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CALBINDIN EXPRESSION IN THE HAMSTER SUPRACHIASMATIC NUCLEUS DEPENDS ON DAY-LENGTH

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Abstract—The mammalian circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus controls many physiological and behavioral rhythms. The SCN is compartmentalized in two functionally distinct subregions: a dorsomedial subregion that rhythmically expresses clock genes, and a ventrolateral subregion which, in contrast, mainly expresses clock genes at a constant level. In the golden hamster, this ventrolateral part of the SCN contains a subpopulation of neurons expressing calbindin D28k. This subpopulation has recently been implicated in the control of locomotor rhythmicity. Because both the pattern and level of locomotor activity are affected by day-length, we investigated whether photoperiod also affects calbindin expression. We show that calbindin expression is negatively correlated to the day-length. The number of calbindin immunopositive neurons and calbindin mRNA levels were markedly increased in hamsters exposed to short photoperiods (light/dark cycle [LD] 6:18 and LD10:14) when compared with hamster exposed to long photoperiods (LD18:6 and LD14:10). This suggests that calbindin neurons are involved in the encoding of seasonal information by the SCN.

Key words: Syrian hamster, photoperiod, immunocytochemistry, *in situ* hybridization.

Many aspects of physiology and behavior exhibit daily rhythms. These include sleep–wake cycle, body temperature, locomotor activity, hormone synthesis (Takahashi et al., 2001). These rhythms are driven by a central circadian clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, which is synchronized to environmental cues (Stephan and Zucker, 1972; Moore and Eichler, 1972). The light/dark cycle (LD) constitutes the main zeitgeber by which the phase of the SCN oscillator is adjusted on a daily basis.

How the circadian clock generates circadian rhythms remains unclear. There is some evidence that SCN cells are autonomous oscillators, since individual dispersed SCN cells still exhibit a circadian rhythm of electrical activity (Welsh et al., 1995; Liu et al., 1997; Herzog et al., 1998). However, this cell-autonomous nature of the SCN oscillator does not extend to all SCN cells, since one quarter of rat SCN neurons does not show a robust circadian rhythm in firing rate (Honma et al., 1998). This discrepancy between rhythmic and non-rhythmic cells could be explained by a compartmentalization within the SCN as is seen for neuropeptide (Moore and Silver, 1998) as well as clock gene (Hamada et al., 2001) content and expression. Hamada et al. (2001) showed that in Syrian hamster, a ventrolateral subregion of neurons expressing the calcium binding protein calbindin D28k (CalB subnucleus or CBsn) does not express *Period* genes rhythmically, whereas cells in the dorsomedial part of the SCN express *Period* genes in a rhythmic fashion. In addition, it has been observed in organotypic slice cultures of rat SCN that the percentage of neurons which exhibit circadian rhythms in spontaneous firing rate is higher in the dorsal SCN compared with the ventral SCN (Nakamura et al., 2001). Recently, Jobst and Allen (2002) showed that CalB expressing neurons in the SCN do not exhibit a circadian variation in spontaneous firing rate, whereas the other neurons in this CBsn do. CalB neurons thus appear to represent a functionally distinct neuronal subpopulation. Their exact role remains however unclear. Because hamsters with lesions restricted to the CBsn lose their locomotor activity rhythm, it has been proposed that the CBsn could be necessary and sufficient for the control of locomotor rhythmicity (LeSauter and Silver, 1999).

Locomotor activity level and pattern are affected by day-length, and specially in photoperiodic animals such as hamsters (Wollnik et al., 1991; Scarbrough et al., 1997). Daily peak levels of locomotor activity are higher in hamsters exposed to a long photoperiod when compared with a short photoperiod, and the length of the activity phase (i.e. α) decreases when photoperiod increases. Mechanisms that regulate photoperiodic variations of the locomotor activity are still unknown. One of the possibilities may be that the SCN would directly control these seasonal changes. The SCN constitutes indeed a clock for all seasons, since number of genes, including *c-fos* and components of the molecular clockwork, show day-length dependent changes in expression in the SCN (Sumova et al., 1995; Vuillez et al., 1996; Messager et al., 1999, 2000; Nuesslein-Hildesheim et al., 2000; Lincoln et al., 2002; Tournier et al., 2003). Moreover, the expression in the rodent SCN of two neuropeptides, vasopressin and vasoactive intestinal polypeptide, also depends on photoperiod (Duncan et al., 1995; Jac et al., 2000).

