Published in final edited form as:

Eur J Neurosci. 2011 August ; 34(3): 478–488. doi:10.1111/j.1460-9568.2011.07762.x.

A role for the habenula in the regulation of locomotor activity cycles

Matthew J. Paul, Premananda Indic, and William J. Schwartz

Department of Neurology, University of Massachusetts Medical School, Worcester, MA 01655, USA

Abstract

While much is known about the regulation of the circadian rest-activity cycle by the hypothalamic suprachiasmatic nucleus in nocturnal rodents, little is known about the neural substrates that regulate the temporal organization of nocturnal activity within the active phase. In this paper, data are presented in Syrian hamsters to implicate the habenula – believed to be involved in motivation, reward, and motor control – as a candidate site for such a role. First, by examining hamsters during the day and night and by introducing a “novel” running wheel in order to induce daytime motor activity, we show that immunoreactive c-Fos expression in the lateral and medial habenula is related to motor activity / arousal. Second, by transecting the habenula’s major efferent pathway (fasciculus retroflexus), we show that the interruption of habenula neural output alters the daily amount of motor activity; lengthens the period of the circadian rest-activity rhythm; and disrupts the species-typical pattern of nocturnal motor activity, measured as either wheel running behavior or general locomotor activity. Instead of the usual pattern of nighttime locomotion, characterized by a prolonged bout of elevated activity in the early night followed by shorter sporadic bouts or the cessation of activity altogether, lesioned animals exhibited a more homogeneous, undifferentiated temporal profile extending across the night. These data suggest a previously unrecognized function of the habenula whereby it regulates the temporal pattern of activity occurring within a circadian rest-activity window set by the suprachiasmatic nucleus.

Keywords

Syrian hamster; c-Fos; circadian rest-activity cycle; wheel running; fasciculus retroflexus

Introduction

The habenula is a periventricular epithalamic structure in the dorso-caudal diencephalon composed of paired medial (MHb) and lateral (LHb) nuclei, with connections that suggest a role as an integration and relay center linking the forebrain limbic system and basal ganglia with the midbrain and hindbrain (Sutherland, 1982). Most habenular afferents arise in the

Address Correspondence to: *Matthew J. Paul, Department of Psychology, University of Massachusetts Amherst, 135 Hicks Way, Amherst, MA 01003, Fax: 413-545-5975, matthew.paul@psych.umass.edu.
*current address

The contents of this report are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

forebrain, coalesce at the rostral border of the thalamus to form the stria medullaris, and travel along the dorsal thalamus to reach the habenula (Herkenham & Nauta, 1977). Habenular efferents, in turn, traverse almost exclusively through the fasciculus retroflexus (FR) to brainstem interpeduncular and monoaminergic nuclei, some of which project back to the habenula via this same fiber bundle (Herkenham & Nauta, 1979; Omelchenko *et al.*, 2009). Consistent with this connectivity, the habenula has been implicated in the regulation of behaviors related to emotion, motivation, behavioral state, and motor activity, including maternal and sex behavior, learning and memory, and reward-based motor control (Sutherland, 1982; Klemm, 2004; Lecourtier & Kelly, 2007; Hikosaka *et al.*, 2008). Destruction of the habenula or its efferents in the FR increases endocrine and behavioral measures of stress (Lee & Huang, 1988; Murray *et al.*, 1994; Murphy *et al.*, 1996), disrupts sleep by shortening sleep bouts and decreasing total REM sleep (Haun *et al.*, 1992; Valjakka *et al.*, 1998), and induces hyperactivity (Nielson & McIver, 1966; Lee & Huang, 1988; Thornton *et al.*, 1994; Murphy *et al.*, 1996).

Each of these functions exhibits highly structured temporal organization on both circadian and ultradian time scales (Gerkema & Daan, 1985; Lavie, 1985; Silver & Lesauter, 2008). Whether the habenula plays a role in their timing is not known, but recent evidence raises this possibility. The rodent LHb exhibits circadian rhythms of gene expression *in vitro* (Guinding *et al.*, 2010) and *in vivo* (Tavakoli-Nezhad & Schwartz, 2006) as well as *in vitro* neuronal discharge activity (Zhao & Rusak, 2005). Neuroanatomical tract-tracing studies have indicated bi-directional, likely multi-synaptic, connections between the habenula and the hypothalamic suprachiasmatic nucleus (SCN), site of the master circadian pacemaker (Kalsbeek *et al.*, 1993; Morin *et al.*, 1994; Krout *et al.*, 2002). Of note, serotonergic function in the median and dorsal raphe is regulated by the habenula (Wang & Aghajanian, 1977; Nishikawa & Scatton, 1985; Kalén *et al.*, 1989), and serotonin-containing fibers from the median and dorsal nuclei project to the SCN and thalamic intergeniculate leaflet, respectively (Meyer-Bernstein & Morin, 1996); the latter regulates photic and non-photoc inputs to the SCN (Harrington, 1997). Few studies have investigated a potential role for the habenula at non-circadian time scales (Valjakka *et al.*, 1998).

