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c-Fos Expression in the Brains of Behaviorally “Split” Hamsters in Constant Light: Calling Attention to a Dorsolateral Region of the Suprachiasmatic Nucleus and the Medial Division of the Lateral Habenula

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Abstract

“Splitting” of circadian activity rhythms in Syrian hamsters maintained in constant light appears to be the consequence of a reorganized SCN, with left and right halves oscillating in antiphase; in split hamsters, high mRNA levels characteristic of day and night are simultaneously expressed on opposite sides of the paired SCN. To visualize the splitting phenomenon at a cellular level, immunohistochemical c-Fos protein expression in the SCN and brains of split hamsters was analyzed. One side of the split SCN exhibited relatively high c-Fos levels, in a pattern resembling that seen in normal, unsplit hamsters during subjective day in constant darkness; the opposite side was labeled only within a central-dorsolateral area of the caudal SCN, in a region that likely coincides with a photo-responsive, glutamate receptor antagonist-insensitive, pERK-expressing cluster of cells previously identified by other laboratories. Outside the SCN, visual inspection revealed an obvious left-right asymmetry of c-Fos expression in the medial preoptic nucleus and subparaventricular zone of split hamsters killed during the inactive phase and in the medial division of the lateral habenula during the active phase (when the hamsters were running in their wheels). Roles for the dorsolateral SCN and the mediolateral habenula in circadian timekeeping are not yet understood.

Keywords

c-Fos; calbindin; circadian; PER; splitting; suprachiasmatic nucleus

Syrian hamsters maintained in constant illumination (LL) for a few months can exhibit a remarkable behavioral state, in which an animal's single daily bout of locomotor (wheel-running) activity dissociates into 2 components that each free-run with different periods until they become stably coupled 180° apart. Pittendrigh and Daan (1976) were the first to systematically describe this phenomenon in hamsters and called it “splitting,” citing it as evidence that the rodent circadian clock must be a complex pacemaker consisting of 2 mutually coupled oscillators. Further experimental analyses revealed that circadian rhythms of drinking (Shibuya et al., 1980), body temperature (Pickard et al., 1984), and luteinizing hormone secretion (Swann and Turek, 1985) could also split and that the 2 oscillators underlying the split state appeared to be functionally equivalent (Lees et al., 1983; Boulos and Morin, 1985; Meijer et al., 1988; Meijer et al., 1990). Pickard and Turek (1982) suggested that the split oscillators might correspond to the left and right sides of the bilaterally paired SCN, based on their observation that unilateral SCN lesions in split hamsters abolished behavioral splitting

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and produced a single bout of locomotion. Interpretation of their findings, however, was confounded by nonspecific surgical effects (Harrington et al., 1990).

Recently, de la Iglesia et al. (2000) were able to assay SCN activity in unlesioned, split hamsters by measuring the expression of the “clock” genes *Per* and *Bmal1* by in situ hybridization using cut tissue sections and film autoradiography. They found that high mRNA levels characteristic of subjective day (*Per1*, *Per2*) and subjective night (*Bmal1*) were simultaneously expressed on opposite sides of the paired SCN, indicating that the left and right sides of the SCN of split hamsters do not oscillate together in phase. The data suggested that each of the SCN's 2 sides continue to cycle with a ~24-h circadian period but that each side is cycling in antiphase, thereby generating the overt ~12-h split rhythm of locomotor activity. When animals were killed at various times across the split circadian cycle, the left-right asymmetry of *Per* expression was observed to peak during the phase of locomotor inactivity, about 6 to 8 h (for *Per1*) or 10 h (for *Per2*) after the offset of the previous locomotor activity bout. The brief, circa-12-h locomotor bouts presumably occur when day-like gene expression is low on both sides of the SCN (i.e., as *Per* is falling on one side and rising on the other). Recently, left-right antiphase oscillations were confirmed in acute SCN slices made from behaviorally-split *Per1*:GFP transgenic mice, in which a side-to-side, opposite oscillation of *Per1*-driven bioluminescence was recorded longitudinally from individual slices in vitro (Ohta et al., 2005).

