Unilateral optic nerve transection alters light response of suprachiasmatic nucleus and intergeniculate leaflet

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Tang, I-Hsiung, Dean M. Murakami, and Charles A. Fuller. Unilateral optic nerve transection alters light response of suprachiasmatic nucleus and intergeniculate leaflet. Am J Physiol Regulatory Integrative Comp Physiol 282: R569–R577, 2002; 10.1152/ajpregu.00412.2001.—The suprachiasmatic nucleus (SCN), the circadian pacemaker, receives photic input directly from the retina to synchronize the pacemaker to the environment. Additionally, the intergeniculate leaflet (IGL), which innervates the SCN, is known to modulate the retinal photic input to the SCN. To further understand the role of the IGL in mediating the photic input to the SCN, this study examined the effects of unilateral optic nerve transection (UONx) on the photic response of the SCN and IGL in adult and neonatal hamsters. UONx led to an overall reduction in light-induced c-Fos expression in the SCN and IGL. The c-Fos expression was greater in the SCN ipsilateral to the remaining eye, despite a symmetrically bilateral retinohypothalamic tract projection as revealed by intraocular injection of horseradish peroxidase. In contrast, UONx led to a greater c-Fos expression in the contralateral IGL. The contralateral IGL of UONx animals also revealed more neuropeptide Y-immunoreactive neurons, while the ipsilateral SCN of these animals exhibited a denser neuropeptide Y terminal field. The neonates with UONx showed a similar pattern with a slight compensation of the photic-induced c-Fos in the SCN. This study suggests that the IGL may have an ipsilateral inhibitory effect in mediating retinal photic input to the SCN.

The circadian rhythms; entrainment; immediate early genes; c-Fos; neuropeptide Y; hamster

THE SUPRACHIASMATIC NUCLEUS (SCN) is the mammalian neural pacemaker (see Ref. 17 for review) that generates circadian rhythms and receives photic zeitgebers to entrain the organism to the 24-h day. Entrainment of the circadian pacemaker by photic zeitgebers resets the circadian clock each day (25). The SCN receives photic input from two different pathways (for reviews, see Refs. 12, 21, and 26). The primary photic pathway, the retinohypothalamic tract (RHT), is a direct projection from retinal ganglion cells to the SCN (16). In hamsters, the ipsilateral and contralateral retinal projection to the SCN is symmetrically bilateral. The second is an indirect pathway in which the retinal ganglion cells project bilaterally to the intergeniculate leaflet (IGL) in the lateral geniculate complex (for review, see Ref. 12). However, the projection pattern for each eye is asymmetric, with a much greater projection to the contralateral IGL. Fibers from the IGL then project to the SCN. This pathway is known as the geniculohypothalamic tract (GHT), and the GHT projection pattern coincides with the RHT terminal field in the SCN.

It has been suggested that the IGL plays a modulatory role in photic entrainment via GHT input to the SCN. Lesions of the IGL attenuated the magnitude of phase advances by light pulses at circadian time (CT) 1800 and CT 2000 (13). In addition, lesions of the IGL reduced the rate of reentrainment to 6-h phase shifts in a 14:10-h light-dark cycle (15). In nocturnal rodents, the free-running period is normally lengthened in constant-light conditions, but IGL lesions significantly reduced this lengthening effect of constant light on the free-running period (32). Furthermore, hamsters with IGL lesions exhibited an enhanced light-masking response, suggesting that the IGL also modulates light-masking effects (34).

The IGL contains several neuronal subtypes, including neuropeptide Y (NPY)-, γ-aminobutyric acid-, and enkephalin-containing neurons (6, 24, 27, 28). Although NPY- and enkephalin-containing neurons project to the SCN, NPY has been identified as the candidate neurotransmitter in the GHT for mediating photic information to the SCN. Microinjection of NPY into the SCN before activity onset induces phase advances but causes phase delays when NPY is administered after the activity onset (1). Administration of NPY in the SCN blocks the photic-induced phase advance (41); however, the phase shift in response to NPY injection is attenuated by a subsequent light exposure (4). Furthermore, injection of NPY antiserum into the SCN increases the amplitude of phase advances in response to phase-shifting light pulses (3). Collectively, these studies suggested that the IGL has modulatory effects on 1) photic entrainment and 2) light-induced phase shifts. However, the underlying mechanism by which the GHT modulates the photic input to the SCN...
is not clear. In addition, the contralateral retinal projection to the IGL is twice as dense as the ipsilateral projection (24), while the GHT projection to the SCN is primarily ipsilateral (6, 31). For example, this anatomic feature suggests that the retinal projection of the left eye primarily activates the IGL on the right side and that this photic input to the right IGL primarily modulates the SCN on the right side of the brain.

