

Research report

Is there a geniculohypothalamic tract in primates? A comparative immunohistochemical study in the circadian system of strepsirrhine and haplorhine species

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Abstract

In rodents, the circadian rhythm generated by the hypothalamic suprachiasmatic nucleus (SCN) is modulated by two types of phenomena: photic phase-shifts, mediated by the retinohypothalamic pathway and non-photoc phase-shifts mediated by the projection of the intergeniculate leaflet (IGL) to the SCN which contains the neuropeptide Y (NPY). In primates, the retinohypothalamic pathway has been well-demonstrated but very little is known about the geniculohypothalamic tract. This prompted us to study NPY immunoreactivity in both the SCN and the IGL in species representative of the three main primate lineages: prosimians (*Microcebus*), New World monkeys (*Callithrix*) and Old World monkeys (*Macacca*). In species studied, we found a region in the pregeniculate nucleus containing both NPY immunopositive cells and substance P immunopositive fibres that we identified as the IGL. During evolution, this structure has moved from a ventral to a dorsomedial position relative to the adjacent dorsal lateral geniculate nucleus. By contrast, NPY-IP fibres in the SCN are dense in prosimians, but are sparse or absent in other primate species. We suggest that either the geniculohypothalamic projection is absent in higher primates as is the case in humans, or is absent in diurnal mammals, or contains a different peptide, or that NPY immunoreactivity varies according to other parameters. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: SCN (Suprachiasmatic Nucleus); IGL (Intergeniculate Leaflet); Circadian rhythms; Comparative anatomy; vLGN (Ventral Lateral Geniculate Nucleus); Neuropeptide Y

1. Introduction

In rodents, the circadian system, which is responsible for both the genesis and the regulation of the biological rhythms of periods close to 24 h, is composed of two main elements: the suprachiasmatic nucleus (SCN) of the hypothalamus and the intergeniculate leaflet (IGL) of the lateral geniculate nucleus of the thalamus. Both these structures receive retinal afferents. These retinal projections convey information concerning environmental irradiance necessary for the photic entrainment of the endogenous rhythm generated within the SCN. The IGL projects to the SCN via the geniculohypothalamic tract (GHT), which contains the neuropeptide Y (NPY) [6,9]. This projection has been shown to be involved in both photic and non-photoc

phase-shifts (induced for example by activity or stress) of the endogenous rhythm generated by the SCN [1,3,4].

By contrast, this geniculohypothalamic projection is reported to be absent in humans [18,25]. Unlike other primates, the human SCN contains a subpopulation of neurons which express NPY [18] and which are assumed to play an analogous function through a local circuit. In man, a subdivision of the pregeniculate nucleus [24,26] has been identified as the IGL [18,19] but its connections and role remain unknown. The existence of a GHT in non-human primates is thus a crucial issue concerning the appropriateness of primate models for the study of human circadian physiology. Moreover, the conservation of this pathway during phylogeny is a question of basic interest for comparative anatomy.

Thus, the present study was undertaken to investigate three important neuropeptides of the circadian system by using NPY, substance P (SP) and enkephalin (ENK) immunohistochemistry in both the SCN and the IGL. Primate species representative of the three main lineages were

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examined: the nocturnal strepsirrhine mouse lemur *Microcebus Murinus* (prosimian), and two diurnal haplorhines: the marmoset *Callithrix Jacchus* (New World monkey) and the macaque monkey *Macacca Fascicularis* (Old World monkey).

2. Materials and methods

Three mouse lemurs, three marmosets and two macaque monkeys, all raised in our laboratory according to INSERM guidelines for animal care have been used for this