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Abbreviations: CalB, calbindin D28k; CBsn, calbindin D28K subnucleus; ir, immunoreactive; LD, light/dark cycle; LLP, very long photoperiod LD18:6; LP, long photoperiod LD14:10; NMDA, *N*-methyl-D-aspartate; PBS, phosphate buffer saline; PCR, polymerase chain reaction; PRC, phase response curve; SCN, suprachiasmatic nucleus; SP, short photoperiod LD10:14; SSP, very short photoperiod LD6:18.

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We thus hypothesized that photoperiodic conditions would affect the SCN subregion regulating locomotor rhythmicity by altering expression of CalB and we tested the influence of photoperiodic background on both protein and mRNA expression of CalB in the SCN.

EXPERIMENTAL PROCEDURES

Animals

Male Syrian hamsters (*Mesocricetus auratus*, originally purchased from Harlan France, Ganat, France) were born in our colony under a long photoperiod with a LD of 14 h of light and 10 h of dark (14:10). The light intensity was approximately 200 lux during daytime and a constant dim red light (<1 lux) was on throughout the experiment. They were given food and water *ad libitum*.

When adults, three groups ($n=10$ per group) were transferred to different light–dark conditions: very short photoperiod LD6:18 (SSP), short photoperiod LD10:14 (SP) and very long photoperiod LP18:6 (LLP). A fourth group ($n=10$) remained in the long photoperiod LD14:10 (LP). Time of lights off was the same (i.e. 6:00 PM) for all groups. After 8 weeks, animals were killed at 3:00 PM (i.e. 3 h before lights off).

Brains were removed and processed for either immunocytochemistry or *in situ* hybridization of CalB D28k. Testes were also removed in order to check short photoperiod induced gonadal regression. Testicular regression was observed in all hamsters exposed to short photoperiods (i.e. SSP and SP), whereas hamsters exposed to long photoperiods had large testes.

All experiments were performed in accordance with National Institutes of Health "Principles of Laboratory Animal Care" (NIH publication no. 86–23, revised 1985) as well as in accordance with the French law. All efforts were made to minimize the number and suffering of animals used.

Immunocytochemistry

Hamsters ($n=5$ per group) were deeply anesthetized with pentobarbital sodium 6% (250 mg/kg, i.p.; Sanofi, Libourne, France). Hamsters were then perfused transcardially with 100–150 ml NaCl 0.9% followed with 250–300 ml of freshly prepared paraformaldehyde 4% in phosphate buffer 0.1 M (pH=7.4). Brains were removed, post-fixed during 4–5 h at 4 °C, and finally rinsed into phosphate buffer saline (PBS) at 4 °C until immunocytochemistry procedure.

Immunocytochemistry was processed in the same run for all animals to minimize variability attributable to handling conditions. Thirty micrometer coronal sections were prepared on a vibratome (Leica Microsystemes SA, Rueil-Malmaison, France) and rinsed into PBS. They were then treated sequentially with ethanol in PBS at concentrations of 10%, 20%, 40%, 20% and 10% (10 min each). This procedure increases penetration of immunological reagents (Eldred et al., 1983). Then, sections were rinsed three times for 10 min with PBS, and incubated for 40 h at 4 °C with monoclonal CalB antibody diluted at 1:20,000 (Sigma, St. Louis, MO, USA) in PBS containing 0.5% Triton X-100 and 1% calf fetal serum. Thereafter, sections were incubated 1 h 15 min with biotinylated secondary antibody (Vector, Burlingame, USA) in PBS 0.5% Triton X-100 and 1% calf fetal serum at room temperature (1/500; rabbit anti-mouse; Dako), followed by ABC reagent (Vector) in PBS Triton X-100 0.5% for 1 h. Peroxidase activity was detected using 0.0125% diaminobenzidine (Sigma) in Tris 0.05 M (pH=7.6) containing 0.0075% H_2O_2. Sections were mounted, dehydrated and coverslipped.

Sections were viewed on a Leica microscope (Leica DMRB; Leica) under bright field microscopy, scanned with an CCD camera (Olympus DP50; Olympus), and displayed using ViewFinder Lite software (version 1.0; Pixera corporation) on a PC computer. All CalB immunoreactive profiles, irrespective of staining intensity, were blind counted in all sections containing the CBsn (i.e. around 10–11 sections per SCN). Because the counting was done on adjacent sections, the number of CalB cells in the SCN would have been overestimated if the number of immunopositive neurons was summed. Thus, in subsequent analysis, the Abercrombie correction factor (Abercrombie, 1946) was applied to the data using the equation $N=n\times(t/t+d)$, where N is the corrected number of CalB-ir cells, n the number of CalB positive neurons which was initially counted, t the thickness of the section (30 μm) and d the diameter of the CalB neurons in micrometers. Diameter was measured in 30 cells from three animals, from each photoperiodic condition, using sections captured by the CCD video camera using Viewfinder Lite sofware, and then Adobe Photoshop software (version7.0). The diameter of CalB-ir neurons, which was consistent with data on the diameter of cells within the ventrolateral SCN (Güldner and Wolff, 1996), was not affected by the photoperiod (SSP=9.67±0.24 μm; SP=9.79±0.18 μm; LP=9.65±0.16 μm; LLP=9.72±0.27 μm). These values were used for the Abercrombie correction factor calculations.