It has been repeatedly demonstrated that light can induce c-Fos expression within rodent SCN neurons (18). In addition, the effectiveness of light to induce c-Fos expression is circadian phase dependent and directly associated with light-induced phase shifts (19). Light also induces c-Fos expression in the IGL, regardless of the animal’s circadian phase (10). Although the function of the light-induced c-Fos in the IGL is not understood, a number of these c-Fos-labeled neurons project to the SCN, indicating that photically activated IGL neurons affect SCN neurons (30). However, it has been difficult to understand how the IGL modulates the photic induction of c-Fos in SCN neurons. Monocular deprivation (enucleation or nerve transection) may be a useful animal model for revealing the effect of IGL neurons in mediating the photic response of the SCN neurons. The bilateral and symmetrical RHT projection suggests that, after monocular enucleation, a light-induced phase shift would induce c-Fos activity equally on both sides. The pattern of retinal-IGL-SCN projection suggests that, after monocular enucleation, the IGL on the remaining eye would be activated more by photic stimulation than the ipsilateral IGL. Therefore, the SCN contralateral to the remaining eye would be modulated most by the GHT after photic stimulation, with the greatest effect on neuronal induction of c-Fos.

For this study, unilateral optic nerve transections (UONx) were performed in hamsters to further understand the underlying neural circuitry and distribution of retinal photic information mediating the changes described previously. Monocular enucleation results in retinal photic information modulating the functional relationship between these two photic pathways. It was hypothesized that, after monocular enucleation, 1) the total photic input (quantified by the number of c-Fos-activated neurons) to the SCN and IGL would be reduced, 2) the bilateral RHT projection to the SCN would remain symmetrical, 3) the contralaterally favoring retinal projection pattern to the IGL would remain, 4) the photic response as indicated by c-Fos-activated neurons in the IGL would be greater on the contralateral side, and 5) the photic induction of c-Fos in the SCN would not be bilaterally symmetrical because of the asymmetric pattern of photic input to the IGL.

MATERIALS AND METHODS

Animals

Male Syrian hamsters (Mesocricetus auratus; Simonsen Laboratories, Gilroy, CA) were provided water and food ad libitum. They were housed in individual cages (16 × 7.5 × 8 in.) within ventilated light-tight enclosures (22.5 × 22.5 × 16 in.) at an ambient temperature of 25 ± 0.5°C and a 14:10-h light-dark cycle. Cages were changed weekly, and animal health was assessed daily. All animal care and surgical procedures were performed according to the National Institutes of Health (NIH) guidelines and under approval by the University of California Davis Animal Care and Use Committee.

Surgery

Animals were divided into three groups according to their surgical preparations. UONx was performed on adult (AONx) and postnatal day 2 (PONx) hamsters. These two groups were compared with control (Con) for possible transynaptic degeneration of SCN neurons after optic nerve transections in adults and the potential synaptic plasticity changes that might occur in early-postnatal hamsters (see DISCUSSION).

UONx on adult animals. Adult (14 to 16 wk old) male hamsters were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed in a stereotaxic frame. A 2-mm incision was made at the lateral canthus for access to the back of the eye; the superior and lateral recti muscles were sectioned to expose the optic nerve, and then the nerve was completely transected. The eye was returned to the orbit, and the eyelid was sutured closed. All these procedures were completed on the anesthetized hamster.

Monocular enucleation on postnatal day 2. Two-day-old hamster pups were anesthetized by induction of hypothermia. Before being removed, one eye was frozen by a freezing steel bar (3-mm diameter) to reduce hemorrhage. The eyelid was closed with biodehesive. After they were weaned on day 21, the animals were housed under the conditions described above. [Neuronal plasticity of the RHT has been reported in neonatal rodent species (20, 39), but the impact of this neuronal plasticity on the photic response in the SCN has not been examined. Thus this study also examines the interaction of UONx and development.]