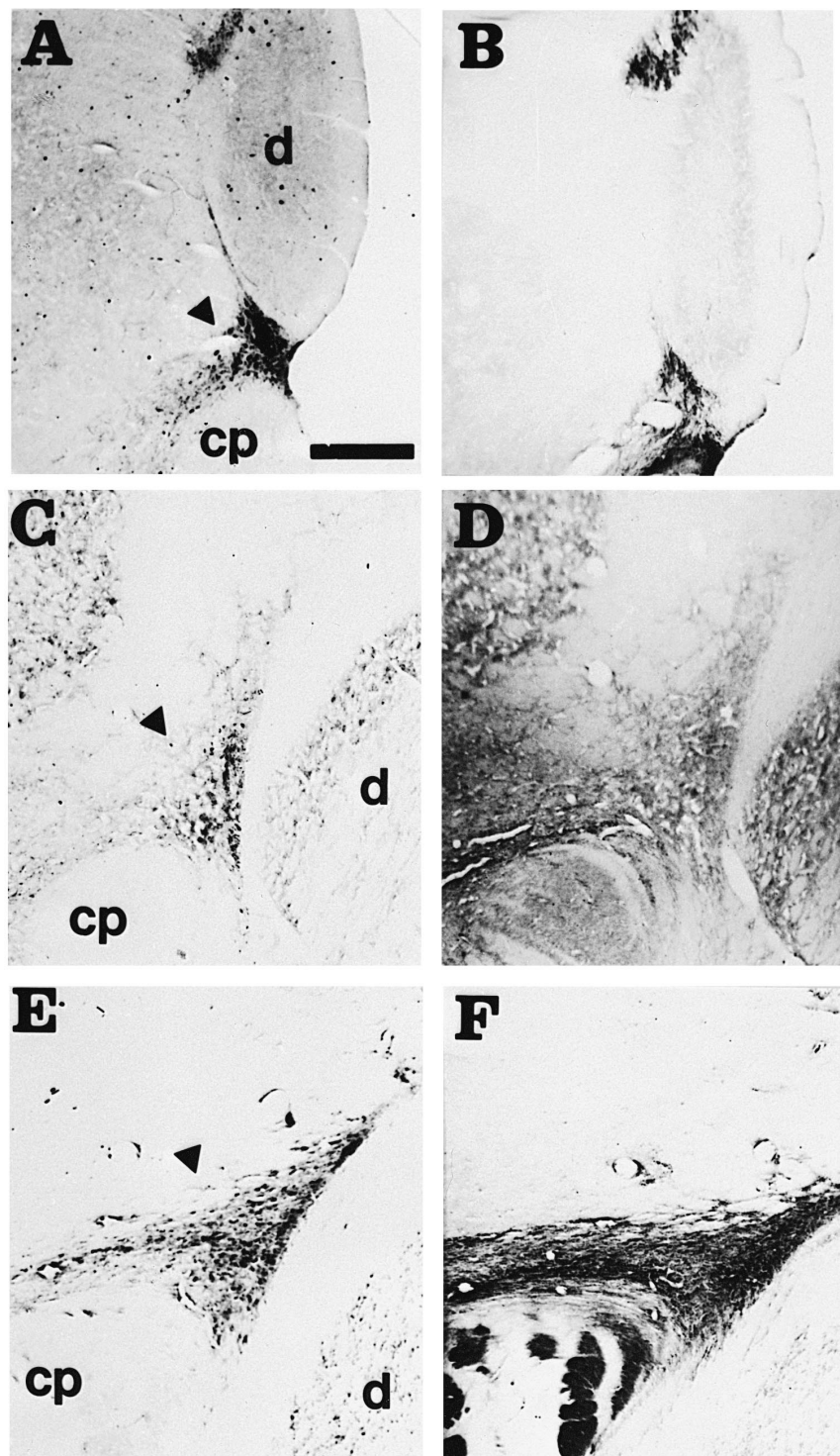


Fig. 1. NPY and SP immunoreactivity at the level of the lateral geniculate body in mouse lemur (A, B), marmoset (C, D) and macaque monkey (E, F). The region (arrowheads) which contains NPY-IP neurons (A, C, E) and SP-IP fibres (B, D, F) includes the primate homologue of the rodent IGL. d: Dorsal Lateral Geniculate Nucleus, cp: Cerebral Peduncle. Scale bar: 300 μ m.

