ding et al., 1998).

Besides c-*fos* and *per*, additional SCN genes exhibit phase-dependent photoinducibility (see Morris et al., 1998). Their roles are uncertain, and some, such as *nur77* and *zif268*, display a photic sensitivity 10- to 100-fold greater than the sensitivity for behavioral phase shifting (Lin et al., 1997) and species differences (Dong et al., 2002). There is also a family of CREB-related factors, including ATF-1, CREM, and ICER (the latter is inducible in the SCN by light at night; Stehle et al., 1996), and there are still more genes that are structurally related to c-*fos*, including *fra-1*, *fra-2*, and *fosB*. The proteins encoded by these genes (c-Fos,

Fra-1, Fra-2, FosB, and ΔFosB) all preferentially bind to a *cis*-regulatory element (the Activator Protein-1 [AP-1] binding site) but only when they are complexed as heterodimers, especially to the proteins of the *jun* gene family (c-Jun, JunB, and JunD). Studies using in situ hybridization, immunohistochemistry, and gel mobility shift assays have shown that *fos* and *jun* gene expression is differentially regulated within individual SCN cells, resulting in the generation of AP-1 binding complexes with constant, as well as variable, protein components. Light acts in the SCN to change the protein composition of these complexes as well as total AP-1 binding activity (Kornhauser et al., 1992; Takeuchi et al., 1993; François-Bellan et al., 1999; Schwartz et al., 2000). The transcriptional regulation of these genes is not governed by any single response element on their promoters, but it relies instead on the cooperative, interdependent activity of multiple control elements (e.g., for c-*fos*, see Robertson et al., 1995).

Implicating Specific SCN Genes as Causal Elements in the Photic Entrainment Pathway Has Been Difficult

Wollnik et al. (1995) reported that the intracerebroventricular injection of antisense oligodeoxynucleotides to both c-*fos* and *junB* prevents light-induced phase delays of the rat locomotor rhythm. However, their observation that photic phase shifts were completely inhibited by a 50% reduction in SCN c-Fos levels is not easily reconciled with previous data (Kornhauser et al., 1990). Interestingly, in studies of dividing Swiss 3T3 fibroblasts, intracellular microinjection of various antibodies against Fos family proteins suggests that the activity of multiple proteins must be inhibited to effectively block DNA synthesis (Kovary and Bravo, 1991). In contrast, similar experiments using antibodies against Jun family proteins indicate that the activity of each single Jun protein is essential for cell cycle progression. Thus, it may be that the inhibition of *junB* is the indispensable part of Wollnik et al.'s (1995) experimental design. Using a similar approach with *per1* antisense, Akiyama et al. (1999) inhibited light- and glutamate-induced phase delays of the locomotor rhythm in mice in vivo and the SCN firing rate rhythm in slices in vitro, respectively, and Wakamatsu et al. (2001) essentially eliminated the delays in vivo by injecting antisense oligos to both *mper1* and *mper2*. However, these authors observed that photic phase shifts were completely blocked by only a 25% reduction in SCN *per* levels, a finding not consonant with previous data (Shigeyoshi et al., 1997).

Mice with targeted disruption of the c-*fos* and *per* genes have been constructed, although the limitations of this approach are well known (sp., that the resultant phenotypes mostly reveal how an animal develops with [and adapts to] the absence of the mutated gene and not necessarily how the intact gene functions normally). Honrado et al. (1996) reported that mice homozygous for a c-*fos* null mutation nevertheless entrain to a light-dark cycle and generate behavioral phase shifts to light pulses. Honrado et al. speculated that other proteins might compensate for the loss of c-Fos in these animals; indeed, Fra-2 and/or FosB would appear to be plausible candidates for such a role (Schwartz et al., 2000). Three lines of mice have now been reported that are genetically deficient in *per1* (all lack Per1 protein expression): one with replacement of exons 4-10, encoding the PAS domain, exhibits a shortened free-running period but normal light-induced delay and advance phase shifts (Cermakian et al., 2001); a second ($mPer1^{Brdm1}$) with replacement of exons 4-18 exhibits an imprecise free-running period (Zheng et al., 2001) and absent phase advances (at ZT 22) but normal phase delays (at ZT 14) to 15-min, 500-lux light pulses (Albrecht et al., 2001); and a third ($mPer1^{ldc}$) with replacement of exons 2-12 becomes gradually arrhythmic after 10 to 14 days in constant darkness (Bae et al., 2001). Clearly, further investigation is needed, and progress should be aided by the ongoing development of conditional, tissue-specific knockouts and methods for RNA interference.

In the Natural World, Dawn and Dusk Presumably Act as Phase-Resetting Signals

When nocturnal locomotor activity onset and offset are used as phase markers, the 2 measures show different kinetics in both rat and hamster (Honma et al., 1985; Elliott and Tamarkin, 1994; Meijer and DeVries, 1995). A phase-advancing light pulse induces transient shifts of activity onset over several cycles but an immediate shift of activity offset; a phase-delaying light pulse has the opposite effect, with a more rapid shift of activity onset than activity offset. Similar differential onset and offset shifts of nocturnal pineal *N*-acetyltransferase activity occur in the rat (Illnerová and Sumová, 1997). Recent data hint that this dynamic behavior may arise within the SCN itself. In hamster slices, the circadian rhythm of the SCN firing rate

exhibits distinct morning and evening peaks when the slices are cut in the horizontal (rather than the usual coronal) plane (Jagota et al., 2000). The morning peak follows projected dawn, while the evening peak occurs around projected dusk, and they are shifted independently in a phase-dependent manner after stimulation with glutamate. Late night glutamate immediately advances the morning peak (without shifting the evening peak), and early night glutamate immediately delays the evening peak (without shifting the morning peak), when recorded during the first poststimulation cycle.