Con group. Animals that did not undergo surgery were used as the Con group for AONx and PONx animals. (Because UONx was done on the right, the left side of the brain is viewed as the contralateral side for all groups.)

Light Treatment

At ~25 wk after surgery, all three surgical groups were further divided into two different light treatments. One series of animals from each surgical group was exposed to a 150-lux, at animals’ eye level, light pulse at CT 1500–1600 and was killed immediately after light exposure: Con/light, AONx/light, and PONx/light groups. The other series of animals was not exposed to light and was also killed at CT 1600: Con/dark, AONx/dark, and PONx/dark groups.

Immunohistological Procedures

Immediately after either light exposure treatment, animals were deeply anesthetized with pentobarbital sodium (50 mg/kg ip) and transcardially perfused with 250 ml of 0.9% saline followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were removed and stored in 4% paraformaldehyde at 4°C. Brains were transferred into 30% sucrose-phosphate buffer for cryoprotection ≥48 h before being processed for c-Fos and NPY immunoreactivity. To minimize the possible variation due to different batches of antibodies or tissue processing, antibodies from the same lot
number were used, and tissues with different surgical or physiological treatments were always processed simultaneously with the same stock of reagents.

**Immunoreactivity of c-Fos.** One set of brains from each group was frozen and coronally sectioned on a microtome at 50 μm. Free-floating brain sections through the SCN and IGL were collected in phosphate-buffered saline (PBS), washed five times for 5 min each in PBS, pH 7.4, and incubated in PBS with 0.4% Triton X-100 (PBS-TX) overnight at 4°C. Tissue was treated with blocking solution containing 2% (vol/vol) normal goat serum, 1% (wt/vol) bovine serum albumin, 3% H₂O₂, and 0.4% Triton X-100 for 1 h. After they were blocked, sections were incubated in 1:10,000 (vol/vol) anti-c-Fos rabbit IgG (100 μg/ml; Oncogene Science) for 24 h at 4°C. After primary antibody incubation, sections were washed five times in PBS-TX and then treated with 1:1,000 biotinylated anti-rabbit goat IgG (1 mg/ml; Vector Laboratory) in PBS-TX for 1 h at room temperature and washed five more times in PBS. Brain slices were then incubated in 1:1,000 horseradish peroxidase (HRP)-conjugated streptavidin (1 mg/ml; Vector Laboratory) for 1 h at room temperature, washed five times in PBS, and reacted for 10 min with TrueBlue (True Blue, Kirkegaard and Perry Laboratories) peroxidase substrate. Sections were washed five times in distilled water, mounted on gelatin-coated slides, and counterstained with neutral red, and coverslips were applied.

**NPY immunoreactivity.** Another set of brains from each group was sectioned, collected as previously described, and processed for NPY immunoreactivity in the SCN and IGL. After they were blocked, sections were incubated in 1:25,000 (vol/vol) anti-NPY rabbit IgG (100 μg/ml; Peninsula Laboratories) for 48 h at 4°C. After primary antibody incubation, sections were washed five times in PBS-TX, treated with 1:1,000 biotinylated anti-rabbit goat IgG (1 mg/ml; Vector Laboratory) in PBS-TX for 1 h at room temperature, and washed five more times in PBS. Brain slices were then incubated in 1:1,000,000 HRP-streptavidin (1 mg/ml; Vector Laboratory) for 1 h at room temperature, washed five times in PBS, and reacted for 20 min with metal-enhanced diaminobenzidine substrate kit (Pierce Chemicals). Sections were washed five times in PBS, mounted on gelatin-coated slides, and counterstained with neutral red, and coverslips were applied.

**Monocular Neuronal Tracer Injection**

Animals, 24–26 wk of age, from each surgery group were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed in a stereotaxic frame. All animals received 10 μl of 30% HRP, an anterograde neuronal tracer, injected into the vitreous chamber of one eye with a 25-μl microsyringe (Hamilton). All animals were allowed to survive for ≥24 h after the HRP injection. Animals were then anesthetized, perfused, and fixed, and brains were removed. Coronal sections at 50 μm were collected throughout the SCN. Slices were reacted with TrueBlue to detect HRP within the terminals of retinal ganglion cells in the SCN. Sections were mounted and cleared with xylene, and coverslips were applied.