study. All animals, of both sexes, were adults. Following administration of lethal doses of pentobarbital, animals were perfused with Zamboni solution (paraformaldehyde 4% plus 15% saturated picric acid in phosphate buffer 0.1 M, pH 7.4) between 10:00 and 17:00 h. Brains were dissected, cryoprotected by an overnight bath in sucrose 30%, and cut into 40 μ m thick sections in the coronal plan with a freezing microtome. Inhibition of endogenous peroxidase was performed by a 1-h incubation in a solution of ethanol 45%, NaCl 0.9%, H₂O₂ 0.3%. After several rinses in phosphate buffer, unspecific antigenic sites were saturated by a 1-h incubation in normal goat serum 1.5% (Vector Laboratories). Sections were then incubated for 3 days at 4°C in the antiserum diluted in normal goat serum 1% in phosphate buffer saline with 0.3 \times Triton (PBST). Two antisera against NPY (Peninsula, anti human, rat NPY, code IHC 7180 at a dilution of 1:20000 and anti porcine NPY, code IHC 7172 at a dilution of 1:5000) have been used for each species and gave similar results. Immunohistochemistry for SP and ENK was performed with the same procedure, using an anti SP serum (Peninsula) at a dilution of 1:10000 and L-ENK serum (INCSTAR) at a dilution of 1:5000. After rinses in PBST and 2 h incubation in a biotinylated anti rabbit antibody (Vector) diluted 1:200 in PBST, revelation of antigen/antibody complexes was performed using an ABC Elite kit (Vector). For revelation, diaminobenzidine (DAB) 0.05% dissolved in Tris (pH 7.6, 50 mM) was used as a chromogen with nickel sulphate (0.2%) intensification. Kinetics of reaction was visually monitored by adding H₂O₂ very progressively. Time of reaction was between 20 and 40 min. Following reaction, sections were rinsed in Tris, mounted,

dehydrated and coverslipped with depex. Specificity of antisera in our hands was established by a 1-h preincubation of the antiserum with a 10⁻⁵ M solution of synthetic antigen (NPY, SP, L-ENK and M-ENK, Sigma) following by the same procedure. Immunopositive labelling was completely absent. Absence of cross-reactivity of the antisera was established by replacing the antiserum by normal serum in the incubation step of the procedure. Absence of both specific and non-specific labelling resulted.

3. Results

In all species studied, we observed a robust population of NPY-IP cells in the caudal part of the pregeniculate nucleus (Fig. 1A,C,E), separated from the adjacent dLGN by a dense capsule of fibres. These neurons with a roughly bipolar morphology were reminiscent of the Golgi impregnated elongated cells described in this region of the pregeniculate nucleus of the macaque [2] and in the rat IGL [20]. This region also contained a few ENK-IP cells and a dense plexus of S-IP fibres (Fig. 1B,D,F). In agreement with other reports [8,18,19] we consider that this area of the primate pregeniculate nucleus is homologous to the rodent IGL.

As depicted in Fig. 2, the position of the IGL has been modified during evolution within the primate group. In the more primitive species, *Microcebus*, the IGL assumes a position ventral to the dorsal lateral geniculate nucleus (dLGN), roughly similar to what is observed in rodents. By contrast, in New and Old World monkeys, the IGL is shifted in position medial or even dorsomedial to the dLGN.

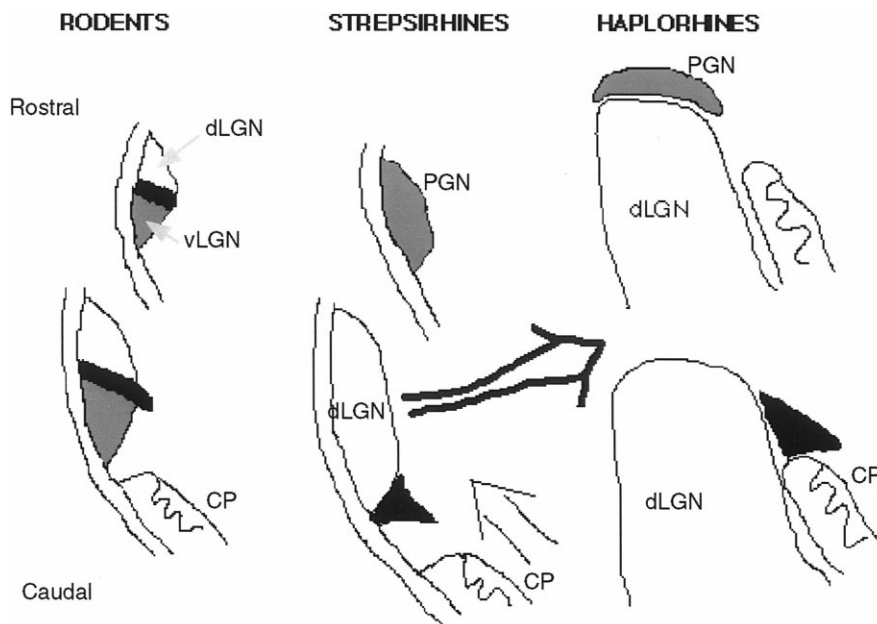


Fig. 2. Evolutionary shift in the position of the IGL. In primates, the pregeniculate nucleus (PGN) is homologous to both the ventral lateral geniculate (grey) and the IGL (black) in rodents. This cartoon illustrates how the growth (thick arrow) of the dorsal lateral geniculate (dLGN) and the expansion (thin arrow) of the cerebral peduncle (CP) may have led to a dorso-medial shift in the position of the IGL (in black) relative to the adjacent structures.