In the experiments described in the present article, we performed c-Fos immunohistochemistry to better visualize how the SCN of split hamsters is reconfigured at a cellular level. We chose c-Fos as a functional marker because it exhibits 2 different, spatially distinct rhythms in the SCN of hamsters (as in other rodents): a rhythm of photoinducibility, with increased gene expression in the caudal and ventral SCN after exposure of animals to light pulses during the subjective night (Kornhauser et al., 1990; Rusak et al., 1990), and a spontaneous circadian rhythm, with increased gene expression in the rostral and dorsal SCN during the subjective day in constant darkness (Chambille et al., 1993; Guido et al., 1999b). In the course of our experiments, we also surveyed the whole brains of split hamsters for any noticeable left-right asymmetries in the number of c-Fos-labeled cells outside the SCN.

MATERIALS AND METHODS

Male Syrian hamsters (Charles River, Kingston, NY), 21 days old at time of delivery to the laboratory, were used in accordance with the regulations of the University of Massachusetts Institutional Animal Care and Use Committee. They were housed individually in clear polycarbonate cages contained within well-ventilated, light-proof environmental cabinets isolated in an animal facility. The conditions within the compartments included 12 air changes per hour, a thermostatically controlled internal temperature of $72^{\circ}\text{F} \pm 1^{\circ}\text{F}$, and a relative humidity matching that of the animal room outside (maintained at 60%). Food and water were freely available and replenished at irregular hours. Light during light-dark cycles, light pulses, or LL was provided by 15-W cool-white fluorescent tubes mounted above the shelves and automatically controlled by a 24-h timer; intensity varied within the cages but was on the order of 300 to 400 lx at the midcage level. No light was present during constant darkness.

Wheel-running activity was continuously recorded, with a microswitch on the outside of each cage activated by the rotation of a 7" metal running wheel. The number of switch closures per 15-min interval was automatically recorded, stored on hard disk by an IBM computer-based data acquisition system, and plotted for each hamster as an actogram (Dataquest; Mini-Mitter Co., Sunriver, OR). About 60% of our hamsters developed stably split activity rhythms after 6 to 8 weeks in LL.

Groups of behaviorally split hamsters were killed in LL at 2 phases, either during a locomotor-inactive interval (about 8 h after the offset of the preceding locomotor activity bout) or during a locomotor bout. Additional unsplit hamsters were killed in constant darkness after they had been entrained to a 14-h:10-h light-dark cycle; sacrifice was either during early subjective day (about 4 h after subjective lights-on), late subjective day (about 2 h before subjective lights-off), early subjective night (about 2 h after subjective lights-off), or after a midsubjective-night light pulse.

For immunohistochemistry, hamsters were deeply anesthetized with an overdose of ketamine/xylazine (70 mg/7 mg/100 g body weight, i.p.) and perfused through the ascending aorta with 25 mL heparinized 0.1 M phosphate-buffered saline (PBS), followed by 100 mL freshly-prepared cold 4% paraformaldehyde in PBS. Brains were removed and immersed in fixative overnight at 4 °C, 50- μ m-thick coronal sections through the SCN were cut on a vibratome, and sections were incubated with rabbit anti-c-Fos₃₋₁₇ antiserum (1:10,000; SC52; Santa Cruz Biotech Inc., Santa Cruz, CA) overnight at 4 °C. Some of the sections were also simultaneously incubated with either mouse anti-calbindin-D_{28K} (1:3,000; C9848; Sigma, St. Louis, MO) or rat anti-mPER1₇₃₁₋₉₆₀ (1:500; generous gift of Drs. Steven Reppert and David Weaver). Sections incubated with the anti-c-Fos antiserum only were then treated with biotinylated goat anti-rabbit secondary antibody and the avidin-biotin method (Vector Laboratories, Burlingame, CA), with diaminobenzidine (Kirkegaard & Perry, Gaithersburg, MD) as the chromogen, mounted on slides, dehydrated, and cover-slipped. Double-label immunofluorescence and confocal microscopy (0.5- μ m thick optical sections) for c-Fos and either calbindin or mPER1 were performed by treating sections with AlexaTM-594 goat anti-rabbit (for c-Fos) and AlexaTM-488 goat anti-mouse (for calbindin), or AlexaTM-488 goat anti-rat (for mPER1) secondary antibodies (1:200; Molecular Probes, Eugene, OR) for 2 h at room temperature, mounted, dehydrated, and cover-slipped in Prolong anti-fade reagent (Molecular Probes). Excitation wavelengths for AlexaTM-594 and -488 were 568 and 488 nm, respectively.