In situ hybridization

Hamsters ($n=5$ per group) were deeply anesthetized as described above. They were then perfused transcardially with 100–150 ml NaCl 0.9%, followed by fixative (4% formaldehyde, 75 mM lysine, 10 mM sodium periodate in 10 mM phosphate buffer, pH 7.4). Brains were removed and post-fixed for 2 h in the fixative at 4 °C. Then, they were rinsed in 50% ethanol and embedded in polyethylene glycol as previously described (Klosen et al., 1993). Eight-micrometer coronal sections were cut and mounted on slides (Superfrost Plus; Menzel-Gläser, Germany).

Hamster CalB D28k cDNA fragments (510 bp) were polymerase chain reaction (PCR) amplified using the following oligonucleotides: 5′-AGCTGCAGAACTTGATCCAG-3′ and 5′-TATCCGTTGCCATTCTGATC-3′. PCR products of the expected size were cloned into the pCRII-TOPO cloning vector (TOPO TA Cloning; Invitrogen). Restriction enzyme digestion and sequencing were performed to assess orientation and identity of the fragments.

Antisense and sense probes were transcribed from the corresponding linearized plasmids using the appropriate polymerase in presence of α-[^{35}S]UTP (1250 Ci/mmol; NEN-Dupond, Zaventem, Belgium) according to the manufacturer's protocol (MAXIscript; Ambion, USA).

Sections were postfixed in 4% phosphate-buffered formaldehyde for 10 min, rinsed in PBS and then treated with 2 mg/ml of proteinase K (Roche, Basel, Switzerland) in PBS for 30 min at 37 °C. Proteinase K digestion was stopped with 2% phosphate-buffered formaldehyde for 5 min on ice. Sections were rinsed in PBS and acetylated twice for 10 min in 100 mM tri-ethanolamine, 0.25% acetic anhydride. Sections were then rinsed in PBS and dehydrated in a graded ethanol series (with 250 mM ammonium acetate, 1 min each) and dried at room temperature.

Sections were hybridized with either antisense or sense cDNA riboprobes (450 pM) in a solution containing 50% deionized formamide, 10% dextran sulfate, 50 mM dithiothreitol, 1× Denhardt's solution, 2× SSC, 1 mg/ml salmon sperm DNA, 1 mg/ml yeast RNA, at 54 °C for 16 h. After incubation, the sections were rinsed twice in 2× SSC for 10 min, before being treated with ribonuclease A (0.02 Kunitz unit/ml; Sigma) in 10 mM Tris, 500 mM NaCl, 10 mM EDTA buffer (30 min at 37 °C). Slides were then rinsed twice and stringency washes were carried out (6×10 min 0.1× SSC, 72 °C). Finally, sections were dehydrated in a graded ethanol series and air-dried. Slides together with ^{35}S standards were exposed to an autoradiographic film (Amersham, Orsay, France) for 1 week. Quantitative analysis of the autoradiograms was performed using a computerized analysis system Biocom-program RAG 200. An area covering approximately the size of one SCN was used to quantify the signal in both SCN of the

three most labeled sections per animal. Specific signal intensity was obtained by subtraction of the background measured in surrounding anterior hypothalamic area where no CalB expression was detected.

Statistical analysis

Statistical comparisons between groups were assessed by analysis of variance using Minitab (Minitab Inc., State College, USA). Post hoc pairwise comparisons were conducted using the Tukey test when significant *F*-ratios were obtained ($P<0.05$). Values are reported as means±S.E.M.

RESULTS

Immunocytochemistry

As described previously (Silver et al., 1996), CalB D28k immunoreactive (CalB-ir) cells in the SCN were restricted to a small ventrolateral subregion and no immunoreactivity could be detected surround this subnucleus (Fig. 1). Moreover, immunostaining was particularly dense in the mid part of the rostro-caudal extent of the SCN.