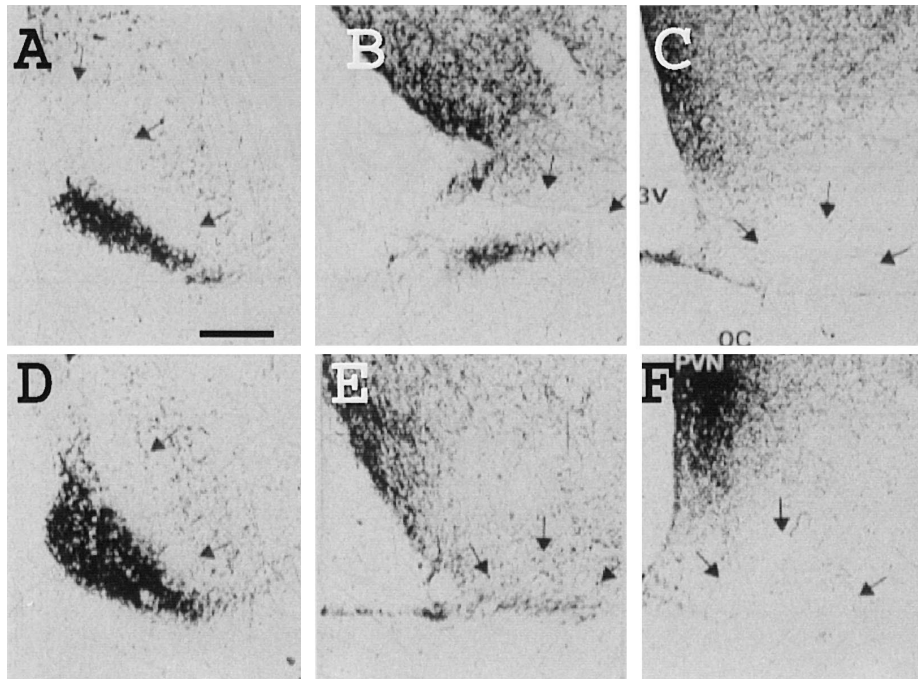


Fig. 3. NPY immunoreactivity in rostral (A, B, C) and caudal (D, E, F) sections of the basal hypothalamus in mouse lemur (A, D), marmoset (B, E) and macaque monkey (C, F). Black arrows indicate the borders of the SCN. In the SCN, NPY-IP fibres form a large and dense ventral core in mouse lemur, a little and mainly rostral core in marmoset and are totally absent in macaque monkey. OC: Optic Chiasm, 3V, Third Ventricle, PVN: Paraventricular Nucleus. Scale bar: 200 μ m.

The density of NPY-IP fibres in the SCN varies among primates. In the SCN of the mouse lemur, we observed a very dense core of NPY-IP fibres situated in the ventral

part of the whole extent of the nucleus (Fig. 3A,D). This pattern overlapped with a dense pattern of SP-IP fibres (Fig. 4A). The remainder of the SCN was devoid of NPY

