ELSEVIER

Brain Research 906 (2001) 176–179

BRAIN RESEARCH

www.elsevier.com/locate/bres

Short communication

# Intergeniculate leaflets lesion delays but does not prevent the integration of photoperiodic change by the suprachiasmatic nuclei

Jérôme Menet, Patrick Vuillez*, Nathalie Jacob, Paul Pévet

*CNRS-UMR 7518, Neurobiologie des Fonctions Rythmiques et Saisonnières, Université Louis Pasteur, 12 rue de l'Université, 67000 Strasbourg, France*

Accepted 3 April 2001

**Abstract**

The duration of the photosensitive phase of the suprachiasmatic nuclei (SCN), as revealed by light-induced Fos protein expression, depends on the photoperiod and is tied to the length of the night. We show here in Syrian hamsters that after a transfer from long to short photoperiod, lengthening of the photosensitive phase of the SCN is significantly delayed but not abolished when the intergeniculate leaflets (IGL) are lesioned. Thus IGL modulate the integration by the SCN of a photoperiodic change. 

*Theme:* Neural basis of behavior

*Topic:* Biological rhythms and sleep

*Keywords:* Circadian clock; Photoperiod; c-fos; Neuropeptide Y

The suprachiasmatic nuclei (SCN), which contain the major endogenous circadian clock, generate rhythms of approximately 24 h in constant conditions. These rhythms are synchronized to exactly 24 h mainly by the light–dark (LD) cycle. Photic information arrives to the SCN directly by the retino-hypothalamic tract and indirectly via fibers coming from the intergeniculate leaflets (IGL) of the lateral geniculate complex [13,3,15,14]. These fibers contain principally neuropeptide Y (NPY) as neurotransmitter. The environmental photoperiodic information bore in the LD cycle appears also to be integrated by the circadian clock. Indeed, using expression of light-induced Fos protein as a cellular activation marker, it has been shown in both rats [23] and hamsters [25] that the photosensitive phase of the SCN is tied to the length of the night. After a photoperiodic change from LD 14:10 (14 h of light and 10 h of darkness per 24 h) to LD 10:14, lengthening of the photosensitive phase takes at least 25 days in golden hamsters [25]. The IGL also integrate this photoperiodic change by increasing the number of NPY-expressing cells [7]. At 25 days a bilateral lesion of this thalamic structure decreases the high expression of Fos protein after the light stimulation at the end of the night [6], indicating that the IGL play a role in the integration of the photoperiod by the SCN. This previous work raises the question of whether IGL lesion prevents the integration of the photoperiod by the SCN or delays it. Therefore, in the present study, we tested the effect of a bilateral IGL lesion on the integration of a photoperiodic change by the SCN after 25 days, to confirm the previous results, and also after 60 days, which is the necessary time for short photoperiod-induced testicular regression in hamsters.

Adult male Syrian hamsters (100–120 g, Harlan, Gannat, France) were housed after their arrival in long photoperiod LD 14:10 (lights on 04:00 h, lights off 18:00 h, light intensity of 200 lux during daytime). Two to four animals were housed per cage with food and water available ad libitum. A constant red dim light was on throughout the experiment.

After at least 3 weeks of exposure to these experimental conditions, hamsters were operated during the light phase. Animals were anesthetized with an i.p. injection of 80 mg/kg zoletil (Virbac, Carros, France) and 10 mg/kg rompun (Bayer Pharma, Puteax, France). They were placed

*Corresponding author. Tel.: +33-3-9024-0555; fax: +33-3-9024-0528.

*E-mail address:* vuillez@neurochem.u-strasbg.fr (P. Vuillez).

0006-8993/01/$ – see front matter 
PII: S0006-8993(01)02518-5

in a Kopf stereotaxic instrument with the incisor bar set at −2 mm. IGL lesions were made bilaterally with a thermic electrode by heating to 80°C during 1 min, with a lesion generator system (Radionics model RFG-4A, Inc., Burlington, USA). IGL lesions were made at three rostro-caudal levels with reference to bregma: −1.1, −1.6, −2.1 posterior to bregma, 3.1, 3.3, 3.1 laterally to bregma and −4.3, −4.6, −5.1 ventral to the dura, respectively. In sham-operated groups, the electrode was lowered at only 2 mm above each level lesion, and no heat was delivered.