For light-microscopic cell counts, labeled cell nuclei (irrespective of the intensity of staining) were counted by one of us (M.T.-N.) without knowledge of the direction of the SCN's side-to-side asymmetry. Counts for each brain structure were conducted within preset circular or oval grids (ranging in area from 0.064 to 0.170 mm², depending on the structure; see Results section) and expressed as average counts from 2 sections/structure/animal.

RESULTS

c-Fos Expression in the Split Hamster SCN

Figure 1 illustrates the pattern of immunoreactive c-Fos expression in coronal sections of the rostral, middle, and caudal SCN from a representative, behaviorally split hamster killed in LL during a locomotor-inactive phase compared with the patterns from normal, unsplit hamsters killed in constant darkness during early subjective day and subjective night, and 60 min after the onset of a midsubjective-night light pulse. As previously described by others (Chambille et al., 1993; Guido et al., 1999b), there is a spontaneous circadian rhythm of SCN c-Fos levels in constant darkness; relatively modest expression is localized mainly rostrally and dorsally during the 1st half of the subjective day and is essentially undetectable during the subjective night. In contrast, photoinducible c-Fos levels result from light stimulation during the subjective night, with very strong expression localized caudally and ventrally (in a retino-recipient distribution) but also at rostral and middle levels, extending beyond the SCN's anatomical borders (Rea, 1992; Guido et al., 1999b).

Split hamsters exhibited a dramatic left-right asymmetry in the levels and distribution of SCN c-Fos. Expression on the relatively "high" side (the right half-SCN in Fig. 1) appeared similar in magnitude and localization to that observed in normal, unsplit hamsters during subjective

day in constant darkness; expression on the opposite “low” side (the left half-SCN in Fig. 1) was comparatively sparse, except for a circumscribed region centrally, extending dorsally and laterally in the caudal SCN. This general left-right pattern was seen in split hamsters killed either during the locomotor-inactive phase (as in Fig. 1) ($n = 4$ animals) or during the active phase (when the hamsters were running in wheels during a locomotor bout) ($n = 6$ animals), although the asymmetry of labeling appeared diminished in the latter group of animals. In the rostral SCN, high-side cell counts were comparable in the 2 groups (242 ± 41 inactive phase vs. 239 ± 27 active phase [$\bar{x} \pm \text{SEM}$; throughout article]; counts/0.110 mm² circular counting grid over the rostral SCN), while low-side counts were nonsignificantly higher in the active group (56 ± 13 inactive phase vs. 84 ± 14 active phase). In the middle SCN, the side-to-side asymmetry was clearly greater during the inactive phase than the active phase (high-side counts, 417 ± 62 inactive phase vs. 281 ± 42 active phase; low-side counts, 50 ± 11 inactive phase vs. 97 ± 12 active phase; counts/0.120 mm² oval counting grid over the middle SCN) (low-side counts significantly different between groups, $p < 0.02$ by 2-tailed Student t test).