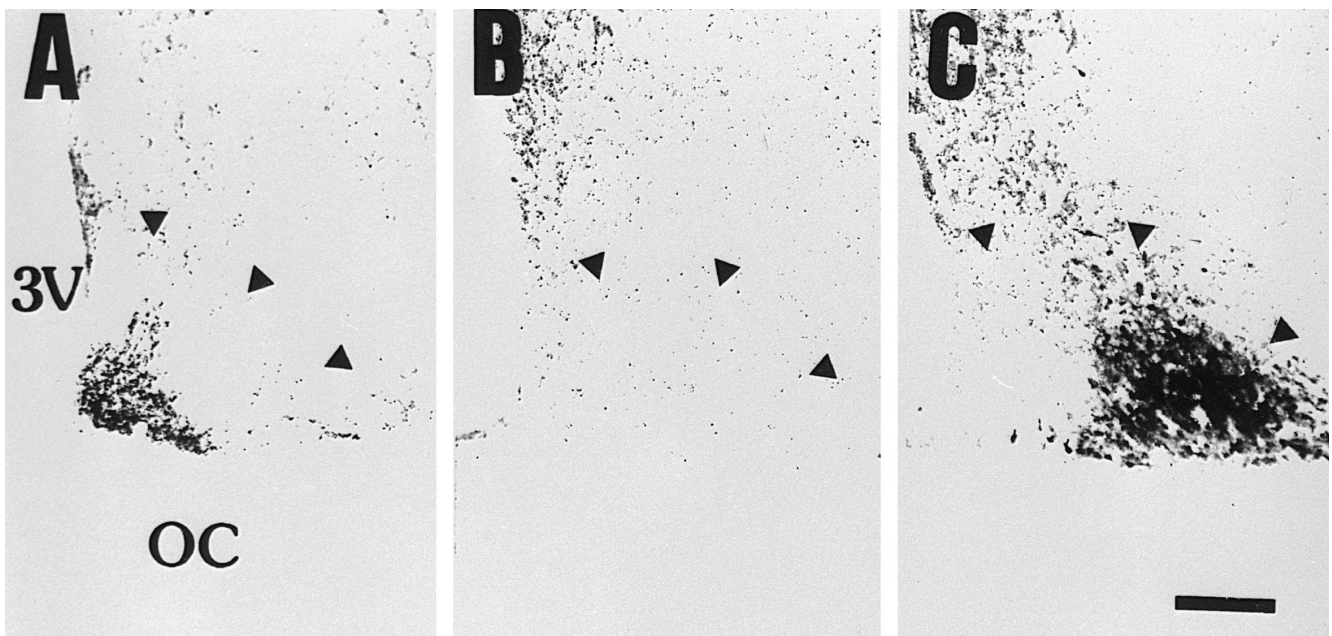


Fig. 4. Substance P immunoreactivity in the mouse lemur (A), marmoset (B) and macaque monkey (C) hypothalamus. Arrowheads indicate the borders of the SCN, as determined from adjacent Nissl stained sections. In mouse lemur, a dense core of SP-IP fibres is observed in the ventral part of the SCN. These fibres are absent in both the marmoset and macaque SCN. In the macaque, a dense population of SP-IP cells is observed in the SCN. OC: Optic Chiasm, 3V, Third Ventricle, Scale bar: 200 μ m.

immunoreactivity whereas adjacent hypothalamic structures contained a moderate plexus of NPY-IP fibres. In the marmoset, the density of NPY-IP fibres in the ventral region of the SCN was comparatively reduced and restricted to the rostral part of the nucleus (Fig. 3B,E). No SP-IP structures could be detected in the marmoset SCN (Fig. 4B). In the macaque monkey, the entire SCN, unlike the paraventricular nucleus and the rest of the basal hypothalamus, was strikingly devoid of any NPY-IP structures (Fig. 3C,F) but contained the previously described [14] population of SP-IP cells (Fig. 4C).

In all species studied, the basal hypothalamus contains a moderate density of ENK-IP varicosities, with ENK-IP cells in the paraventricular nucleus. The SCN of all species is devoid of ENK immunoreactivity.

4. Discussion

Our immunohistochemical study shows the presence of NPY-IP cells in the primate homologue of the rodent IGL in all species studied. In contrast, the density of NPY-IP fibres in the ventral SCN decreases from prosimians to New World and Old World monkeys. To our knowledge, the present study is the first to describe the IGL in several non-rodent mammals. Since the presence of NPY-IP cells and SP-IP fibres are only two of the five criteria retained by Morin et al. [21] for the identification of the IGL, it should be emphasised that IGL itself is likely to be a subregion of the NPY-IP cell/SP-IP fibres area. Among primates, the IGL has undergone a ventro-dorsal displacement, relative to the dLGN, which is interpreted as a consequence of the dramatic expansion of adjacent structures, namely the cerebral peduncle and the dLGN.

Our results concerning NPY immunoreactivity in the SCN are in agreement with previous observations in mouse lemur [5] and in the New World squirrel monkey [30]. However, considerable contradiction exists in the literature regarding the presence of NPY immunoreactivity in the SCN of macaque monkeys: NPY-IP fibres density has been reported to be either high [18], moderate [33] or absent [34]. Our results clearly confirm this latter report and these contradictory results will be discussed below.