Number of CalB-ir cells within the whole SCN was markedly affected by photoperiod ($F_{3,16}=27.79$; $P<0.001$; Table 1). Number of CalB-ir cells was maximal in the two short photoperiods (SSP and SP), and reduced when day-length increased. In the SCN of hamsters exposed to LP, CBsn contained significantly more cells when compared with those exposed to LLP group, but less cells when compared with those exposed to SP and SSP groups. This increase was not due to a change in the size of the CBsn in rostro-caudal extents, which was similar whatever the photoperiodic condition and measuring around 270–300 μm (Fig. 2A). Increase of CalB expression was mainly due to an increase of the number of CalB-ir neurons at the mid level of the subnucleus. The area of the subnucleus was not markedly changed, however, and there was an increased density of CalB cells within the subnucleus (Fig. 2B).

Structures including the bed nucleus of the stria terminalis and the intergeniculate leaflets also exhibited CalB immunoreactivity, but CalB expression was not affected by the photoperiodic conditions in these structures (data not shown).

In situ hybridization

Representative patterns of *CalB* mRNA expression in hamsters exposed to LLP or SP are presented on Fig. 3. mRNA expression overlaps protein immunoreactivity in forebrain structures (data not shown). In the SCN, intensity of *CalB* mRNA expression was relatively low compared with other forebrain structures.

The *CalB* mRNA expression was significantly affected by photoperiod ($F_{3,16}=3.59$; $P<0.05$; Table 2). mRNA levels appeared to be higher in animals exposed to short photoperiods; however, post hoc analysis revealed only a significant difference between SP and LLP group ($P<0.05$).

DISCUSSION

We show here for the first time that the number of CalB-ir cells in the SCN of golden hamster is inversely correlated to the day-length. Similar variations also occur at the mRNA level, which suggests that photoperiod impacts on CalB transcript. Moreover, these changes seem to be specific to the SCN since no difference could be observed in the bed nucleus of the stria terminalis or the intergeniculate leaflets. As demonstrated for neuropeptide mRNA expression in the SCN, assessment of seasonal variations is complicated by concurrent circadian variations (Freeman et al., 2002). Since CalB expression does not exhibit circadian variation in terms of cell number and amount of CalB protein (LeSauter et al., 1999), these difficulties are circumvented and the seasonal variations observed here are not likely to be due to comparisons made at different circadian phases.

LeSauter et al. (1999) demonstrated that an increase in CalB protein occurs in golden hamsters exposed to several weeks in constant darkness. The authors suggested that light could affect the number of CalB-ir neurons by decreasing CalB expression. The present study agrees with this and extends this result by showing there is a strong relationship between the duration of day-length and the number of CalB expression in the SCN. The mechanisms involved in these changes of CalB expression could be directly dependent on the retinal afferences which convey light information to the circadian clock. In support of this, CalB is robustly expressed in the dorsomedial part of the SCN in neonatal mice at postnatal day 3, and a reduction in CalB expression in this dorsomedial part parallels the establishment of connections between the retinohypothalamic tract and the SCN neurons (Ikeda and Allen, 2003).

What could be the role of this day-length influence on CalB expression?

First, photoperiod-dependent changes in the number of CalB-ir cells in the SCN might be related to the photoperiod-dependent pattern and/or level of locomotor activity. Indeed, CalB cells are thought to be of importance in the mechanisms regulating locomotor rhythmicity. Partial SCN lesions that destroy CBsn lead to arrhythmicity of locomotor activity whereas hamsters bearing partial SCN lesion that spare this subregion continue to show circadian locomotor rhythmicity (LeSauter and Silver, 1999). Grafts of fetal SCN in complete SCN-lesioned hamsters restored rhythmicity only if CalB cells were present in the grafts. The efficiency of the graft in restoring rhythmicity was furthermore correlated with the number of CalB-ir cells (LeSauter and Silver, 1999). Moreover, exposure to constant light that disrupted circadian rhythmicity in rats, decreased the number of CalB positive cells in the SCN (Arvanitogiannis et al., 2000). Thus, the photoperiodic changes in the organization and level of the locomotor activity, observed in Syrian hamsters (Scarbrough et al., 1997) as well as in the European hamsters (Wollnik et al., 1991) could be a con-