**Data Analysis**

**Immunohistochemistry.** The SCN and IGL were examined under light microscopy at ×200 with a charge-coupled device color videocamera (model 3CCD, Optronics Engineering). The boundary of the SCN was determined by Nissl counterstaining. The IGL was identified by Nissl staining and NPY-immunoreactive neurons. All images were digitized as 24-bit images into a microcomputer (PowerMacintosh 7500/100) via a 24-bit video-digitizing card (Targa 2000, True Vision). The c-Fos-immunoreactive neurons throughout the SCN and IGL and NPY cells in the IGL were quantified for each group (Con, AONx, and PONx) and each side (ipsilateral and contralateral). The photic responses of the ipsilateral vs. contralateral SCN and IGL for each group were statistically analyzed with a between-within-group ANOVA (StatView 5.0). The average optical density of NPY terminals in the SCN was calculated by scanning (NIH Image) the entire nucleus of each animal. The effect of UONx on the IGL NPY projection in the SCN was analyzed with a between-within-group ANOVA as stated above.

**RHT projection.** Coronal sections encompassing the whole SCN were collected. The RHT projection revealed by HRP was examined under a light microscope. Serial sections were visually examined for their projection symmetry. Images throughout the SCN were captured and digitized as previously described. Average optical density of each SCN of every animal was analyzed by NIH Image. The optical density differences between the ipsilateral and contralateral SCN for each group were analyzed with a between-within-group ANOVA.

**RESULTS**

**Effects of UONx on Light-Induced c-Fos Expression in the SCN**

Con/light animals exhibited a symmetrical bilateral distribution of light-induced c-Fos-immunoreactive neurons primarily in the caudal half of the SCN. The c-Fos expression was mainly confined to the ventrolateral subdivision of the nucleus, with c-Fos-labeled neurons scattered at the dorsolateral border of the nucleus (Fig. 1A). UONx reduced the number of light-induced c-Fos-immunoreactive neurons in the SCN and produced a predominant expression in the SCN ipsilateral to the remaining eye (Fig. 1, B and C). Con/light animals exhibited the highest total average number of c-Fos-positive neurons in the SCN, while AONx/light and PONx/light animals exhibited a reduced number of c-Fos-positive SCN neurons (Table 1). There was a significant difference in c-Fos expression between Con/light and both AONx/light and PONx/light hamsters [Table 1; F(2,15) = 59.248, P < 0.01, Tukey’s post hoc test]. In addition, the number of c-Fos-positive neurons in the SCN was significantly greater in PONx/light than in AONx hamsters [Table 1; F(2,15) = 59.248, P < 0.05, Tukey’s post hoc test]. On the other hand, Con/dark, AONx/dark, and PONx/dark animals, which were not exposed to light at CT 1500, exhibited no c-Fos induction in the SCN.

AONx/light exhibited c-Fos-positive neurons that were more confined to the caudal area with less expression in the mid and rostral section of the nucleus (Fig. 1B). The c-Fos neurons in the dorsal area extended into the rostral SCN, similar to the pattern of the intact animals. The optic chiasm ipsilateral to the remaining eye also became thinner than the contralateral half of the chiasm. The ventral part of the ipsilateral SCN directly above the thinned chiasm appeared to expand toward the chiasm. In addition, AONx/light (Fig. 1B) and PONx/light (Fig. 1C) hamsters did not exhibit the
PONx/light hamsters exhibited less asymmetry in c-Fos neuron distribution than did the AONx/light animals.

Effects of UONx on Symmetry of the RHT Projection to the SCN

Monocular injection of HRP revealed bilateral projections in Con, AONx, and PONx animals. No significant difference (Fig. 2) in the symmetry of projection could be determined visually or by optical density measurements between intact and transected animals. Optical density measurements of the HRP deposit showed no statistical difference between two SCN nuclei of the intact animals [Table 1; \(F(2,6) = 1.616\)]. In both UONx conditions, the optical density measurement indicated that the bilaterally symmetrical RHT projection remained unchanged.