After the surgical procedure, hamsters were placed back in their cage. To extend the photosensitive phase of the SCN, hamsters were transferred 6 days after the surgery to a short photoperiod LD 10:14 (lights on 08:00 h, lights off 18:00 h). In order to test the maximal lengthening of the photosensitive phase of the SCN, the technique already described [25] was used. In the short photoperiod, hamsters received a 15-min light stimulation (200 lux) 13 h after the onset of dark either the 25th or 60th night after the photoperiodic change. One hour after the beginning of the light stimulation, they were deeply anesthetized with pentobarbital sodium 6% (0.8 ml/kg, i.p., Sanofi, Libourne, France) and perfused transcardially with 100–150 ml NaCl 0.9% followed with 250–300 ml of 4% freshly prepared paraformaldehyde in 0.1 M phosphate buffer (pH 7.4).

Brains were removed and post-fixed during 4–5 h at 4°C. Perfused testes were also removed and weighed. Fifty-micrometer coronal sections of the SCN and IGL were prepared on a vibratome, rinsed in 0.1 M phosphate-buffered saline (PBS) and then processed for immunohistochemistry. Half of the SCN sections was used for Fos protein detection, and the second half of the SCN and all of the IGL sections were used to test the quality of IGL lesions through NPY immunoreactivity detection. Sections were incubated overnight at 4°C with the primary antibody in PBS containing 0.5% Triton X-100 (sheep anti-Fos, 1/5000, Calbiochem, Cambridge, USA; rabbit anti-NPY, 1/6000, gift from Prof. Buijs, Institute for Brain Research, Amsterdam, Netherlands [2]). Thereafter, sections were incubated 1 h 15 min with biotinylated secondary antibody in PBS–Triton 0.5% and 1% of appropriate normal serum (Vector, Burlingame, USA) at room temperature (1/500, rabbit anti-sheep; 1/500, goat anti-rabbit, respectively, followed by ABC reagent (Vector) in PBS–Triton 0.5% for 1 h. Peroxidase activity was detected using 0.0125% diaminobenzidine (Sigma, St. Louis, USA) in 0.05 M Tris (pH 7.6) containing 0.0075% $H_2O_2$, and only for the Fos immunohistochemistry using 0.5% nickel ammonium sulfate. Sections were mounted, dehydrated and coverslipped for microscopic analysis.

Despite tissue not being processed in the same run, stark and reproducible NPY immunohistochemistry permits the use of disappearance of NPY-immunoreactive fibers as a criterion of quality of IGL lesion. Only animals with sustained complete bilateral ablation of the entire rostro-caudal extent of the IGL and acute NPYergic denervation in the SCN were selected ($n=6$ per group, noted IGL-X animals). Fos-labeled cells were blind-counted on four rostro-caudal levels of the SCN, using a monitoring video coupled to a microscope (Leica). Cells inside SCN and in the hypothalamic area immediately adjacent to the dorso-lateral boundaries of the nuclei which have been described to be sensitive to light [24] were counted. Fos-immunoreactive (-ir) cells densely immunostained, as well as more weakly stained cells being taken into account. Results are expressed as mean±S.E.M. The analysis of the data was performed using a two-way analysis of variance. Pairwise post hoc comparisons of significant effects ($P<0.05$) were made using the Student–Newman–Keuls test.

In selected IGL-X animals, lesions extended a little out the ventral lateral geniculate nuclei and dorsal lateral geniculate nuclei. Nevertheless, damage to the hippocampus was negligible. These lesions triggered a sharp decrease of NPY immunoreactivity in the SCN and, as described before, only a scarce number of fibers persisted in the ventral SCN [5,6,11,17].

At 25 days, the number of Fos-ir cells was weaker in IGL-X than in sham animals ($P<0.05$) (Fig. 1). This confirms our previous observation [6]. Moreover, at 60 days, the expression was the same between IGL-X and sham groups. Irrespective of the lesion treatment, the number of Fos-ir cells in the two groups was lower after 25 days than after 60 days in the short photoperiod ($P<0.01$).

The number of Fos-ir cells was higher in caudal than in rostral SCN. This rostro-caudal variation of expression in the SCN could be observed in all of the groups. Short photoperiod-induced gonadal atrophy was not achieved after 25 days, but was completed after 60 days of exposure to LD10:14 ($P<0.001$). We did not observe an effect of IGL lesion on this time course ($P>0.05$) (Fig. 2).