In this paper, we present findings that implicate the habenula in the regulation of activity cycles, measured in groups of Syrian hamsters as either wheel running behavior (WR) or general locomotor activity (GLA). We determined the effects of inducing daytime motor activity on habenula c-Fos expression by introducing hamsters to “novel” running wheels, and we analyzed the activity profiles of hamsters with disrupted habenular output by transecting the FR (FRx) using microknives. Our data show that the normal timing and amount of nocturnal motor activity depends upon the integrity of the FR.

Materials and Methods

Animals and housing conditions

Male Syrian hamsters (*Mesocricetus auratus*, HsdHan:AURA) were purchased from Harlan Laboratories (Indianapolis, IN) and housed singly in clear polypropylene cages (19" × 10.5" × 9") contained within well-ventilated, light-proof environmental cabinets with ambient temperature and relative humidity maintained at 23 ± 3°C and 40 ± 20%, respectively.

Hamsters were provided with food and tap water *ad libitum* and kept under a 14:10 h light:dark cycle (lights off at 1800 EST), unless otherwise noted. All procedures were approved by the University of Massachusetts Medical School Institutional Animal Care and Use Committee.

Experimental design

Experiment 1, Novel wheel running and c-Fos activation in the habenula—Four hours before the scheduled onset of darkness (zeitgeber time (ZT) 8; ZT12 = dark onset of 14:10 h light:dark cycle), lights were turned off manually, and hamsters, aged 13 – 15 wks, were placed either in an unlocked novel wheel (Unlocked Wheel – Day; n=12) or in a locked novel wheel that would not rotate (Locked Wheel; n=8). Controls remained in their home cage, also in the dark, but without a running wheel (No Wheel; n=4). One to two hours later, animals were sacrificed and their brains processed for c-Fos immunohistochemistry. For day/night comparisons, a separate set of hamsters, aged 27–28 wks, with free access to a running wheel in their home cage were sacrificed ~1 h after onset of their nighttime wheel running under the 14:10 h light:dark cycle (ZT13.5 – ZT14.5; Unlocked Wheel – Night; n=6). Because experimental procedures were not conducted concurrently, statistical comparisons were not made between this set and the groups sacrificed during the day.

Experiment 2, Wheel running behavior (Exp. 2a) and general locomotor activity (Exp. 2b) after transection of the fasciculus retroflexus—Hamsters in Exp. 2a were housed with free access to a running wheel placed inside their home cage, and WR was recorded continuously. In Exp. 2b, GLA was continuously recorded by passive infrared detectors suspended above the home cage; these hamsters did not have a running wheel in their home cage. At 10 – 11 wks of age, hamsters underwent FR transections (FRx; n = 9 and 6 for Exp. 2a and 2b, respectively) or control surgery (Control; n = 3 and 6 for Exp. 2a and 2b, respectively). The light:dark cycle was changed to constant darkness (DD) ~2.5 – 4 wks after surgery and maintained for 2 – 4 wks to assess free-running rhythms of WR and GLA. Baseline and post-surgical behavioral measures were calculated for the final 10 days prior to surgery and transfer to DD, respectively. The mean phase of nighttime WR and GLA, weighted for the amount of activity over time (center of gravity, in hours after activity onset), was computed for each animal by determining the mean activity in each 15-min nighttime bin over the 10 day interval, determining the time average of all bin means

over the night, and calculating the center of gravity as $\frac{\int tA(t)dt}{\int A(t)dt}$, where t = time in hours, A = activity, and $A(t)$ = activity as a function of time (Kreyszig, 1993). Each animal's endogenous free-running circadian period (tau, τ) was determined by drawing an eye-fitted regression line over activity onsets spanning the entire DD interval using the τ cursor function in the Actiwatch program (Mini-Mitter Co., Inc., Oregon). At the end of the experiment, hamsters were sacrificed and brains were processed for the immunohistochemical detection of glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) by light-microscopic double immunofluorescence, in order to assess the location of the knife cuts (GFAP) and to confirm degradation of the FR pathway (MBP).

Experiment 3, Novel wheel running after fasciculus retroflexus transections—

Hamsters from Exp. 2b were returned to the original 14:10 h light:dark cycle for 2 wks to allow sufficient time for re-entrainment. Following re-entrainment, the light cycle was changed back to DD by the extension of the dark phase. On either the second or third day in DD, hamsters were placed in a novel wheel for 3 h (10:00 – 13:00 h; projected ZT4-7) and then returned to their home cage. Hamsters were maintained in DD for a further 10 days to assess potential phase shifts in GLA. Phase shifts were calculated as the difference between the actual onset of GLA on the day prior to novel wheel exposure and the predicted onset, calculated by backward extrapolation of an eye-fitted regression line drawn over post-novel wheel activity onsets; positive and negative values represent phase advances and delays, respectively. Calculating phase shifts of GLA is difficult, and thus we conducted this analysis twice, with several months between each analysis. A mean phase shift was calculated for each hamster and used for statistical analyses.