Effects of UONx on Light-Induced c-Fos Expression in the IGL

Light exposure during CT 1500–1600 induced symmetrical bilateral c-Fos expression in the IGL of the Con/light animals (Fig. 3, A and B). UONx reduced the number of light-induced c-Fos-positive neurons in contralateral 55

Table 1. Summary of c-Fos- and NPY-immunoreactive cell count and HRP and NPY optical density in the SCN and IGL

<table>
<thead>
<tr>
<th></th>
<th>Cell Count</th>
<th>Optical Density (n = 3)</th>
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<tbody>
<tr>
<td></td>
<td>c-Fos</td>
<td>NPY</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HRF</td>
</tr>
<tr>
<td><strong>SCN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>Total 1,423 ± 133(6)</td>
<td>106 ± 10.39 98.7 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>Contralateral 713 ± 68</td>
<td>97.93 ± 4.09 99.3 ± 3.3</td>
</tr>
<tr>
<td>AONx</td>
<td>Total 164 ± 21†(6)</td>
<td>94.41 ± 5.94 84.3 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>Contralateral 55 ± 4</td>
<td>93.29 ± 6.02 95.3 ± 1.3*</td>
</tr>
<tr>
<td>PONx</td>
<td>Total 445 ± 64* (6)</td>
<td>108.86 ± 2.75 76.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Contralateral 164 ± 15</td>
<td>110.76 ± 3.01 92.2 ± 3.2†</td>
</tr>
<tr>
<td></td>
<td>Ipsilateral 251 ± 52*</td>
<td></td>
</tr>
<tr>
<td><strong>IGL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>Total 1,666 ± 91(4)</td>
<td>207 ± 11(4)</td>
</tr>
<tr>
<td></td>
<td>Contralateral 854 ± 54</td>
<td>105 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>Ipsilateral 812 ± 39</td>
<td>102 ± 5</td>
</tr>
<tr>
<td>AONx</td>
<td>Total 619 ± 111†(7)</td>
<td>168 ± 11†(4)</td>
</tr>
<tr>
<td></td>
<td>Contralateral 428 ± 74*</td>
<td>106 ± 7</td>
</tr>
<tr>
<td></td>
<td>Ipsilateral 191 ± 40</td>
<td>62 ± 9†</td>
</tr>
<tr>
<td>PONx</td>
<td>Total 348 ± 63†(6)</td>
<td>149 ± 5†(3)</td>
</tr>
<tr>
<td></td>
<td>Contralateral 239 ± 37*</td>
<td>101 ± 4</td>
</tr>
<tr>
<td></td>
<td>Ipsilateral 119 ± 30</td>
<td>48 ± 2†</td>
</tr>
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</table>

Values are means ± SE of number (n) of animals in parentheses.

NPY, neuropeptide Y; HRP, horseradish peroxidase; SCN, suprachiasmatic nucleus; IGL, intergeniculate leaflet; Con, control; AONx, adult animals subjected to unilateral optic nerve transection; PONx, animals subjected to unilateral optic nerve transections on postnatal day 2. *P < 0.05; †P < 0.01.
the IGL and resulted in a predominant expression in the IGL contralateral to the remaining eye (Fig. 3, C–F). Con/light hamsters exhibited more robust symmetrical c-Fos expression than both groups of UONx animals [Table 1; \(F(2,14) = 42.007, P < 0.01\), Tukey’s post hoc test]. UONx caused a three- and a fivefold light-induced c-Fos expression in the IGL of AONx and PONx animals, respectively [Table 1; \(F(2,14) = 42.007, P < 0.01\), Tukey’s post hoc test]. Furthermore, the IGL contralateral to the remaining eye revealed twice as many c-Fos-positive neurons in AONx and PONx hamsters [Table 1; \(F(2,14) = 42.007, P < 0.01\), Tukey’s post hoc test]. Unlike the light-induced c-Fos expression in the SCN, PONx animals exhibited the least c-Fos-positive neurons in the IGL with the same amount of asymmetry as in IGL c-Fos expression in AONx animals.