The present study confirms that an IGL lesion reduces light-induced Fos expression in the SCN of hamsters

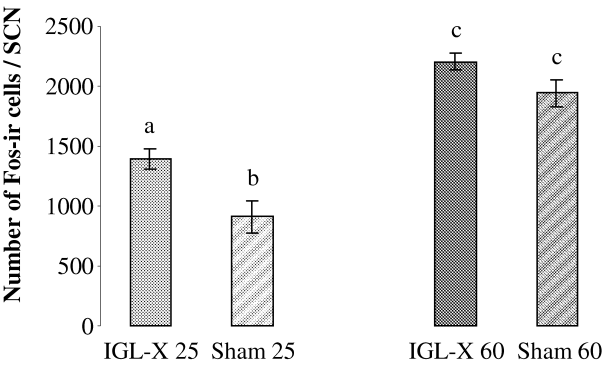


Fig. 1. Expression of Fos protein in the SCN after a light stimulation 13 h after the beginning of the night, of Syrian hamsters with IGL lesion (IGL-X) or sham-operated (Sham), transferred from LD 14:10 to LD 10:14 for 25 or 60 days. Results represent mean±S.E.M. Groups with no letters in common are significantly different from one another ($P<0.05$).

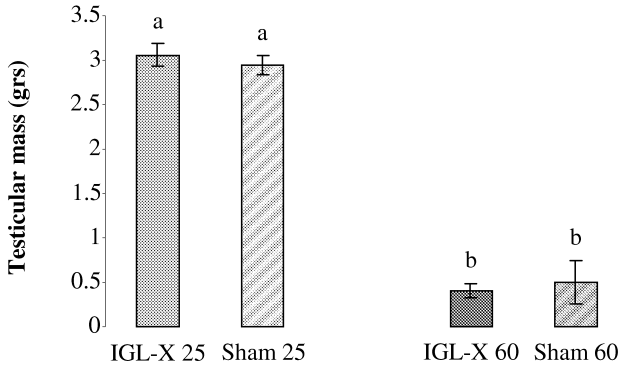


Fig. 2. Testis mass of Syrian hamsters with IGL lesion (IGL-X) or sham-operated (Sham), transferred from LD 14:10 to LD 10:14 for 25 or 60 days. Results represent mean±S.E.M. Groups with no letters in common are significantly different from one another ($P<0.05$).

exposed to a short photoperiod for 25 days. In addition, we show that this reduction is no longer detectable after 60 days in the short photoperiod. Moreover, both groups present similar gonadal time-course atrophy. Thus, IGL lesion impairs but does not prevent the integration of a photoperiodic change by the SCN.

As previously shown [8], the increase of the light-induced Fos protein after a photoperiodic change is not linear. The number of labeled cells is still very low at 20 days and close to the maximum at 25 days. In the present study, even if hamsters were in the same conditions as those of Jacob et al. [6], they have not fully integrated at 25 days the short photoperiod. The integration of the photoperiodic change has been delayed for few days. It should be considered that the integration of the short photoperiod LD 10:14 takes about 4 weeks in golden hamsters. Nevertheless, despite the sub-maximal Fos expression in the sham group at 25 days, the present IGL-X 25 hamsters have a similar 35% decrease in the Fos-ir cells as compared to previous data [6]. Moreover, at 60 days, the number of Fos-ir cells is the same between the two groups. So, IGL lesion delays but does not prevent the lengthening of the phase of light sensitivity in the SCN.

We found no difference in the mass of the testis between the two groups, neither 25 nor 60 days after the photoperiodic change. This is further evidence that the IGL lesion does not prevent the integration of the photoperiodic change, here at a higher physiological level. Such a result is in accordance with the findings of Smale and Morin [22] who showed that a lesion strictly restricted to the IGL has no effect on SP-induced testis regression, contrary to extended IGL lesion with lateral damage to the hippocampus. Nevertheless, our data clearly demonstrate that the IGL themselves play a role in the integration of a photoperiodic change by the circadian clock.