Pittendrigh and Daan (1976) modeled the rodent circadian clock as a complex pacemaker consisting of 2 mutually-coupled oscillators: a morning oscillator (*M*) accelerated by light and synchronized to dawn and an evening oscillator (*E*) decelerated by light and synchronized to dusk. A phase-advancing light pulse would immediately advance *M* (and so the morning peak of SCN electrical activity and the offset of nocturnal locomotor activity) with a later shift of *E*; while a phase-delaying light pulse would immediately delay *E* (and so the evening peak of SCN electrical acitivity and the onset of nocturnal locomotor activity) with a later shift of *M*. Recently, Daan et al. (2001) hypothesized that *per1* is part of *M* and *per2* is part of *E*. Indeed, in the mouse and rat, light-induced elevation of *per1* mRNA appears somewhat more robust after light is administered during the late subjective night, whereas the elevation of *per2* mRNA appears generally greater after light during the early subjective night (Zylka et al., 1998; Miyake et al., 2000); in the mouse, light-induced advance and delay shifts at these late and early night phases are correlated with the appearance of *per1* and *per2* hybridization signals, respectively, in the dorsomedial SCN (Yan and Silver, 2002). The behavior of $mPer1^{Brdm1}$ and $mPer2^{Brdm1}$ mutant mice after nocturnal light pulses (Albrecht et al., 2001) and in constant light (Steinlechner et al., 2002) is consistent with a *per1*/*M* and *per2*/*E* hypothesis. However, alternative hypotheses have been proposed for the molecular identity of *M* and *E* (Hastings, 2001), and further testing is under way (Lincoln et al., 2002).

Additional Visual Functions Involve Additional Neural Substrates

Although not covered in this review, there are other circadian visual phenomena besides phase resetting and photoentrainment (e.g., light-induced melatonin suppression and masking of locomotor activity) that seem to involve additional mechanisms that are not well characterized. For example, even though pineal melatonin rhythmicity is driven by the SCN, nighttime light suppression of melatonin in the hamster appears substantially more sensitive to irradiance than does phase shifting (Nelson and Takahashi, 1991a), implying a difference in the organization of the responsible input pathways.

Masking (the direct inhibition of nocturnal locomotor activity by light) is preserved in SCN-lesioned hamsters (Redlin and Mrosovsky, 1999); in mice rendered behaviorally arrhythmic by various mutations of clock genes, including those lacking photoinducible c-*fos* and *per* expression in the SCN (Harmar et al., 2002); and in mice genetically deficient in melanopsin, despite strongly attenuated light-induced phase shifting (Panda et al., 2002). Of note, triple mutant mice (*rd/rd;mcry1*$^{-}$*/mcry1*$^{-}$*;mcry2*$^{-}$/mcry2^{-}) exhibit little or no masking, even though masking is preserved in *rd/rd* or *cry*-deficiency alone (Selby et al., 2000). Additional genetic models should help to dissect the mechanisms for masking from those for photoentrainment: *waved-2* mice (with a loss-of-function mutation in the epidermal growth factor receptor) entrain, but masking is impaired (Kramer et al., 2001); while a line of California mice (*Peromyscus californicus*) mask, but entrainment is impaired (de Groot and Rusak, 2002). The SCN is part of an extensive, nonimage-forming, subcortical visual system, including the IGL and visual midbrain (Morin and Blanchard, 1998; Moore et al., 2000), and it is likely that multiple structures in this network will be found that contribute to behavioral and physiological rhythmicity.

STILL THE GOAL: DECONSTRUCTING THE PHOTIC PHASE RESPONSE CURVE

Our mechanistic understanding of the neurobiological basis for the circadian pacemaker's dualistic response to light during the night, and its "dead zone" during most of the day, remains incomplete. We do know that the phase-dependent "gate" that restricts responsiveness to the night is specific to and originates in the SCN. In other retino-recipient areas, light-induced c-*fos* gene expression does not depend on the circadian phase of stimulation (e.g., IGL; Peters et al., 1996). Phase-dependent control of hamster c-Fos protein expression and mouse *per1* transcription persists when the SCN is isolated in a

brain slice and either the attached optic nerves are electrically stimulated (Bennett et al., 1996) or NMDA is applied to the bath (Asai et al., 2001), respectively, but we do not know how NMDA receptor activity is enhanced during the night. We are beginning to understand how bidirectional phase shifts might result from the unidirectional induction of clock gene transcription on the rising or falling phases of an endogenous oscillation. But we still need to know much more about the regulation of phospho-CREB (e.g., its interaction with CBP and its active dephosphorylation), photic effects on clock proteins (Tamaru et al., 2000), dynamic interactions among clock genes (Reddy et al., 2002), and SCN organization at the tissue level (Yan and Silver, 2002). Tackling these problems remains a challenge, but it also surely represents an unparalleled opportunity to illuminate the links between genes, brain, and behavior.

REFERENCES

Abe H, Honma S, Shinohara K, and Honma K (1996) Substance P receptor regulates the photic induction of Fos-like protein in the suprachiasmatic nucleus of Syrian hamsters. Brain Res 708:135-142.

Abrahamson EE and Moore RY (2001) Suprachiasmatic nucleus in the mouse: Retinal innervation, intrinsic organization and efferent projections. Brain Res 916:172-191.

Aggelopoulos NC and Meissl H (2000) Responses of neurons of the rat suprachiasmatic nucleus to retinal illumination under photopic and scotopic conditions. J Physiol 523:211-222.

Aida R, Moriya T, Araki M, Akiyama M, Wada K, Wada E, and Shibata S (2002) Gastrin-releasing peptide mediates photic entrainable signals to dorsal subsets of suprachiasmatic nucleus via induction of *Period* gene in mice. Mol Pharmacol 61:26-34.

Aïoun J, Chambille I, Peytevin J, and Martinet L (1998) Neurons containing gastrin-releasing peptide and vasoactive intestinal polypeptide are involved in the reception of the photic signal in the suprachiasmatic nucleus of the Syrian hamster: An immunocytochemical ultrastructural study. Cell Tissue Res 291:239-253.

Akiyama M, Kouzu Y, Takahashi S, Wakamatsu H, Moriya T, Maetani M, Watanabe S, Tei H, Sakaki Y, and Shibata S (1999) Inhibition of light- or glutamate-induced *mPer1* expression represses the phase shifts into the mouse circadian locomotor and suprachiasmatic firing rhythms. J Neurosci 19:1115-1121.