Effects of UON on NPY-Immunoreactive Neurons in the IGL

Con/light animals exhibited a bilaterally symmetrical distribution of NPY-immunoreactive neurons in the IGL (Fig. 4, A and B), while in AONx (Fig. 4, C and D) and PONx (Fig. 4, E and F) animals, distribution of the NPY-immunoreactive neurons was predominantly contralateral to the remaining eye. The average total number of NPY-labeled neurons in both UONx groups was also significantly reduced compared with that in Con/light hamsters [Table 1; \(F(2,8) = 7.982, P < 0.05\), Tukey’s post hoc test]. There was a greater reduction of NPY-labeled neurons in the IGL in PONx animals. There were twice as many NPY-labeled neurons in IGLs contralateral to the remaining eye of both UONx groups than on the ipsilateral side [Table 1; \(F(2,8) = 12.674, P < 0.01\), Tukey’s post hoc test].
Effects of UONx on the NPY Terminal Field in the SCN

In the intact Con animals, the distribution of NPY terminals in the SCN exhibited a bilaterally symmetrical pattern that was coexistent with the distribution of the light-induced c-Fos-immunoreactive neurons in the SCN (Fig. 5A). In the AONx animals, the NPY terminals were distributed in the midanterior SCN ipsilateral to the remaining eye (Fig. 5B). The caudal SCN exhibited a bilaterally symmetrical NPY terminal field. The PONx animals exhibited an NPY terminal distribution similar to the AONx animals, ipsilateral in the midanterior SCN (Fig. 5C) and bilaterally symmetrical in the caudal SCN. Optical density measurements showed bilateral NPY terminal distribution in Con animals (Table 1) and ipsilateral distribution in AONx (Table 1; $F(2,9) = 9.982, P < 0.01$, Tukey’s post hoc test) and PONx (Table 1; $F(2,9) = 9.982, P < 0.01$, Tukey’s post hoc test) animals.

Fig. 5. Light photomicrographs of coronal sections of the hamster SCN illustrating the effect of UONx on the NPY terminal field in the SCN. A: bilaterally symmetrical distribution of the NPY terminal in the Con hamster SCN. B: distribution in the midanterior SCN ipsilateral to the remaining eye of the AONx hamster. C: enhanced NPY terminal field in the midanterior SCN ipsilateral to the remaining eye of PONx hamsters. Scale bar, 100 μm.

DISCUSSION

This study revealed that control (Con/light) hamsters exhibited a bilaterally symmetrical distribution of light-induced c-Fos- and NPY-immunoreactive neurons in the ventrolateral SCN and IGL. UONx in adults (AONx) reduced light-induced c-Fos in nuclei and NPY-immunoreactive neurons in the IGL. In addition, the AONx animals exhibited a loss of symmetry. The SCN ipsilateral to the remaining eye revealed a greater number of light-induced c-Fos-immunoreactive neurons than the contralateral SCN. In contrast, the IGL contralateral to the remaining eye showed more light-induced c-Fos-immunoreactive neurons and more NPY-immunoreactive neurons. Compared with the AONx animals, the PONx animals had a further reduction in the numbers of light-induced c-Fos- and
NPY-immunoreactive neurons in the IGL; the SCN exhibited less reduction of c-Fos expression in these animals. The bilateral distribution of NPY-containing terminals in the SCN overlaps with the distribution of light-induced c-Fos-immunoreactive neurons. This relationship remained unchanged after monocular transection. Monocular HRP injection in Con animals revealed a bilaterally symmetrical RHT projection to the SCN. This symmetry remained unchanged in AONx and PONx animals. Therefore, the anatomic distribution of the RHT in AONx and PONx animals does not coincide with photic responsiveness as revealed by c-Fos.

Light-induced c-Fos-immunoreactive neurons were mainly localized to the ventrolateral area in the mid to caudal portion of the SCN (Fig. 1A). This pattern was consistent with other studies (2, 33, 35, 37). However, the number of SCN and IGL neurons expressing light-induced c-Fos in the AONx animals was reduced (Fig. 1B). The reduction of light-induced c-Fos expression could be due to the decrease of photic input to these nuclei after transection. The excitatory amino acid N-acetylaspartylglutamate has been suggested to be the primary neural transmitter mediating retinal input to the SCN and IGL (22). Bilateral optic nerve transection caused a 50% decrease of N-acetylaspartylglutamate immunoreactivity in the SCN and IGL of rats (23). This would suggest that the reduction of light-induced c-Fos expression in the SCN seen in our study could be due to a decrease in excitatory amino acid neurotransmitter after UONx.