We notice that the effect of the lesion on light-induced Fos expression can be seen in both rostral and caudal parts of the SCN. Because NPY fibers in the SCN of intact animals are mainly located in the caudal part of the nuclei, the decrease of Fos-labeled cells could not be strictly correlated to the disappearance of the NPY projections from the IGL to the SCN. Nevertheless, Jacob et al. have shown that the number of cells expressing NPY mRNA in the median IGL is higher in LD10:14 than in LD 14:10 [7], and that the increase occurs in 4 days [16]. Photoperiodic-dependent changes in the IGL have been observed in several other species. Seasonal variations in NPY-ir in the SCN are found in the jerboa [10]. NPY levels in the SCN of Djungarian hamster are augmented during nighttime under a long photoperiod, but not under short-photoperiod conditions [19]. Moreover, Shinohara et al. [21] showed in rats under a LD 12:12 cycle that there is a bimodal rhythm of NPY in the SCN, with peaks at both light–dark and dark–light transitions. Thus, we can suppose that there are photoperiodic-dependent nycthemeral profiles of NPY liberation in the SCN, which facilitate the integration of a new photoperiod by the SCN.

Moreover, in numerous circadian studies, the IGL and especially their NPY projections to the SCN are related to non-photic synchronization of the clock [9] or to modulation of photic entrainment [1]. The mainly studied non-photic zeitgeber cue is a change in locomotor activity. Different parameters of locomotor behavior depend on photoperiodic environment [20]. Thus, photoperiodic-related changes in locomotor activity might act on the integration of photoperiodic change through the IGL and especially their NPY fibers.

The IGL and the SCN receive separate serotonergic projections from the dorsal and the median raphe nuclei, respectively [12]. However, Dudley et al. [4] have shown that dorsal as well as median raphe nuclei neurons can contribute to the regulation of serotonin release in the hamster SCN. Thus thalamic lesion, in destroying serotonergic terminals in the IGL, may affect neurons of the dorsal raphe and then the median raphe–SCN pathway. Following the first depletion of IGL projections to the SCN in IGL-X animals we cannot assume that such an indirect mechanism is not at least partly involved in our present results.

Despite the modulatory role of the IGL, our present data indicate that the SCN is able by itself to built a photoperiodic message, which is in accordance with the theoretical model of a biological clock proposed almost 25 years ago by Pittendrigh and Daan [18].

It is now commonly accepted that some of the zeitgebers, mainly the non-photic ones, are ‘processed’ in the IGL and then modulate the direct synchronization, mainly photic, of the circadian clock. We believe that IGL play a similar role for photoperiod and photoperiodic-dependent cues in modulating the seasonal functioning of the circadian clock.

## Acknowledgements

We wish to thank Dr. R.M. Buijs, Institute for Brain Research, Amsterdam, Netherlands, for the gift of NPY antiserum, as well as. D. Bonn and A. Senser for their assistance in taking care of the animals and E. Challet for comments.