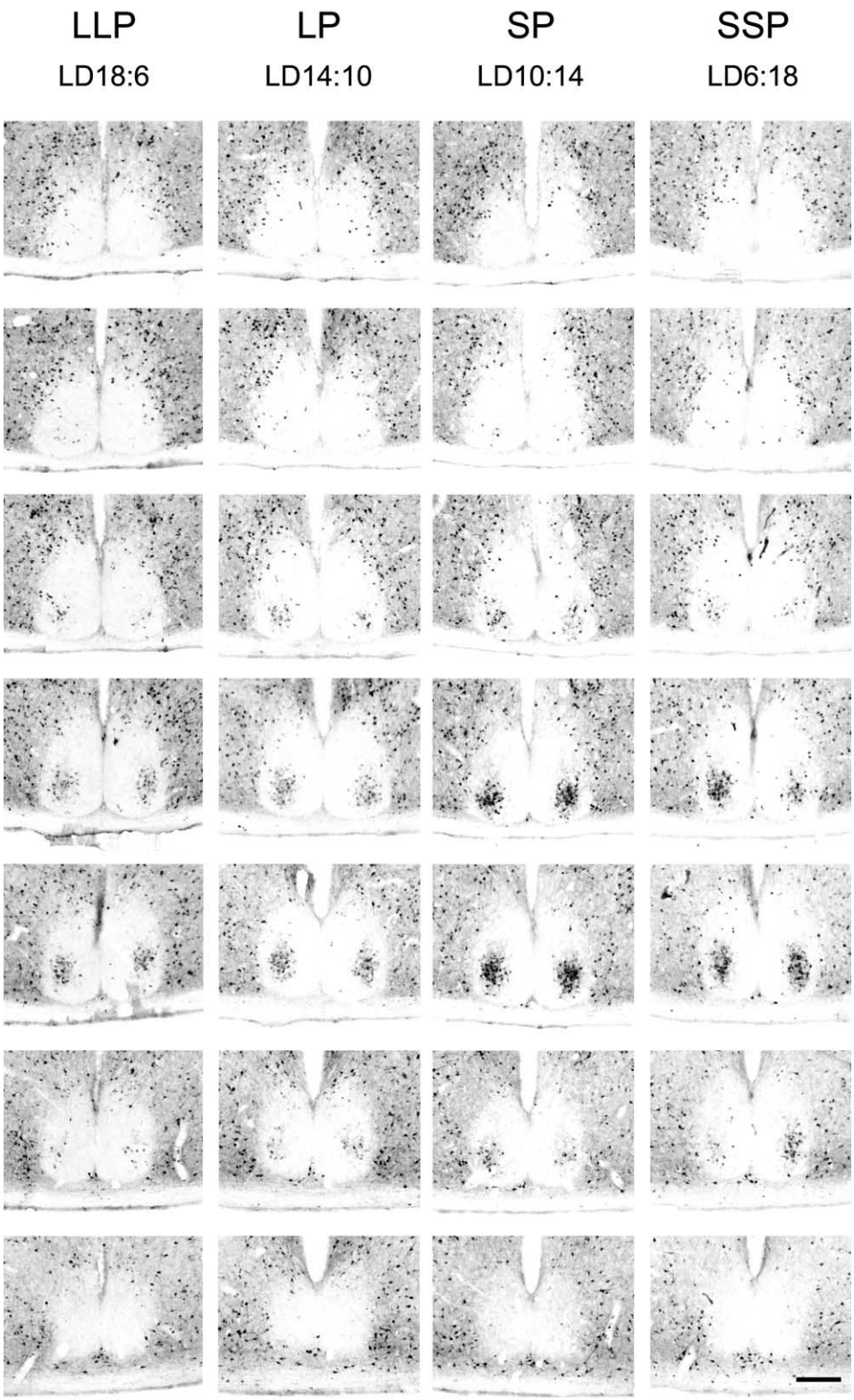

Fig. 1. Distribution of CalB immunoreactivity through the SCN of hamsters exposed to different photoperiodic conditions. Sections come from representative hamsters exposed either to a LLP, a LP, a SP, or a SSP. Serial 30 μm coronal sections are arranged from rostral (top) to caudal (bottom) extent of the SCN and one every two sections are presented. Calbindin expression delimits a subpopulation of neurons in the ventrolateral part and at the medial–caudal extent of the hamster SCN. Decreasing day-length induces an increase of calbindin immunoreactivity in this subnucleus. Scale bar=200 μm.

Table 1. Quantification of CalB immunoreactivity in the SCN with respect to photoperiod*

	Number of CalB-ir cells
LLP	470±16[a]
LP	557±28[b]
SP	736±19[c]
SSP	746±22[c]

* Calbindin cells were counted throughout the SCN of hamsters exposed to LLP, LP, SP, or SSP. Values are group mean±S.E.M. (n=5 per group). Groups bearing different superscripts are significantly different (P<0.05).

sequence of the photoperiodic change of CalB expression within the SCN.