**Effects on the RHT**

The retinohypothalamic projection to the SCN in hamsters is bilateral, with a slightly greater contralateral density (16). Results from this study showed that UONx resulted in a predominant light-induced c-Fos expression in the SCN ipsilateral to the remaining eye (Fig. 1, B and C). This observation was not expected on the basis of the RHT projection to the SCN in hamsters. It was anticipated that there would be a relatively bilateral or slightly contralateral c-Fos induction pattern in these transected animals.

It is tempting to speculate that neural plasticity of the RHT and SCN may play a role in the unexpected asymmetric changes in light-induced c-Fos expression after UONx. Adult hamsters, with RHT lesions, revealed extensive axonal sprouting of retinal fibers in the SCN and proximally located hypothalamic areas (14). Rats monocularly enucleated on postnatal day 2 exhibited an increase in the ipsilateral RHT projection to the SCN (39). Monocular enucleation at birth also causes an increase of vasoactive intestinal peptide/peptide histidine isoleucine in the SCN ipsilateral to the remaining eye of the rat (9). This change in RHT distribution might be due to a redirection or sprouting of terminals in the SCN ipsilateral to the remaining eye. Another study, on the effects of early postnatal monocular enucleation on the visual system in the wallaby, a marsupial, reported similar results (20).

The wallaby has a highly asymmetric pattern of RHT projection. There is a very dense retinal projection to the contralateral SCN and a sparse projection to the ipsilateral SCN. However, after early monocular enucleation, there was a dramatic increase in the density of the ipsilateral projection (20). The terminal density, in some cases, approached the density of the contralateral projection. Finally, after monocular enucleation at birth, physiological recording from the superior colliculus of adult hamsters indicated greater physiological responses to visual stimulation from the ipsilateral eye (7). Collectively, these studies demonstrate significant ipsilateral changes in the RHT after monocular enucleation and are consistent with the greater c-Fos induction in the ipsilateral SCN seen in this study. However, this study did not find a significant change in the pattern of RHT projection after AONx or PONx (Table 1). Thus the pattern of RHT projection revealed by the anterograde transport of HRP likely does not represent the extent of functional changes that occur after monocular enucleation. To this end, we believe that other factors may mediate the enhanced c-Fos induction in the ipsilateral SCN of unilaterally transected animals.

**Effects on the IGL and GHT**

In addition to the RHT input, the SCN also receives indirect photic input from the IGL via the GHT. In hamsters, the IGL receives bilateral retinal input; however, the retinal projection to the contralateral IGL is much denser (28). In this study, photic stimulation of both eyes of intact animals resulted in an equal induction of c-Fos within neurons in both IGLs (Table 1). However, the unilaterally transected animals exhibited less overall light-induced c-Fos expression, suggesting a reduction of photic responsiveness in the IGL (Table 1). In addition, the IGL contralateral to the remaining eye of the transected animals expressed twice as many light-induced c-Fos-positive neurons as the ipsilateral IGL, consistent with the retinal pattern of projection to the IGL (31).

This enhanced contralateral response to photic input in the IGL of the UONx animals could lead to the predominant ipsilateral response of light-induced c-Fos expression in the SCN resulting from the reduced inhibitory input from the ipsilateral IGL. This hypothesis was based on the following observations: 1) the IGL efferent input to the SCN was bilateral but with a projection to the ipsilateral SCN that was two to three times denser (24, 31), and 2) the GHT innervation coincided with the RHT projection in the SCN (27, 28).