## References

[1] S.M. Biello, D.A. Golombek, M.E. Harrington, Neuropeptide Y and glutamate block each other's phase shifts in the suprachiasmatic nucleus in vitro, Neuroscience 77 (1997) 1049–1057.
[2] R.M. Buijs, C.W. Pool, J.J. van Heerikhuize, A.A. Sluiter, P.J. Van der Sluis, M. Ramkema, T.P. van der Woude, E. Van Der Beek, Antibodies to small transmitter molecules and peptides: production and application of antibodies to dopamine, serotonin, GABA, vasopressin, vasoactive intestinal peptide, neuropeptide Y, somatostatin and substance P, Biomed. Res. 10 (1989) 213–221.
[3] J.P. Card, R.Y. Moore, Organization of lateral geniculate–hypothalamic connections in the rat, J. Comp. Neurol. 284 (1989) 135–147.
[4] T.E. Dudley, L.A. Dinardo, J.D. Glass, In vivo assessment of midbrain raphe nuclear regulation of serotonin release in the hamster suprachiasmatic nucleus, J. Neurophysiol. 81 (1999) 1469–1477.
[5] M.E. Harrington, D.M. Nance, B. Rusak, Neuropeptide Y immunoreactivity in the hamster geniculo-suprachiasmatic tract, Brain Res. Bull. 15 (1985) 465–472.
[6] N. Jacob, P. Vuillez, N. Lakdhar-Ghazal, P. Pévet, Does the intergeniculate leaflet play a role in the integration of the photoperiod by the suprachiasmatic nucleus? Brain Res. 828 (1999) 83–90.
[7] N. Jacob, P. Vuillez, M. Møller, P. Pévet, Photoperiodic dependent changes in the number of neurons containing mRNA encoding neuropeptide Y in the intergeniculate leaflet of the Syrian hamster, Brain Res. 813 (1998) 160–166.
[8] N. Jacob, P. Vuillez, P. Pévet, Photoperiod does not act on the suprachiasmatic nucleus photosensitive phase through the endogenous melatonin, in the Syrian hamster, Neurosci. Lett. 229 (1997) 117–120.
[9] D. Janik, J.D. Mikkelsen, N. Mrosovsky, Cellular colocalization of Fos and neuropeptide Y in the intergeniculate leaflet after nonphotic phase-shifting events, Brain Res. 698 (1995) 137–145.
[10] N. Lakhdar-Ghazal, R. Oukouchoud, P. Pévet, Seasonal variation in NPY immunoreactivity in the suprachiasmatic nucleus of the jerboa (*Jaculus orientalis*), a desert hibernator, Neurosci. Lett. 193 (1995) 49–52.
[11] E.S. Maywood, E. Smith, S.J. Hall, M.H. Hastings, A thalamic contribution to arousal-induced, non-photic entrainment of the circadian clock of the Syrian hamster, Eur. J. Neurosci. 9 (1997) 1739–1747.
[12] E.L. Meyer-Bernstein, L.P. Morin, Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation, J. Neurosci. 16 (6) (1996) 2097–2111.
[13] R.Y. Moore, J.P. Card, Intergeniculate leaflet: an anatomically and functionally distinct subdivision of the lateral geniculate complex, J. Comp Neurol. 344 (1994) 403–430.
[14] L.P. Morin, J. Blanchard, Organization of the hamster intergeniculate leaflet: NPY and ENK projections to the suprachiasmatic nucleus, intergeniculate leaflet and posterior limitans nucleus, Vis. Neurosci. 12 (1995) 57–67.
[15] L.P. Morin, J. Blanchard, R.Y. Moore, Intergeniculate leaflet and suprachiasmatic nucleus organization and connections in the golden hamster, Vis. Neurosci. 8 (1992) 219–230.
[16] P. Pevet, N. Jacob, N. Lakhdar-Ghazal, P. Vuillez, How do the suprachiasmatic nuclei of the hypothalamus integrate photoperiodic information? Biol. Cell 89 (1997) 569–577.
[17] G.E. Pickard, M.R. Ralph, M. Menaker, The intergeniculate leaflet partially mediates effects of light on circadian rhythms, J. Biol. Rhythms 2 (1987) 35–56.
[18] C.S. Pittendrigh, S. Daan, A functional analysis of circadian pacemakers in nocturnal rodents, J. Comp. Physiol. A 106 (1976) 333–355.
[19] S. Reuss, J. Olcese, Neuropeptide Y: distribution of immunoreactivity and quantitative analysis in diencephalic structures and cerebral cortex of dwarf hamsters under different photoperiods, Neuroendocrinology 61 (1995) 337–347.
[20] K. Scarbrough, S. Losee-Olson, E.P. Wallen, F.W. Turek, Aging and photoperiod affect entrainment and quantitative aspects of locomotor behavior in Syrian hamsters, Am. J. Physiol. 272 (1997) R1219–R1225.
[21] K. Shinohara, K. Tominaga, Y. Isobe, S.T. Inouye, Photic regulation of peptides located in the ventrolateral subdivision of the suprachiasmatic nucleus of the rat: daily variations of vasoactive intestinal polypeptide, gastrin-releasing peptide, and neuropeptide Y, J. Neurosci. 13 (1993) 793–800.
[22] L. Smale, L.P. Morin, Photoperiodic responsiveness of hamsters with lesions of the lateral geniculate nucleus is related to hippocampal damage, Brain Res. Bull. 24 (1990) 185–190.
[23] A. Sumova, Z. Travnickova, R. Peters, W.J. Schwartz, H. Illnerova, The rat suprachiasmatic nucleus is a clock for all seasons, Proc. Natl. Acad. Sci. USA 92 (1995) 7754–7758.
[24] R. Teclemariam-Mesbah, P. Vuillez, A. Van Rossum, P. Pévet, Time course of neuronal sensitivity to light in the circadian timing system of the golden hamster, Neurosci. Lett. 201 (1995) 5–8.
[25] P. Vuillez, N. Jacob, R. Teclemariam-Mesbah, P. Pévet, In Syrian and European hamsters, the duration of sensitive phase to light of the suprachiasmatic nuclei depends on the photoperiod, Neurosci. Lett. 208 (1996) 37–40.