Albrecht U, Sun ZS, Eichele G, and Lee CC (1997) A differential response of two putative mammalian circadian regulators, *mper1* and *mper2*, to light. Cell 91:1055-1064.

Albrecht U, Zheng B, Larkin D, Sun ZS, and Lee CC (2001) *mPer1* and *mPer2* are essential for normal resetting of the circadian clock. J Biol Rhythms 16:100-104.

Asai M, Yamaguchi S, Isejima H, Jonouchi M, Moriya T, Shibata S, Kobayashi M, and Okamura H (2001) Visualization of *mPer1* transcription in vitro: NMDA induces a rapid phase shift of *mPer1* gene in cultured SCN. Curr Biol 11:1524-1527.

Bae K, Jin X, Maywood ES, Hastings MH, Reppert SM, and Weaver DR (2001) Differential functions of *mPer1*, *mPer2*, and *mPer3* in the SCN circadian clock. Neuron 30:525-536.

Belenky MA and Pickard GE (2001) Subcellular distribution of 5-HT(1B) and 5-HT(7) receptors in the mouse suprachiasmatic nucleus. J Comp Neurol 432:371-388.

Bennett MR, Aronin N, and Schwartz WJ (1996) In vitro stimulation of c-Fos protein expression in the suprachiasmatic nucleus of hypothalamic slices. Mol Brain Res 42:140-144.

Best JD, Maywood ES, Smith KL, and Hastings MH (1999) Rapid resetting of the mammalian circadian clock. J Neurosci 19:828-835.

Bos NPA and Mirmiran M (1993) Effects of excitatory and inhibitory amino acids on neuronal discharges in the cultured suprachiasmatic nucleus. Brain Res Bull 31:67-72.

Bryant DN, LeSauter J, Silver R, and Romero M-T (2000) Retinal innervation of calbindin-D_{28K} cells in the hamster suprachiasmatic nucleus: Ultrastructural characterization. J Biol Rhythms 15:103-111.

Butcher GQ, Dziema H, Collamore M, Burgoon PW, and Obrietan K (2002) The p42/44 mitogen-activated protein kinase pathway couples photic input to circadian clock entrainment. J Biol Chem 277:29519-29525.

Castel M, Belenky M, Cohen S, Wagner S, and Schwartz WJ (1997) Light-induced c-Fos expression in the mouse suprachiasmatic nucleus: Immuno-electron microscopy reveals co-localization in multiple cell types. Eur J Neurosci 9:1950-1960.

Cermakian N, Monaco L, Pando MP, Dierich A, and Sassone-Corsi P (2001) Altered behavioral rhythms and clock gene expression in mice with a targeted mutation in the *Period1* gene. EMBO J 20:3967-3974.

Challet E, Dugovic C, Turek FW, and Van Reeth O (2001) The selective neurokinin 1 receptor antagonist R116301 modulates photic responses of the hamster circadian system. Neuropharmacology 40:408-415.

Chen D, Buchanan GF, Ding JM, Hannibal J, and Gillette MU (1999) Pituitary adenylate cyclase-activating peptide: A pivotal modulator of glutamatergic regulation of the suprachiasmatic circadian clock. Proc Natl Acad Sci U S A 96:13468-13473.

Colwell CS (2001) NMDA-evoked calcium transients and currents in the suprachiasmatic nucleus: Gating by the circadian system. Eur J Neurosci 13:1420-1428.

Crosio C, Cermakian N, Allis CD, and Sassone-Corsi P (2000) Light induces chromatin modification in cells of the mammalian circadian clock. Nat Neurosci 3:1241-1247.

Cui L-N and Dyball REJ (1996) Synaptic input from the retina to the suprachiasmatic nucleus changes with the light-dark cycle in the Syrian hamster. J Physiol 492:483-493.

Daan S, Albrecht U, van der Horst GTJ, Illnerová H, Roenneberg T, Wehr TA, and Schwartz WJ (2001) Assembling a clock for all seasons: Are there M and E oscillators in the genes? J Biol Rhythms 16:105-116.

de Groot MHM and Rusak B (2002) Entrainment impaired, masking spared: An apparent genetic abnormality that prevents circadian rhythm entrainment to 24-h lighting cycles in California mice. Neurosci Lett 327:203-207.

de Jeu M, Hermes M, and Pennartz CM (1998) Circadian modulation of membrane properties in slices of rat suprachiasmatic nucleus. NeuroReport 9:3725-3729.

de la Iglesia HO and Schwartz WJ (2002) A subpopulation of efferent neurons in the mouse suprachiasmatic nucleus is also light-responsive. NeuroReport 13:857-860.

Ding JM, Buchanan GF, Tischkau SA, Chen D, Kuriashkina L, Fairman LE, Alster JM, McPherson PS, Campbell KP, and Gillette MU (1998) A neuronal ryanodine receptor mediates light-induced phase delays of the circadian clock. Nature 394:381-384.

Ding JM, Chen D, Weber ET, Fairman LE, Rea MA, and Gillette MU (1994) Resetting the biological clock: Mediation of nocturnal circadian shifts by glutamate and NO. Science 266:1713-1717.

Ding JM, Fairman LE, Hurst WJ, Kuriashkina LR, and Gillette MU (1997) Resetting the biological clock: Mediation of nocturnal CREB phosphorylation via light, glutamate, and nitric oxide. J Neurosci 17:667-675.

Dkhissi-Benyahya O, Sicard B, and Cooper HM (2000) Effects of irradiance and stimulus duration on early gene expression (Fos) in the suprachiasmatic nucleus: Temporal summation and reciprocity. J Neurosci 20:7790-7797.

Dolmetsch RE, Pajvani U, Fife K, Spotts JM, and Greenberg ME (2001) Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. Science 294:333-339.

Dong YN, Goguen D, Robertson HA, and Rusak B (2002) Anatomical and temporal differences in the regulation of ZIF268 (NGFI-A) protein in the hamster and mouse suprachiasmatic nucleus. Neuroscience 111:567-574.