Second, the photoperiodic change of CalB expression could be important for the synchronization of the SCN to the seasonal variation of the environment, since an involvement of CalB neurons in photic signaling has also been proposed. CBsn receives dense retinal innervation (Bryant et al., 2000), and light stimulation during the night induces Fos expression in about 75% of the CalB-ir cells in the SCN (Silver et al., 1996). Moreover, a correlation has been found between magnitude of light-induced phase shifts in DD and the level of CalB expression in the SCN (LeSauter et al., 1999). The number of CalB positive cells in the SCN is significantly higher in tau mutant hamsters than in wild type hamsters (LeSauter et al., 1999). LeSauter et al. have proposed that this difference of CalB expression in the SCN could be responsible for the increased amplitude of phase shifts in tau mutants compared with wild type hamsters (Shimomura and Menaker, 1994; Scarbrough and Turek, 1996). Thus, magnitude of light-induced phase shifts could be positively correlated to the number of CalB cells in the SCN. Daan and Pittendrigh (1976) proposed a model of entrainment following a seasonal changing photoperiod. To conserve a stable phase angle (ψ) when photoperiod shortens, nocturnal pacemaker behavior is associated with an increased amplitude of the phase response curve (PRC) to light. This theoretical model was experimentally confirmed by Pittendrigh and co-workers (1984) who measured portions of PRCs (circadian times