Our subjective-day assignment to the half-SCN with high c-Fos expression is consistent with previous *in situ* hybridization data on the split hamster SCN (de la Iglesia et al., 2000), which showed that *Bmal1* mRNA levels were high in the half-SCN contralateral to the half with high c-fos and *Per1* levels (as expected for *Bmal1*, a gene characteristic of subjective night). Double-label immunofluorescence for c-Fos and PER1 proteins confirmed that they were both expressed ipsilaterally on the same (subjective day) side of the split SCN (the right half-SCN in Fig. 2A). Of note, however, was a difference in their localization, with PER1 expressed less rostrally and more caudally in a non-retino-recipient, dorsomedial distribution. Even in the middle SCN, where the 2 immunoreactivities overlapped at least somewhat at the tissue level, counts of double-labeled cell nuclei by confocal microscopy revealed that only 5% of the c-Fos nuclei and 14% of the PER1 nuclei coexpressed the other protein ($n = 4$ sections; 263 c-Fos⁺ nuclei and 100 PER1⁺ nuclei counted).

The opposite (subjective night) side of the split SCN exhibited little or no c-Fos and PER1 expression—despite the continued presence of ambient light in LL—except in the aforementioned central-dorsolateral region in the caudal SCN (the left half-SCN in Fig. 2A). In this region, counts of double-labeled cell nuclei by confocal microscopy revealed that 20% of the c-Fos nuclei and 40% of the PER1 nuclei coexpressed the other protein (Fig. 2B) ($n = 5$ sections; 96 c-Fos⁺ nuclei and 48 PER1⁺ nuclei counted). Double-label immunofluorescence for c-Fos and calbindin revealed that the region formed a dorsal and lateral canopy that was adjacent to but largely separated from the calbindin-rich zone (Fig. 2C). We found no instance of c-Fos/calbindin colocalization ($n = 7$ sections, each from a different hamster; 171 c-Fos⁺ nuclei and 243 calbindin⁺ cell bodies counted).

c-Fos Expression in the Split Hamster Brain

Examination of the whole brain (Morin and Wood, 2001) of split hamsters was most notable for the overall left-right symmetry of c-Fos-expressing cell nuclei. Although no attempt was made to comprehensively quantify cell counts in all structures throughout the brain, side-to-side differences in c-Fos expression were clearly visible in periventricular hypothalamic regions neighboring the SCN, specifically in the medial preoptic nucleus (MPN) and the subparaventricular zone (SPZ) of split hamsters killed during their inactive phase (Fig. 3). Higher cell counts in the MPN were on the side contralateral to high SCN c-Fos expression (49 ± 5 vs. 22 ± 5 ; counts/0.133 mm² oval counting grid over the MPN; $n = 4$ animals; $p < 0.005$ by 1-tailed Student t test), whereas higher counts in the SPZ were on the side ipsilateral to high SCN c-Fos expression (67 ± 4 vs. 39 ± 12 ; counts/0.170 mm² oval counting grid over the SPZ; $n = 4$ animals; $p < 0.05$ by 1-tailed Student t test). Statistically significant asymmetries were not present in the MPN and SPZ of split hamsters killed during the active phase (when

they were running in wheels). Instead, split hamsters killed during their active phase exhibited an unexpected but obvious side-to-side asymmetry in the medial division of the lateral habenula (LHbM) (Fig. 4A), with higher cell counts on the side contralateral to high SCN c-Fos expression (65 ± 4 vs. 31 ± 3 ; counts/0.064 mm² oval counting grid over the habenula; $n = 6$ animals; $p < 0.001$ by 2-tailed Student *t* test). Split hamsters killed during their inactive phase displayed little or no c-Fos labeling in either the left or right LHbM. We also analyzed a separate group of normal, unsplit hamsters killed in constant darkness; they showed a robust circadian rhythm of c-Fos expression in the LHbM, with high levels during the subjective night and virtually undetectable levels during the subjective day (Fig. 4B). This rhythm was present regardless of whether running wheels were available in the cages.