Dziema H and Obrietan K (2002) PACAP potentiates L-type calcium channel conductance in suprachiasmatic nucleus neurons by actvating the MAPK pathway. J Neurophysiol 88:1374-1386.

Earnest DJ, DiGeorgio S, and Olschowka JA (1993) Light induces expression of fos-related proteins within gastrin-releasing peptide neurons in the rat suprachiasmatic nucleus. Brain Res 627:205-209.

Ebling FJP (1996) The role of glutamate in the photic regulation of the suprachiasmatic nucleus. Prog Neurobiol 50:109-132.

Elliott JA and Tamarkin L (1994) Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. J Comp Physiol A 174:469-484.

Field MD, Maywood ES, O'Brien JA, Weaver DR, Reppert SM, and Hastings MH (2000) Analysis of clock proteins in mouse SCN demonstrates phylogenetic divergence of the circadian clockwork and resetting mechanisms. Neuron 25:437-447.

François-Bellan AM, Deprez P, and Becquet D (1999) Light-induced variations in AP-1 binding activity and composition in the rat suprachiasmatic nucleus. J Neurochem 72:841-847.

Gau D, Lemberger T, von Gall C, Kretz O, Le Minh N, Gass P, Schmid W, Schibler U, Korf HW, and Schütz G (2002) Phosphorylation of CREB Ser142 regulates light-induced phase shifts of the circadian clock. Neuron 34:245-253.

Gillespie CF, van der Beek EM, Mintz EM, Mickley NC, Jasnow AM, Huhman KL, and Albers HE (1999) GABAergic regulation of light-induced c-Fos immunoreactivity within the suprachiasmatic nucleus. J Comp Neurol 411:683-692.

Gillette MU and Mitchell JW (2002) Signaling in the suprachiasmatic nucleus: Selectively responsive and integrative. Cell Tissue Res 309:99-107.

Ginty DD, Kornhauser JM, Thompson MA, Bading H, Mayo KE, Takahashi JS, and Greenberg ME (1993) Regulation of CREB phosphorylation in the suprachiasmatic nucleus by light and a circadian clock. Science 260:238-241.

Golombek DA and Ralph MR (1994) KN-62, an inhibitor of Ca^{2+}/calmodulin kinase II, attenuates circadian responses to light. NeuroReport 5:1638-1640.

Groos GA and Mason R (1980) The visual properties of rat and cat suprachiasmatic neurons. J Comp Physiol A 135:349-356.

Guido ME, de Guido LB, Goguen D, Robertson HA, and Rusak B (1999a) Daily rhythm of spontaneous immediate-early gene expression in the rat suprachiasmatic nucleus. J Biol Rhythms 14:275-280.

Guido ME, Goguen D, de Guido L, Robertson HA, and Rusak B (1999b) Circadian and photic regulation of immediate-early gene expression in the hamster suprachiasmatic nucleus. Neuroscience 90:555-571.

Hallworth R, Cato M, Colbert C, and Rea MA (2002) Presynaptic adenosine A1 receptors regulate retinohypothalamic neurotransmission in the hamster suprachiasmatic nucleus. J Neurobiol 52:230-240.

Hamada T, Le Sauter J, Venuti JM, and Silver R (2001) Expression of *Period* genes: Rhythmic and non-rhythmic compartments of the suprachiasmatic nucleus pacemaker. J Neurosci 19:7742-7750.

Hamada T, Yamanouchi S, Watanabe A, Shibata S, and Watanabe S (1999) Involvement of glutamate release in substance P-induced phase delays of suprachiasmatic neuron activity rhythm in vitro. Brain Res 836:190-193.

Hannibal J and Fahrenkrug J (2002) Immunoreactive substance P is not part of the retinohypothalamic tract in the rat. Cell Tissue Res 309:293-299.

Hannibal J, Jamen F, Nielsen HS, Journot L, Brabet P, and Fahrenkrug J (2001) Dissociation between light-induced phase shift of the circadian rhythm and clock gene expression in mice lacking the pituitary adenylate cyclase activating polypeptide type 1 receptor. J Neurosci 21:4883-4890.

Hannibal J, Møller M, Ottersen OP, and Fahrenkrug J (2000) PACAP and glutamate are co-stored in the retinohypothalamic tract. J Comp Neurol 418:147-155.

Harmar AJ, Marston HM, Shen S, Spratt C, West KM, Sheward WJ, Morrison CF, Dorin JR, Piggins HD, Reubi J-C, et al. (2002) The $VPAC_2$ receptor is essential for circadian function in the mouse suprachiasmatic nuclei. Cell 109:497-508.

Harrington ME (1997) The ventral lateral geniculate nucleus and the intergeniculate leaflet: Interrelated structures in the visual and circadian systems. Neurosci Biobehav Rev 21:705-727.

Harrington ME, Hoque S, Hall A, Golombek D, and Biello S (1999) Pituitary adenylate cyclase activating peptide phase shifts circadian rhythms in a manner similar to light. J Neurosci 19:6637-6642.

Hastings MH (2001) Modelling the molecular calendar. J Biol Rhythms 16:117-123.

Hendrickson AE, Wagoner N, and Cowan WM (1972) An autoradiographic and electron microscopic study of retino-hypothalamic connections. Z Zellforsch Mikrosk Anat 135:1-26.

Honma K-I, Honma S, and Hiroshige T (1985) Response curve, free-running period, and activity time in circadian locomotor rhythm of rats. Jpn J Physiol 35:643-658.

Honrado GI, Johnson RS, Golombek DA, Spiegelman BM, Papaioannou V, and Ralph MR (1996) The circadian system of c-*fos* deficient mice. J Comp Physiol A 178:563-570.

Illnerová H and Sumová A (1997) Photic entrainment of the mammalian rhythm in melatonin production. J Biol Rhythms 12:547-555.

Jagota A, de la Iglesia HO, and Schwartz WJ (2000) Morning and evening circadian oscillations in the suprachiasmatic nucleus *in vitro*. Nat Neurosci 3:372-376.

Jiang Z-G, Yang YQ, Liu Z-P, and Allen CN (1997) Membrane properties and synaptic inputs of suprachiasmatic nucleus neurons in rat brain slices. J Physiol 499:141-159.