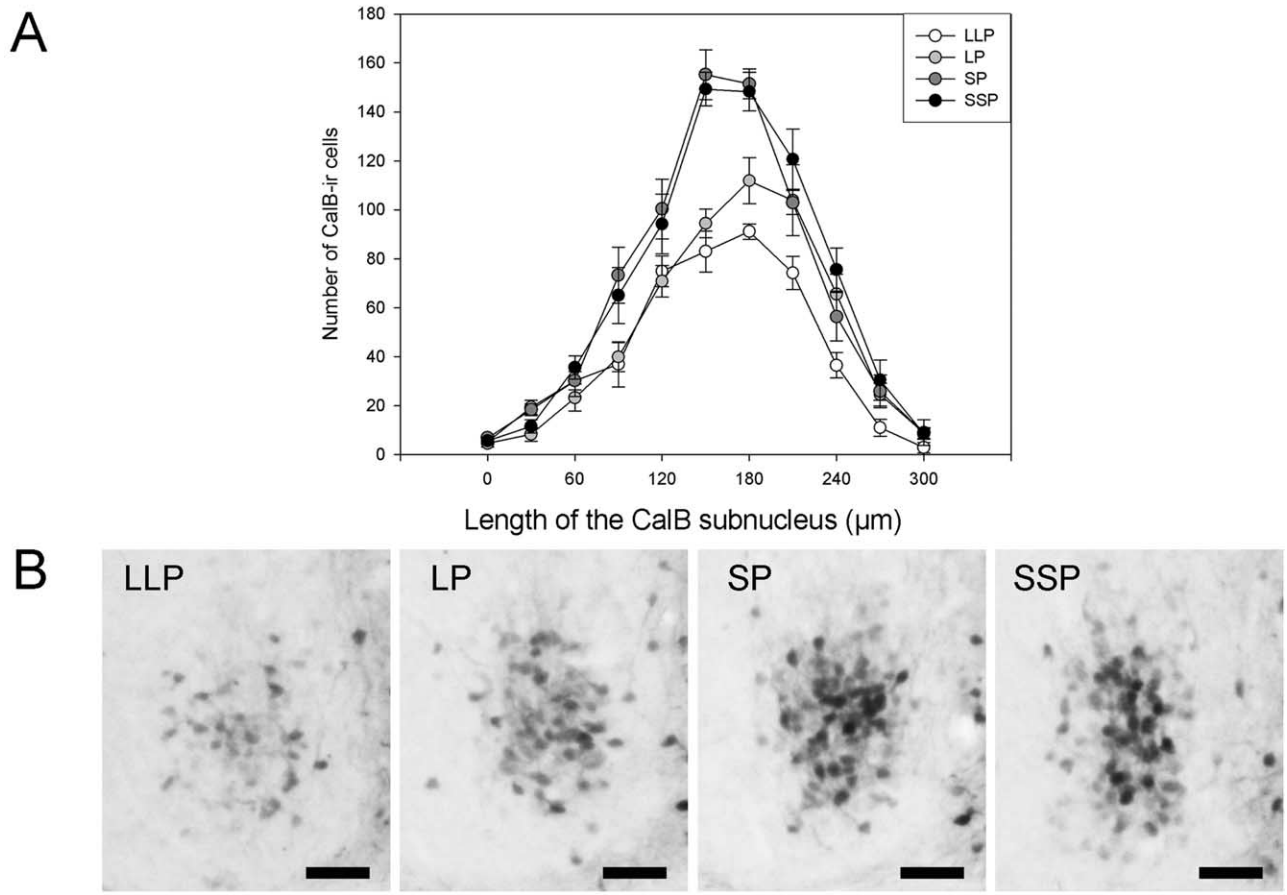

Fig. 2. Effect of day-length on the density of CalB-ir neurons in the SCN of hamsters exposed to different photoperiodic conditions. A: Number of CalB-ir neurons on adjacent sections of 30 μm thickness in the CBsn of hamsters exposed either to a LLP, a LP, a SP, or a SSP. The length of the CBsn, which is around 270–300 μm in each photoperiodic condition, does not change in response to day-length. In contrast, the number of CalB-ir neurons is increased in the mid extent of the CBsn. B: Photomicrographs at the mid extent of the CBsn of hamsters exposed to different photoperiodic conditions. Day-length does not affect the size of the CBsn (dorsoventral or mediolateral), but increases the density of neurons immunostained for CalB. Scale bar=50 μm.

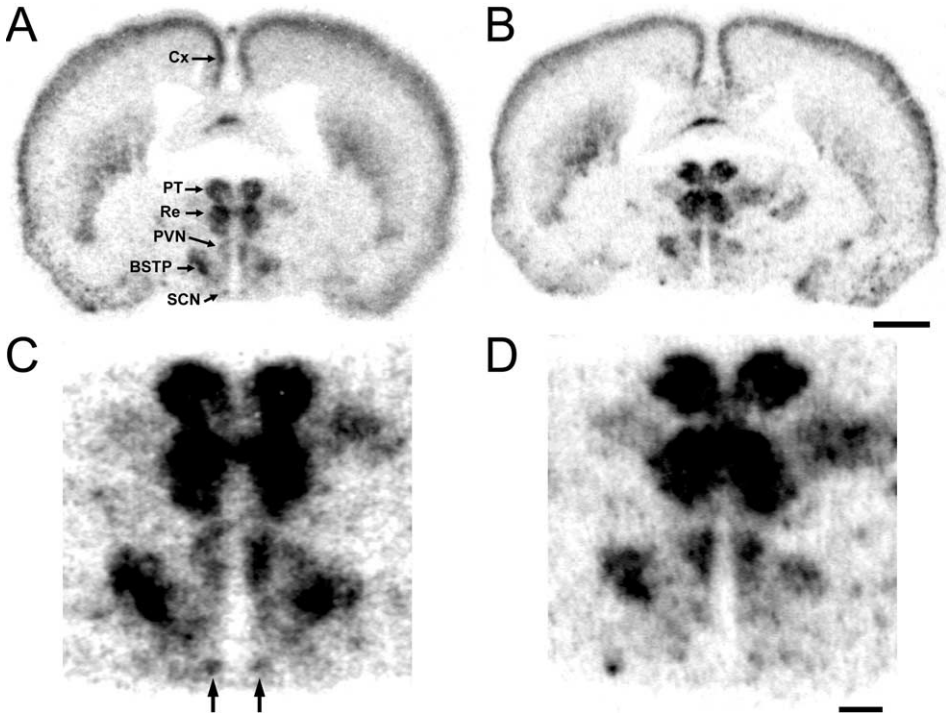

Fig. 3. CalB mRNA expression detected by radioactive *in situ* hybridization with ^{35}S-labeled probes on coronal sections at the level of the suprachiasmatic nuclei of hamsters exposed to different photoperiods. A and B: Coronal sections at low magnification of one hamster exposed to the SP (A) and one hamster exposed to the LLP (B). Calbindin mRNA is expressed in several structures other than the suprachiasmatic nuclei. Cx, cortex; PT, paratenial thalamic nucleus; Re, reuniens thalamic nucleus; PVN, paraventricular hypothalamic nucleus; BSTP, bed nucleus of the stria terminalis, posterior part. Scale bar=2 mm. C and D: higher magnification of A and B, respectively. Calbindin mRNA expression is restricted to the ventrolateral part of the SCN (arrows). Note the difference of expression between hamster exposed to SP (C) to that exposed to the LLP (D). Scale bar=500 μm. The difference observed for labeling of the BSTP between the two sections is solely due to a difference in the angle of section: in reference to the golden hamster brain atlas of Morin and Wood (2001); the BSTP is present on sections which are 0.3 mm posterior to bregma but not on section 0.6 mm posterior to bregma, whereas the SCN are present from 0.3–0.9 posterior to bregma.