The hypothesis that the discrepancies are due to technical differences between different investigators can be eliminated since most studies ([18,33], present study) used the same antibody. Moreover, we obtained exactly similar results with two different antibodies. Thus, the IGL of all primates contains NPY-IP cells whereas NPY-IP fibres of the GHT are not present in the SCN of all species. Four hypotheses can be proposed to explain these different results: (1) The GHT does not exist in higher primates; (2) The GHT does not exist in diurnal primates; (3) The GHT does not contain NPY in higher primates; (4) The neurochemical content of the GHT is variable and depends on chronobiological or other parameters.

The absence of a GHT in higher primates such as the macaque would not be unexpected, since the GHT does not exist in man [18]. In addition, in the tree shrew (*Tupaia*), which has been considered to be phylogenically related to both insectivores and primates, this projection has not been found despite careful investigation [7]. Moreover, it has been reported that phase-shifts induced by triazolam, a paradigm of non-photic phase-shifts mediated by the NPY in the hamster [35], have distinct features in primates. In the hamster, injection of triazolam shifts the phase of the circadian rhythms through an increase of locomotor activity. Triazolam also produces phase-shifts in monkeys (as well as in human) but in contrast is a powerful sedative and leads to a decrease in activity. Thus the phase-shifting effect of triazolam depends on different mechanisms in monkeys and hamsters and could be due to the decreased exposure to light observed in treated animals [17]. This absence of pure non-photic phase-shifts in monkey is consistent with the suggestion of an absence of the GHT tract in higher primates. The sparse NPY-IP plexus observed in the ventral part of the SCN in marmoset may arise from another source, and could be similar to the residual NPY-IP fibres observed after IGL ablation in hamster [21], which clearly do not belong to the GHT. Instead of projecting to the SCN, we suggest that NPY-IP cells of the IGL in higher primates could project to the pineal organ and/or the pretectum. The IGL-pineal organ projection has been demonstrated in rodents [15] and NPY-IP fibres have been reported in the pineal organ of primates [5,16]. The reciprocal connection between IGL and pretectum has been established in mammals including macaque monkeys [23], and shown to contain NPY in hamster [22]. Thus alternative circuitry involving other brain areas known to be involved in circadian physiology could exist in the circadian system of higher primates, including humans.

However, primate species used in the present study are representative not only from different lineages but also from different activity rhythms, i.e., the mouse lemur is nocturnal while both marmoset and macaque are diurnal. Therefore, it is possible that the difference in NPY immunoreactivity in the SCN described in the present report actually reflects the different organisation of the circadian system in diurnal and nocturnal mammals. Our experiments clearly need to be repeated in diurnal prosimians, such as the *Propithecus* (Sifika) as well as in nocturnal haplorhine primates such as the *Tarsius* (owl monkey). However, it should be noted that immunohistochemical evidences for a GHT have been provided in diurnal rodents such as the ground squirrel [29], suggesting a wide conservation of this projection.

There are currently few arguments in favour of the hypothesis of an absence of NPY in the GHT. We describe a population of enkephalinergic cells in the IGL of all primate species studied, but we do not observe enkephalinergic fibres in the SCN. Thus, unlike in hamster [21]

enkephalin does not appear to constitute a candidate neuropeptide of the GHT in higher primates. However, it should be mentioned that rigorous cell-counts in the IGL of rodents have provided evidence for the existence of a third non NPY-IP, non ENK-IP population that, if present in primates could be responsible for the projection to the SCN [20]. The same situation applies in cat. In this species, the existence of a GHT has been convincingly established [27,32]. Yet, the SCN does not contain NPY-IP fibres [13].

However, objections to both these hypotheses are raised by the observation of NPY-IP fibres in the macaque SCN by some authors [18,33]. Thus, a final possibility is that the NPY contents of the GHT varies temporally, for instance according to the phase of the light cycles [10]. This would be in agreement with in vitro studies which have established that SCN sensitivity to NPY is restricted to certain circadian times [11] and with in vivo studies showing that the concentration of NPY in the SCN highly depends on the light cycle [28]. In addition to this circadian dependence, circannual [12] or age-related differences could exist, similar to reports for vasopressinergic cells in the human SCN [31].

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