Jiao Y, Lee TM, and Rusak B (1999) Photic responses of suprachiasmatic area neurons in diurnal degus (*Octodon degus*) and nocturnal rats (*Rattus norvegicus*). Brain Res 817:93-103.

Johnson RF, Moore RY, and Morin LP (1988) Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. Brain Res 460:297-313.

Kim DY, Kang H-C, Shin HC, Lee KJ, Yoon YW, Han HC, Na HS, Hong SK, and Kim YI (2001) Substance P plays a critical role in photic resetting of the circadian pacemaker in the rat hypothalamus. J Neurosci 21:4026-4031.

Kim YI and Dudek FE (1991) Intracellular electrophysiological study of suprachiasmatic nucleus neurons in rodents: Excitatory synaptic mechanisms. J Physiol 444:269-287.

Kim YI and Dudek FE (1993) Membrane properties of rat suprachiasmatic nucleus neurons receiving optic nerve input. J Physiol 464:229-243.

Kopp MDA, Meissl H, Dehghani F, and Korf H-W (2001) Pituitary adenylate cyclase-activating polypeptide modulates glutamatergic calcium signalling: Investigations on rat suprachiasmatic nucleus neurons. J Neurochem 79:161-171.

Kornhauser JM, Nelson DE, Mayo KE, and Takahashi JS (1990) Photic and circadian regulation of c-*fos* gene expression in the hamster suprachiasmatic nucleus. Neuron 5:127-134.

Kornhauser JM, Nelson DE, Mayo KE, and Takahashi JS (1992) Regulation of *jun-B* messenger RNA and AP-1 activity by light and a circadian clock. Science 255:1581-1584.

Kovary K and Bravo R (1991) The Jun and Fos protein families are both required for cell cycle progression in fibroblasts. Mol Cell Biol 11:4466-4472.

Kramer A, Yang F-C, Snodgrass P, Li X, Scammell TE, Davis FC, and Weitz CJ (2001) Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. Science 294:2511-2515.

Liang F-Q, Allen G, and Earnest D (2000) Role of brain-derived neurotrophic factor in the circadian regulation of the suprachiasmatic pacemaker by light. J Neurosci 20:2978-2987.

Lin JT, Kornhauser JM, Singh NP, Mayo KE, and Takahashi JS (1997) Visual sensitivities of *nur77* (NGFI-B) and *zif268* (NGFI-A) induction in the suprachiasmatic nucleus are dissociated from c-*fos* induction and behavioral phase-shifting responses. Mol Brain Res 46:303-310.

Lincoln G, Messager S, Andersson H, and Hazlerigg D (2002) Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: Evidence for an internal coincidence timer. Proc Natl Acad Sci U S A 99:13890-13895.

Liu C and Reppert SM (2000) GABA synchronizes clock cells within the suprachiasmatic circadian clock. Neuron 25:123-128.

Lonze BE and Ginty DD (2002) Function and regulation of CREB family transcription factors in the nervous system. Neuron 35:605-623.

Manning G, Whyte DB, Martinez R, Hunter T, and Sudarsanam S (2002) The protein kinase complement of the human genome. Science 298:1912-1934.

Mathur A, Golombek DA, and Ralph MR (1996) CGMP-dependent protein kinase inhibitors block light-induced phase advances of circadian rhythms in vivo. Am J Physiol 270:R1031-1036.

McArthur AJ, Coogan AN, Ajpru S, Sugden D, Biello SM, and Piggins HD (2000) Gastrin-releasing peptide phase-shifts suprachiasmatic nuclei neuronal rhythms in vitro. J Neurosci 20:5496-5502.

McNulty S, Schurov IL, Sloper PJ, and Hastings MH (1998) Stimuli which entrain the circadian clock of the neonatal Syrian hamster *in vivo* regulate the phosphorylation of the transcription factor CREB in the suprachiasmatic nucleus *in vitro*. Eur J Neurosci 10:1063-1072.

Meijer JH, Albus H, Weidema F, and Ravesloot JH (1993) The effects of glutamate on membrane potential and discharge rate of suprachiasmatic neurons. Brain Res 603:284-288.

Meijer JH and DeVries MJ (1995) Light-induced phase shifts in onset and offset of running-wheel activity in the Syrian hamster. J Biol Rhythms 10:4-16.

Meijer JH, Groos GA, and Rusak B (1986) Luminance coding in a circadian pacemaker: The suprachiasmatic nucleus of the rat and the hamster. Brain Res 382:109-118.

Meijer JH, Rusak B, and Harrington ME (1989) Photically responsive neurons in the hypothalamus of a diurnal ground squirrel. Brain Res 501:315-323.

Meijer JH, Watanabe K, Schaap J, Albus H, and Détári L (1998) Light responsiveness of the suprachiasmatic nucleus: Long-term multiunit and single-unit recordings in freely moving rats. J Neurosci 18:9078-9087.

Meyer JL, Hall AC, and Harrington ME (1998) Histamine phase shifts the hamster circadian pacemaker via an NMDA dependent mechanism. J Biol Rhythms 13:288-295.

Michel S, Itri J, and Colwell CS (2002) Excitatory mechanisms in the suprachiasmatic nucleus:RThe role of AMPA/KA glutamate receptors. J Neurophysiol 88:817-828.

Mikkelsen JD and Vrang N (1994) A direct pretecto-suprachiasmatic projection in the rat. Neuroscience 62: 497-505.

Minami Y, Furuno K, Akiyama M, Moriya T, and Shibata S (2002) Pituitary adenylate cyclase-activating polypeptide produces a phase shift associated with induction of *mPer* expression in the mouse suprachiasmatic nucleus. Neuroscience 113:37-45.

Mintz EM, Marvel CL, Gillespie CF, Price KM, and Albers HE (1999) Activation of NMDA receptors in the suprachiasmatic nucleus produces light-like phase shifts of the circadian clock in vivo. J Neurosci 19:5124-5130.