12–24) in hamsters pre-exposed to LD18:6 or LD10:14. Pre-treatment with LD18:6 reduced the amplitude of phase responses to 31% compared with pre-exposure to LD10:14. It has been also shown, using pineal *N*-acetyl transferase rhythm as parameter, that the amplitude of phase delay was larger under short photoperiod than in long photoperiod (Illnerova, 1991; Humlova and Illnerova, 1992; Travnickova et al., 1996). This theoretical model, the published experimental data (see also Binkley and Mosher, 1986; Elliott, 1994) and the present results are consistent with the hypothesis that the amplitude of light-induced phase shift could be dependent on the CalB expressing cells: the more CalB cells are expressed in the SCN, the higher is the amplitude of light-induced phase-shifts.

Table 2. Quantification of *Calbindin D28k* mRNA expression in the SCN with respect to photoperiod*

	Relative mRNA levels
LLP	1.6 ± 0.6^{a}
LP	$2.7\pm1.3^{a,b}$
SP	7.8 ± 2.5^{b}
SSP	$5.6\pm0.9^{a,b}$

* SCN sections of hamsters exposed to LLP, LP, SP, or SSP were hybridized with ^{35}S-labelled probes for *Calbindin D28k*. Results (relative levels of desintegration per minute) are presented as mean±S.E.M. ($n=5$ per group). Groups bearing different superscripts are significantly different ($P<0.05$).

At a cellular level, an increase of CalB expression is likely to induce changes in intracellular calcium signaling. Light information conveyed by retinal ganglion cells reaches the ventrolateral part of the SCN (for review, Hannibal, 2002). Interestingly, CalB positive cells are direct targets of retinal terminals (Bryant et al., 2000). The primary neurotransmitter of the retinohypothalamic tract is glutamate (Castel et al., 1993; van den Pol and Dudek, 1993; Ebling, 1996), and this transiently increases intracellular calcium concentration in cultured SCN neurons (van den Pol et al., 1992). Other reports are consistent with the idea that the effect of light within the SCN involves activation of calcium-requiring transduction mechanisms (Golombek and Ralph, 1994; Shibata and Moore, 1994; Kako et al., 1996; von Gall et al., 1998). Moreover, a direct implication of CalB in glutamatergic neurotransmission has been described in the hippocampus. In CalB-deficient mice, *N*-methyl-D-aspartate (NMDA) receptor-mediated responses were decreased while non-NMDA receptor-mediated responses increased (Jouvenceau et al., 1999). Altogether, these data suggest that CalB may modify amplitude of phase

shifts through its effects on calcium signaling. Our finding that CalB expression changes with photoperiod may help to explain the observed effects of prior photoperiodic exposure on the amplitude of phase shift responses to light pulses.

One interesting feature of CalB cells is their circadian arrhythmicity in spontaneous firing frequency *in vitro* (Jobst and Allen, 2002). In addition, they delimit a subpopulation of neurons in the ventrolateral part of the SCN in which clock genes are expressed at a constant level (Hamada et al., 2001). CalB cells within the SCN represent thus a functionally distinct neuronal subpopulation, and they may have a distinctive role. Since long term potentiation in the hippocampus, used as a model for synaptic plasticity, is impaired in mice lacking CalB (Jouvenceau et al., 1999), one role for CalB cells in the SCN may be the control of synaptic plasticity. The short photoperiod-induced increase of the number of CalB cells may therefore induce changes in synaptic connectivity between SCN neurons and as a consequence, connections and functioning of the non-rhythmic ventrolateral part of the SCN may be photoperiod-dependent. Moreover, since almost all CalB cells receive dense appositions from retinal ganglion cells, neuropeptide Y, serotonin and vasoactive intestinal peptide fibers (Bryant et al., 2000; LeSauter et al., 2002), integration of synchronizing cues by the ventrolateral part of the SCN may also depend on day-length.

In conclusion, we report that expression of a calcium binding protein, the CalB D28k, is correlated with day-length. As the CalB cells do not oscillate in a circadian manner but are markedly affected by the photoperiod, they represent a good candidate, at least in Syrian hamsters, as a neuronal subpopulation directly implicated in the building of the seasonal message by the circadian clock.

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