NPY has been identified as one neurotransmitter of the GHT terminals in the SCN and has been suggested to have a role in modulation of photic input to the SCN (3, 4). Administration of NPY into the SCN before a phase-advancing light pulse blocked the light-induced phase shift (41). The amplitude of a light-induced phase shift can be enhanced by injecting anti-NPY serum into the SCN area before exposure to a phase-shifting light pulse (4). More importantly, NPY administration in the SCN causes an inhibition of spontane-
ous firing activity of the SCN neurons (8, 11, 36). In addition, glutamate-induced phase advances and delays in SCN neuron spontaneous activity can be blocked by application of NPY. If this assumption is correct, photic stimulation of monocularly enucleated hamsters would primarily activate the contralateral IGL, leading to an inhibition in the SCN on the same side. The greater reduction of NPY-containing neurons in the IGL of PONx animals (Table 1) coupled with the increase in c-Fos-labeled cells in the SCN further supports the hypothesis that IGL NPY neurons negatively modulate photic activation of the SCN. Furthermore, the IGL would produce a predominant ipsilateral inhibition of the c-Fos induction in the SCN that is contralateral to the remaining eye. Thus this ipsilateral inhibition from the IGL may contribute to the ipsilaterally induced c-Fos in the SCN of the UONx animals.

A model was developed to account for the two effects of UONx on the functions of RHT and GHT (Fig. 6). This model suggests that UONx produces an overall reduction in photic input to the SCN and IGL and induces an asymmetric shift favoring light-induced c-Fos expression in the SCN ipsilateral to the remaining eye as a result of enhanced inhibition from the IGL to the SCN contralateral to the remaining eye. This unilateral IGL inhibition to the SCN results from a predominant contralateral retinal projection to the IGL from the remaining eye. The light-induced c-Fos and NPY neurons in the IGL contralateral to the remaining eye reflect this contralateral retinal projection. The disruption of symmetry of light-induced c-Fos expression in the SCN and IGL may reveal how the photic signal is integrated between the RHT and GHT. The IGL also has reciprocal projections between the two IGLs (24, 31). In this model, it is not clear what role these reciprocal IGL projections may have in mediating the photic input to the SCN and, furthermore, what impact the UONx may have on these reciprocal projections. The direct proportional changes in the numbers of c-Fos- and NPY-positive neurons in the IGL suggest a functional linkage between these two cell populations. On the basis of a prior double-labeling experiment (c-Fos and NPY), we know that >90% of the cells are uniquely c-Fos or NPY positive (40).

Neurotransmitters such as substance P, enkephalin, and γ-aminobutyric acid have been identified in the SCN and IGL and shown to have important roles in mediating photic input in the SCN (12, 17, 26–28). These neurotransmitters may contribute to the observations revealed in this study. However, how these neurotransmitters may impinge on the modulation from the IGL to the SCN is not understood.

Because AONx and PONx animals exhibited more NPY-containing neurons in the contralateral IGL (Table 1) and a predominant ipsilateral projection to the SCN, we expected to find a greater density of NPY immunoreactivity in the SCN that is contralateral to the remaining eye. Surprisingly, AONx and PONx animals exhibited greater NPY immunoreactivity in the ipsilateral SCN (Table 1, Fig. 5). This observation was not only unexpected but is also inconsistent with the NPY-containing neuron results from AONx and PONx animals. Because the IGL is considered the sole source of NPY input to the SCN, there is no direct or indirect evidence from this study to elucidate the functional significance of this observation. Although various possibilities such as terminal sprouting or nuclear elongation could explain such changes, resolution of this issue requires further study.

**Perspectives**

As a result of unbalancing the retinal input by UONx, this study suggests how the photic signal is processed in the two primary nuclei, the SCN and IGL, of the circadian timing system. These data further support the inhibitory role of the IGL in mediating the photic input to the SCN and functionally validated previous observations on the anatomy of the IGL and GHT. The unilaterally light-induced c-Fos expression in the SCN of the UONx animals suggests that the IGL modulates the photic input to the SCN mainly through ipsilateral inhibition. On the other hand, UONx did not seem to have a significant impact on the RHT innervation as revealed by the tract tracing results. Nonetheless, RHT plasticity has been suggested in several studies. However, the functional significance of the postulated RHT plasticity in relation to photic input is not understood. Results from this study suggest that RHT plasticity, if it exists in hamsters, has a greater
impact on PONx than on AONx animals. Although there seems to be a correlation between the number of light-induced c-Fos neurons in the SCN and IGL, the function of c-Fos-positive neurons in the IGL in modulating the SCN remains to be determined. The functional identification of the light-induced c-Fos-positive neurons in the IGL will also help us understand how the IGL modulates SCN function.

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