Miyake S, Sumi Y, Yan L, Takekida S, Fukuyama T, Ishida Y, Yamaguchi S, Yagita K, and Okamura H (2000) Phase-dependent responses of *Per1* and *Per2* genes to a light-stimulus in the suprachiasmatic nucleus of the rat. Neurosci Lett 294:41-44.

Moga MM and Moore RY (1996) Putative excitatory amino acid projections to the suprachiasmatic nucleus in the rat. Brain Res 743:171-177.

Moore RY and Lenn NJ (1972) A retinohypothalamic projection in the rat. J Comp Neurol 146:1-14.

Moore RY, Weis R, and Moga MM (2000) Efferent projections of the intergeniculate leaflet and the ventral lateral geniculate nucleus in the rat. J Comp Neurol 420:398-418.

Morin LP (1994) The circadian visual system. Brain Res Rev 67:102-127.

Morin LP and Blanchard JH (1998) Interconnections among nuclei of the subcortical visual shell: The intergeniculate leaflet is a major constituent of the hamster subcortical visual system. J Comp Neurol 396:288-309.

Moriya T, Horikawa K, Akiyama M, and Shibata S (2000) Correlative association between *N*-methyl-D-aspartate receptor-mediated expression of *Period* genes in the suprachiasmatic nucleus and phase shifts in behavior with photic entrainment of clock in hamsters. Mol Pharmacol 58:1554-1562.

Morris ME, Viswanathan N, Kuhlman S, Davis FC, and Weitz CJ (1998) A screen for genes induced in the suprachiasmatic nucleus by light. Science 279:1544-1547.

Munch IC, Møller M, Larsen PJ, and Vrang N (2002) Light-induced c-Fos expression in suprachiasmatic nuclei neurons targeting the paraventricular nucleus of the hamster hypothalamus: Phase dependence and immunochemical identification. J Comp Neurol 442:48-62.

Nelson DE and Takahashi JS (1991a) Comparison of visual sensitivity for suppression of pineal melatonin and circadian phase-shifting in the golden hamster. Brain Res 554:272-277.

Nelson DE and Takahashi JS (1991b) Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). J Physiol 439:115-145.

Nielsen HS, Hannibal J, and Fahrenkrug J (2002) Vasoactive intestinal polypeptide induces *per1* and *per2* gene expression in the rat suprachiasmatic nucleus late at night. Eur J Neurosci 15:570-574.

Nielsen HS, Hannibal J, Knudsen SM, and Fahrenkrug J (2001) Pituitary adenylate cyclase activating polypeptide induces *period1* and *period2* gene expression in the rat suprachiasmatic nucleus during late night. Neuroscience 103:433-441.

Novak CM and Albers HE (2002) *N*-methyl-D-aspartate microinjected into the suprachiasmatic nucleus mimics the phase-shifting effects of light in the diurnal Nile grass rat (*Arvicanthis niloticus*). Brain Res 951:255-263.

Obrietan K, Impey S, Smith D, Athos J, and Storm DR (1999) Circadian regulation of cAMP response element-mediated gene expression in the suprachiasmatic nuclei. J Biol Chem 274:17748-17756.

Obrietan K, Impey S, and Storm DR (1998) Light and circadian rhythmicity regulate MAP kinase activation in the suprachiasmatic nuclei. Nat Neurosci 1:693-700.

Panda S, Sato TK, Castrucci AM, Rollag MD, DeGrip WJ, Hogenesch JB, Provencio I, and Kay SA (2002) Melanopsin (*Opn4*) requirement for normal light-induced circadian phase shifting. Science 498:2213-2216.

Pennartz CMA, Hamstra R, and Geurtsen AMS (2001) Enhanced NMDA receptor activity in retinal inputs to the rat suprachiasmatic nucleus during the subjective night. J Physiol 532:181-194.

Peters RV, Aronin N, and Schwartz WJ (1996) c-Fos expression in the rat intergeniculate leaflet: Photic regulation, co-localization with Fos B, and cellular identification. Brain Res 728:231-241.

Piggins HD, Antle MC, and Rusak B (1995) Neuropeptides phase shift the mammalian circadian pacemaker. J Neurosci 15:5612-5622.

Piggins HD and Rusak B (1997) Effects of microinjections of substance P into the suprachiasmatic nucleus region on hamster wheel running rhythms. Brain Res Bull 42:451-455.

Piggins HD, Samuels RE, Coogan AN, and Cutler DJ (2001) Distribution of substance P and neurokinin-1 receptor immunoreactivity in the suprachiasmatic nuclei and intergeniculate leaflet of hamster, mouse, and rat. J Comp Neurol 438:50-65.

Pittendrigh CS and Daan S (1976) A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: A clock for all seasons. J Comp Physiol A 106:333-355.

Pu M (2000) Physiological response properties of cat retinal ganglion cells projecting to suprachiasmatic nucleus. J Biol Rhythms 15:31-36.

Rea MA and Pickard GE (2000) Serotonergic modulation of photic entrainment in the Syrian hamster. Biol Rhythm Res 31:284-314.

Redlin U and Mrosovsky N (1999) Masking by light in hamsters with SCN lesions. J Comp Physiol A 184:439-448.

Reddy AB, Field MD, Maywood ES, and Hastings MH (2002) Differential resynchronisation of circadian clock gene expression within the suprachiasmatic nuclei of mice subjected to experimental jet lag. J Neurosci 22:7326-7330.

Reed HE, Meyer-Spasche A, Cutler DJ, Coen CW, and Piggins HD (2001) Vasoactive intestinal polypeptide (VIP) phase-shifts the rat suprachiasmatic nucleus clock in vitro. Eur J Neurosci 13:839-843.

Reuss S, Decker K, Robeler L, Layes E, Schollmayer A, and Spessert R (1995) Nitric oxide synthase in the hypothalamic suprachiasmatic nucleus of rat: Evidence from histochemistry, immunohistochemistry and Western blot; and colocalization with VIP. Brain Res 695:257-262.

Robertson LM, Kerppola TK, Vendrell M, Luk D, Smeyne RJ, Bocchiaro C, Morgan JI, and Curran T (1995) Regulation of c-*fos* expression in transgenic mice requires multiple interdependent transcription control elements. Neuron 14:241-252.