DISCUSSION

LL-induced splitting is associated with a remarkable reconfiguration in the coupling of cellular oscillators within SCN tissue. The SCN's left and right sides cycle in antiphase, and high levels of *c-fos*, *Per1*, and *Per2* mRNA are all expressed unilaterally and opposite to high levels of *Bmal1* mRNA (de la Iglesia et al., 2000). The present results extend these data to the protein level and also show that the split SCN is more complex than merely 2 out-of-phase, otherwise intact halves.

We show that immunoreactive c-Fos and PER1 proteins are expressed asymmetrically in the split SCN, with an immunohistochemical appearance on the “high” side, most closely resembling that seen in the normal, unsplit SCN during subjective day in constant darkness. Interestingly, the tissue distribution of the immunoreactive proteins is not identical, especially in the rostro-caudal plane. One possible interpretation of this observation is that c-Fos and PER1 might be marking some of the different cell populations known to oscillate in the SCN (Quintero et al., 2003; Saeb-Parsy and Dyball, 2003; Schaap et al., 2003; Yamaguchi et al., 2003). An alternative possibility is that both c-Fos and PER1 might be expressed in a common cell population but at different times of the day; levels of the proteins do peak at different phases of the subjective day in constant darkness—during the 1st half for c-Fos (Guido et al., 1999b) and the 2nd half for PER1 (Maywood et al., 1999), and gene expression (at least for *Per1*) apparently “spreads” initially from a dorsomedial location and then laterally, ventrally, and rostrally (Yan and Okamura, 2002 [in rat]; Hamada et al., 2004 [in hamster]).

On the other hand, relatively little c-Fos and PER1 are expressed on the SCN's contralateral (subjective night) side, consistent with the absence of photoinducible *c-fos* and *Per1* mRNA on that side (de la Iglesia et al., 2000). The unknown molecular mechanism(s) responsible for the photic unresponsiveness of the split SCN in LL are likely to be critical for the generation and maintenance of the split condition. However, here we show the presence of a discrete c-Fos⁺/PER1⁺ region caudally and centro-dorsolaterally on the SCN's subjective-night side. It does not match the usual dorsomedial “shell” or ventrolateral “core” subdivisions of the nucleus and likely corresponds to an anatomically and functionally distinct area previously noted in the hamster SCN by other laboratories under various circumstances. It was first described as a site where photically-induced c-Fos was *not* blocked by NMDA (Abe et al., 1991; Ebling et al., 1991; Rea et al., 1993; Vuillez et al., 1998; Guido et al., 1999a) or non-NMDA (Abe et al., 1992; Vindlacheruvu et al., 1992; Rea et al., 1993) glutamatergic receptor antagonists that were effective elsewhere in the SCN. This region may be innervated predominantly by ganglion cells from the contralateral retina (Muscat et al., 2003) that may also project to the intergeniculate leaflet (Pickard, 1985; Treep et al., 1995; Morin et al., 2003). It is the site of a cluster of cells expressing the phosphorylated MAP kinases ERK1 and ERK2 during subjective night (Lee et al., 2003); like c-Fos, pERK1/2 expression in this region is resistant to treatment with the NMDA receptor blocker MK-801 administered before a light pulse (Coogan and Piggins, 2003). Antle et al. (2005) have activated pERK cells in this region by intraventricular

injections of gastrin-releasing peptide (GRP), naming them “cap” cells and suggesting that GRP may link them to light-responsive cells in the underlying calbindin-rich zone. We suspect that this photo-responsive, NMDA and non-NMDA antagonist-insensitive, GRP-sensitive, pERK-expressing central-dorsolateral region coincides with the c-Fos- and PER1-expressing region that we have observed on the subjective-night side of the split SCN.* Apparently, some degree of night-like photosensitivity persists in the split SCN, is putatively nonglutamatergic, appears independent of c-Fos expression in calbindin cells, and does not preclude maintenance of the split state.