Rollag et al. (2003) Melanopsin, ganglion cell photoreceptors, and mammilian photo entrainment. J Biol Rhythms 18:227-234.

Romijn HJ, Sluiter AA, Pool CW, Wortel J, and Buijs RM (1996) Differences in co-localization between Fos and PHI, GRP, VIP and VP in neurons of the rat suprachiasmatic nucleus after a light stimulus during the phase delay versus the phase advance period of the night. J Comp Neurol 372:1-8.

Schaap J, Bos NPA, de Jeu MTG, Geurtsen AMS, Meijer JH, and Pennartz CMA (1999) Neurons of the rat suprachiasmatic nucleus show a circadian rhythm in membrane properties that is lost during prolonged whole-cell recording. Brain Res 815:154-166.

Schurov IL, McNulty S, Best JD, Sloper PJ, and Hastings MH (1999) Glutamatergic induction of CREB phosphorylation and Fos expression in primary cultures of the suprachiasmatic hypothalamus in vitro is mediated by co-ordinate activity of NMDA and non-NMDA receptors. J Neuroendocrinol 11:43-51.

Schwartz WJ, Aronin N, Takeuchi J, Bennett MR, and Peters RV (1995) Towards a molecular biology of the suprachiasmatic nucleus: Photic and temporal regulation of c-*fos* gene expression. Seminars Neurosci 7:53-60.

Schwartz WJ, Carpino A Jr, de la Iglesia HO, Baler R, Klein DC, Nakabeppu Y, and Aronin N (2000) Differential regulation of *fos* family genes in the ventrolateral and dorsomedial subdivisions of the rat suprachiasmatic nucleus. Neuroscience 98:535-547.

Selby CP, Thompson C, Schmitz TM, Van Gelder RN, and Sancar A (2000) Functional redundancy of cryptochromes and classical photoreceptors for nonvisual ocular photoreception in mice. Proc Natl Acad Sci U S A 97:14697-14702.

Shearman LP, Zylka MJ, Weaver DR, Kolakowski LF Jr, and Reppert SM (1997) Two *period* homologs: Circadian expression and photic regulation in the suprachiasmatic nuclei. Neuron 19:1261-1269.

Shibata S and Moore RY (1993) Neuropeptide Y and optic chiasm stimulation affect suprachiasmatic nucleus circadian function in vitro. Brain Res 615:95-100.

Shibata S, Tsuneyoshi A, Hamada T, Tominaga K, and Watanabe S (1992) Effect of substance P on circadian rhythms of firing activity and the 2-deoxyglucose uptake in the rat suprachiasmatic nucleus in vitro. Brain Res 597:257-263.

Shigeyoshi Y, Taguchi K, Yamamoto S, Takekida S, Yan L, Tei H, Moriya T, Shibata S, Loros JJ, Dunlap JC, et al. (1997) Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the *mper1* transcript. Cell 91:1043-1053.

Shimomura K, Kornhauser JM, Wisor JP, Umezu T, Yamazaki S, Ihara NL, Takahashi JS, and Menaker M (1998) Circadian behavior and plasticity of light-induced c-*fos* expression in SCN of *tau* mutant hamsters. J Biol Rhythms 13:305-314.

Silver R, Romero M-T, Besmer HR, Leak R, Nunez JM, and LeSauter JM (1996) Calbindin-D_{28K} cells in the hamster SCN express light-induced Fos. NeuroReport 7:1224-1228.

Smith BN, Sollars PJ, Dudek FE, and Pickard GE (2001) Serotonergic modulation of retinal input to the mouse suprachiasmatic nucleus mediated by 5-HT_{1B} and 5-HT_7 receptors. J Biol Rhythms 16:25-38.

Stehle JH, Pfeffer M, Kühn R, and Korf H-W (1996) Light-induced expression of transcription factor ICER (inducible cAMP early repressor) in rat suprachiasmatic nucleus is phase-restricted. Neurosci Lett 217:169-172.

Steinlechner S, Jacobmeier B, Scherbarth F, Dernbach H, Kruse F, and Albrecht U (2002) Robust circadian rhythmicity of *Per1* and *Per2* mutant mice in constant light, and dynamics of *Per1* and *Per2* gene expression under long and short photoperiods. J Biol Rhythms 17:202-209.

Sumová A, Trávnícková Z, Mikkelsen JD, and Illnerová H (1998) Spontaneous rhythm in c-Fos immunoreactivity in the dorsomedial part of the rat suprachiasmatic nucleus. Brain Res 801:254-258.

Takahashi JS, Decoursey PJ, Bauman L, and Menaker M (1984) Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. Nature 308:186-188.

Takeuchi J, Shannon W, Aronin N, and Schwartz WJ (1993) Compositional changes of AP-1 DNA-binding proteins are regulated by light in a mammalian circadian clock. Neuron 11:825-836.

Takumi T, Taguchi K, Miyake S, Sakakida Y, Takashima N, Matsubara C, Maebayashi Y, Okumura K, Takekida S, Yamamoto S, et al. (1998) A light-independent oscillatory gene *mPer3* in mouse SCN and OVLT. EMBO J 17:4753-4759.

Tamaru T, Isojima Y, Yamada T, Okada M, Nagai K, and Takamatsu K (2000) Light and glutamate-induced degradation of the circadian oscillating protein BMAL1 during the mammalian clock resetting. J Neurosci 20:7525-7530.

Tanaka M, Ichitani Y, Okamura H, Tanaka Y, and Ibata Y (1993) The direct retinal projection to VIP neuronal elements in the rat SCN. Brain Res Bull 31:637-640.

Tierno A, Fiore P, and Gannon RL (2002) Delta opioid inhibition of light-induced phase advances in hamster circadian activity rhythms. Brain Res 937:66-73.

Tischkau SA, Gallman EA, Buchanan GF, and Gillette MU (2000) Differential cAMP gating of glutamatergic signaling regulates long-term state changes in the suprachiasmatic circadian clock. J Neurosci 20:7830-7837.

Tischkau SA, Mitchell JW, Tyan S-H, Buchanan GF, and Gillette MU (2003) Ca^{2+}/cAMP response element-binding protein (CREB)-dependent activation of *Per1* is required for light-induced signaling in the suprachiasmatic nucleus circadian clock. J Biol Chem 278:718-723.

Tominaga K, Geusz ME, Michel S, and Inouye ST (1994) Calcium imaging in organotypic cultures of the rat suprachiasmatic nucleus. NeuroReport 5:1901-1905.

Trávnícková Z, Sumová A, Peters R, Schwartz WJ, and Illnerová H (1996) Photoperiod-dependent correlation between light-induced SCN c-*fos* expression and resetting of circadian phase. Am J Physiol 271:R825-R831.

Trávnícková-Bendova Z, Cermakian N, Reppert SM, and Sassone-Corsi P (2002) Bimodal regulation of mPeriod promoters by CREB-dependent signaling and CLOCK/BMAL1 activity. Proc Natl Acad Sci U S A 99:7728-7733.

van den Pol AN, Finkbeiner SM, and Cornell-Bell AH (1992) Calcium excitability and oscillations in suprachiasmatic nucleus neurons and glia in vitro. J Neurosci 12:2648-2664.

von Gall C, Duffield GE, Hastings MH, Kopp MDA, Dehghani F, Korf H-W, and Stehle JH (1998) CREB in the mouse SCN: A molecular interface coding the phase-adjusting stimuli light, glutamate, PACAP, and melatonin for clockwork access. J Neurosci 18:10389-10397.

Wakamatsu H, Takahashi S, Moriya T, Inouye ST, Okamura H, Akiyama M, and Shibata S (2001) Additive effect of *mPer1* and *mPer2* antisense oligonucleotides on light-induced phase shift. NeuroReport 12:127-131.

Wang H and Morris JF (1996) Presence of neuronal nitric oxide synthase in the suprachiasmatic nuclei of mouse and rat. Neuroscience 74:1059-1068.

Watanabe K, Deboer T, and Meijer JH (2001) Light-induced resetting of the circadian pacemaker: Quantitative analysis of transient versus steady-state phase shifts. J Biol Rhythms 16:564-573.

Watanabe A, Hamada T, Shibata S, and Watanabe S (1994) Effects of nitric oxide synthase inhibitors on N-methyl-D-aspartate-induced phase delay of circadian rhythm of neuronal activity in the rat suprachiasmatic nucleus in vitro. Brain Res 646:161-164.

Watanabe A, Ono M, Shibata S, and Watanabe S (1995) Effect of a nitric oxide synthase inhibitor, N-nitro-L-arginine methylester, on light-induced phase delay of circadian rhythm of wheel-running activity in golden hamsters. Neurosci Lett 192:25-28.

Weber ET, Gannon RL, Michel AM, Gillette MU, and Rea MA (1995) Nitric oxide synthase inhibitor blocks light-induced phase shifts of the circadian activity rhythm, but not c-*fos* expression in the suprachiasmatic nucleus of the Syrian hamster. Brain Res 692:137-142.

West AE, Chen WG, Dalva MB, Dolmetsch RE, Kornhauser JM, Shaywitz AJ, Takasu MA, Tao X, and Greenberg ME (2001) Calcium regulation of neuronal gene expression. Proc Natl Acad Sci U S A 98:11024-11031.

Wollnik F, Brysch W, Uhlmann E, Gillardon F, Bravo R, Zimmermann M, Schlingensiepen KH, and Herdegen T (1995) Block of c-Fos and JunB expression by antisense oligonucleotides inhibits light-induced phase shifts of the mammalian circadian clock. Eur J Neurosci 7:388-393.

Wu G-Y, Deisseroth K, and Tsien RW (2001) Activity-dependent CREB phosphorylation: Convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. Proc Natl Acad Sci U S A 98:2808-2813.

Yan L and Okamura H (2002) Gradients in the circadian expression of *Per1* and *Per2* genes in the rat suprachiasmatic nucleus. Eur J Neurosci 15:1153-1162.

Yan L and Silver R (2002) Differential induction and localization of mPer1 and mPer2 during advancing and delaying phase shifts. Eur J Neurosci 16:1531-1540.

Yan L, Takekida S, Shigeyoshi Y, and Okamura H (1999) *Per1* and *Per2* gene expression in the rat suprachiasmatic nucleus: Circadian profile and the compartment-specific response to light. Neuroscience 94:141-150.

Yannielli PC and Harrington ME (2001a) Neuropeptide Y in the mammalian circadian system: Effects on light-induced circadian responses. Peptides 22:547-556.

Yannielli PC and Harrington ME (2001b) The neuropeptide Y Y5 receptor mediates the blockade of "photic-like" NMDA-induced phase shifts in the golden hamster. J Neurosci 21:5367-5373.

Yokota S, Yamamoto M, Moriya T, Akiyama M, Fukunaga K, Miyamoto E, and Shibata S (2001) Involvement of calcium-calmodulin protein kinase but not mitogen-activated protein kinase in light-induced phase delays and *Per* gene expression in the suprachiasmatic nucleus of the hamster. J Neurochem 77:618-627.

Zhang Y, Kornhauser JM, Zee PC, Mayo KE, Takahashi JS, and Turek FW (1996a) Effects of aging on light-induced phase-shifting of circadian behavioral rhythms: Fos expression and CREB phosphorylation in the hamster suprachiasmatic nucleus. Neuroscience 70:951-961.

Zhang Y, Takahashi JS, and Turek FW (1996b) Critical period for cycloheximide blockade of light-induced phase advances of the circadian locomotor activity rhythm in golden hamsters. Brain Res 740:285-290.

Zheng B, Albrecht U, Kaasik K, Sage M, Lu W, Vaishnav S, Li Q, Sun ZS, Eichele G, Bradley A, and Lee CC (2001)

Nonredundant roles of the *mPer1* and *mPer2* genes in the mammalian circadian clock. Cell 105:683-694.

Zylka MJ, Shearman LP, Weaver DR, and Reppert SM (1998) Three *period* homologs in mammals: Differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain. Neuron 20:1103-1110.