With regard to c-Fos expression outside the split SCN, visual inspection revealed no gross side-to-side differences in multiple brain areas already known to be innervated by SCN efferents, such as the lateral septum, paraventricular and dorsomedial hypothalamic nuclei, paraventricular thalamic nucleus, and intergeniculate leaflet (Kalsbeek et al., 1993; Morin et al., 1994; Kriegsfeld et al., 2004). While detailed quantification might disclose some subtle left-right asymmetries in these regions, there are more tangible reasons why our c-Fos maps would not correspond to anatomical tract-tracing data. In particular, asymmetries might be revealed only at certain phases and only within functionally or morphologically identified neuronal subsets. In fact, de la Iglesia et al. (2003) have demonstrated a marked left-right asymmetry of c-Fos expression in the septal/preoptic area, but only within labeled luteinizing hormone releasing hormone-containing neurons in ovariectomized and estrogen-treated split females, and only when the animals were killed 1 h before locomotor activity onset. Of course, some of the SCN's targets, even when excited via ipsilateral connections, may not express c-Fos. Perhaps some of these “silent” neurons elaborate transcriptional regulatory proteins of different classes; for example, in long-term potentiation, *zif/268*, of the class defined by the “zinc finger” motif, appears to be more reliably induced than *c-fos* (Cole et al., 1989; Wisden et al., 1990).

Notwithstanding these limitations, we did observe a clear side-to-side asymmetry of c-Fos expression in 2 nearby periventricular regions that receive dense, mainly unilateral SCN projections. The MPN, involved in sexual and parenting behaviors when animals are awake, exhibits relatively higher c-Fos cell counts ipsilateral to the split SCN's subjective-night side, whereas the SPZ shows the opposite pattern, with higher c-Fos levels ipsilateral to the split SCN's subjective-day side. In normal, unsplit rats, the SPZ exhibits a daily rhythm of c-Fos expression that mirrors the rhythm in the SCN; levels are high soon after lights-on and decline over the 1st half of the light phase (Nunez et al., 1999). Unlike in the SCN, however, the SPZ rhythm appears to be lost after animals are held for a few weeks in constant darkness (Schwartz et al., 2004).

We also observed a remarkable and unexpected asymmetry of c-Fos expression in the LHbM of split animals, but only during their active phase (when they were running in wheels). High LHbM c-Fos levels are ipsilateral to the split SCN's subjective-night side, just as high levels are present in normal, unsplit hamsters during subjective night in constant darkness.

The function(s) of the habenular complex remain elusive. Limbic and striatal efferents appear to converge in the lateral habenula, which then projects mainly to the raphe nuclei, ventral tegmental area and substantia nigra, central gray, and lateral preoptic/hypothalamic regions; thus, the nucleus has been hypothesized to “play a prominent role in the control of movements by motivational processes” (Sutherland, 1982, p 2). In LHbM, c-Fos expression is activated by various forms of stress (Chastrette et al., 1991; Wirtshafter et al., 1994). While the lateral habenula is intimately associated with the pineal gland in lower vertebrates, it has not been

*We were unable to confirm a c-Fos/pERK coexpression in our material, as the immunohistochemical pERK signal was undetectable in the SCN of split hamsters in constant light (M.T.-N. and W.J.S., personal observations).

considered to be part of the circadian system in mammals. Nevertheless, there are anatomical tract-tracing studies that have suggested a putative SCN-habenula connection in rodents. The lateral habenula appears to be sparsely innervated by the SCN in the hamster using anterograde tracing with *Phaseolus vulgaris* leucoagglutinin (Kalsbeek et al., 1993; Morin et al., 1994), although a direct projection was not confirmed in the rat using retrograde transport of true blue (Watts and Swanson, 1987). Fine-caliber vasopressinergic fibers believed to originate from the SCN are present in the LHbM in the rat, mouse, and guinea pig (Sofroniew and Weindl, 1978), although SCN lesions in the rat failed to eliminate the fiber staining (Hoorneman and Buijs, 1982). In the rat, the SCN and LHbM are both innervated by at least 1 common structure, the nucleus incertus (Olucha-Bordonau et al., 2003). Recently, electrophysiological studies of the rat lateral habenula have revealed populations of photoreponsive and rhythmic cells in vivo; a circadian rhythm of firing rate in habenula slices persists for at least 2 cycles in vitro (Zhao and Rusak, 2005). The functional relationship between oscillators in the SCN and habenula is unknown. Interestingly, the Syrian hamster LHbM specifically expresses high-affinity melatonin receptors (Weaver et al., 1989), the function of which is not understood.

Locomotor activity rhythms are not driven by a unitary circadian output mechanism (Davis and Menaker, 1980; Vogelbaum and Menaker, 1992). In a model proposed by Davis and Menaker (1980) for hamsters, there is a “window” oscillator output that determines a time for activity (subjective night) and a time for rest (subjective day), interacting with a “bout” oscillator output that controls the expression of locomotion per se. We wonder if 1 of these outputs might be diffusable (Kramer et al., 2001; Cheng et al., 2002) and speculate that the other might be neural, with lateralized axonal projections involving the LHbM. For the future, split hamsters should be a valuable model for dissecting the neural substrates of multimodal SCN outputs and their regulation of behavioral state.

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Figure 1.

Immunohistochemical c-Fos protein expression in rostral (top row), middle (center row), and caudal (bottom row) coronal sections of the SCN from individual representative hamsters: behaviorally split in LL and killed during the locomotor-inactive phase (left column), unsplit in constant darkness and killed during subjective day or subjective night (middle columns), or unsplit in constant darkness and killed 60 min after the onset of a midsubjective-night light pulse (right column). Arrows on the split SCN show that c-Fos expression in the right half-SCN appears similar to that observed in the unsplit hamster during subjective day in constant darkness, while expression in the left half-SCN is low except for a circumscribed region centro-dorsolaterally in the caudal SCN.

Figure 2.

(A) Immunohistochemical c-Fos and PER1 protein expression in rostral, middle, and caudal coronal sections of the SCN from a representative split hamster. Individual sections are processed for double-label immunofluorescence, and each is viewed using excitation wavelengths of 568 nm (red, for c-Fos) and 488 nm (green, for PER1). (B) Immunohistochemical c-Fos and PER1 protein expression in the central-dorsolateral region in the caudal SCN of a split hamster; c-Fos (red) and PER1 (green) immunoreactivities are colocalized in some cell nuclei (yellow). (C) Immunohistochemical c-Fos and calbindin protein expression in a caudal coronal section of the SCN from a representative split hamster. Double-label immunofluorescence is viewed using excitation wavelengths of 568 nm (red, for c-Fos) and 488 nm (green, for calbindin).

Figure 3.

Asymmetric immunohistochemical c-Fos protein expression in coronal sections from a representative split hamster killed during the locomotor inactive phase. High c-Fos levels in the MPN (arrow, B) are on the side contralateral to high c-Fos levels in the SCN (arrow, A), whereas high levels in the SPZ (arrow, C) are on the side ipsilateral to high levels in the SCN.

A. Split in Constant Light

B. Constant Darkness

Figure 4.

(A) Asymmetric immunohistochemical c-Fos protein expression in coronal sections from a representative split hamster killed during the locomotor active phase. High c-Fos levels in the lateral habenula (arrow, bottom) are on the side contralateral to high c-Fos levels in the SCN (arrow, top). (B) Immunohistochemical c-Fos protein expression in coronal sections from representative normal, unsplit hamsters in constant darkness and killed during subjective day (top) or subjective night (bottom).