Spontaneous motor activity

For WR, cages were equipped with a 7" diameter metal running wheel, the rotation of which activated a microswitch on the outside of the cage. For GLA, passive infrared motion detectors were suspended above the animal's home cage; movement across any 1 of 27 zones deflected an infrared beam, which activated a closed-contact relay. The number of wheel revolutions (for WR) or infrared deflections (for GLA) was tallied, binned every 15 min, and stored on a hard disk using the DataQuest software acquisition system (Mini Mitter, Sunriver, OR).

For novel wheel running, hamsters with no prior wheel running experience were transferred to running wheels. These 'novel' wheels were similar to those described above (7" diameter, metal) except that (a) plastic mesh lined the outer rungs to facilitate running and (b) plastic walls enclosed the novel wheels to restrict the animal to the wheel.

Immunohistochemistry

Animals were deeply anesthetized with a 0.7 ml ip injection of ketamine (100 mg/ml) and xylazine (10 mg/ml) solution and perfused intracardially with heparinized Dulbecco's Phosphate Buffered Saline followed by 4% paraformaldehyde. For hamsters sacrificed in the dark (Exp. 1), anesthesia was administered under dim red light, and once the animal was anesthetized, the head was covered with aluminum foil until the end of paraformaldehyde perfusion. Brains were removed and immersed in fixative overnight at 4°C. The following day, 40 µm sections were cut on a vibratome and stored at -20°C in cryoprotectant until staining. For c-Fos staining on free-floating sections, unreacted aldehydes were first blocked with 1% NaBH₄ (S9125; Sigma-Aldrich, St. Louis, MO), after which sections were incubated at 4°C overnight with a rabbit polyclonal c-Fos antibody (1:2,000; SC52; Santa Cruz Biotech Inc., Santa Cruz, CA) and treated with biotinylated anti-rabbit secondary antibody (BA-1000; Vector Laboratories, Burlingame, CA) and the avidin-biotin method (Vectastain Elite ABC Kit; PK-6100; Vector Labs) with 3,3'-diaminobenzidine tetrahydrochloride (DAB; D5637; Sigma-Aldrich) as the chromagen. For GFAP and MBP immunofluorescence, free-floating sections were blocked in 10% normal goat serum (S-1000; Vector Labs), incubated in the primary antibodies overnight at 4°C (rabbit

polyclonal GFAP antibody, 10555, MP Biomedicals, Solon, OH; mouse monoclonal MBP antibody, SMI-99P, Sternberger Monoclonals Inc., Lutherville, MD; 1:1,000 for both antibodies), and then incubated in Alexa Fluor 488 goat anti-rabbit (for GFAP) and Alexa Fluor 594 goat anti-mouse (for MBP) secondary antibodies (1:200; Molecular Probes, Eugene, OR) for 2h at room temperature in the dark. Between steps, sections were washed several times in either potassium phosphate buffered saline (for c-Fos staining) or Tris buffered saline with 2% normal goat serum and 0.4% Triton X-100 (for GFAP and MBP staining). Sections were mounted on slides, dehydrated, and coverslipped.

For c-Fos quantification, alternate sections through the habenula (from plate 25 – 29 in Morin & Wood, 2001) were visualized using a Leica DM IRB microscope (Leica Microsystems, Deerfield, IL). Stained cell nuclei in the MHb and LHb labeled above background were counted without knowledge of group assignments; the borders of the MHb and LHb were determined by differences in background DAB staining (see Figure 1A). Cell counts were averaged across sections (from 11 – 15 sections/animal) and expressed as the mean number of c-Fos-labeled cells/section.

Fasciculus retroflexus transections and control surgeries

An L-shaped microsurgical knife was custom made by clipping a spinal needle (22G x 3.5 inches; Monoject, St. Louis, MO) to 5.0 cm in length. The metal stylet, inserted through the needle, was bent at the top and bottom to form the handle and blade, respectively. The blade was then sanded to a length of 1.3mm and sharpened. At surgery, hamsters were anesthetized with ketamine (100mg/kg) and xylazine (10 mg/kg) ip and placed in a stereotaxic instrument with the surgical site shaved and cleaned with Betadine followed by saline. The dorsal aspect of the parietal bones was exposed, and two parasagittal holes were drilled with a dremel tool. The knife was lowered just ventral to the habenula and aimed at the FR on each side (coordinates: 0.9 mm posterior to bregma, 4.4 mm ventral to dura, 1.4 mm lateral to midline, skull flat), with the blade facing caudally. In FRx animals, the knife was rotated medially 180° to intersect the FR; in Control animals, the knife was either not rotated (in Exp. 2A) or rotated laterally 180° (in Exp. 2B). The skin incision was apposed with wound clips and treated with antibiotic ointment (Neomycin & Polymyxin B Sulfates & Bacitracin Zinc Ointment USP). Buprenorphine (0.05 mg/kg) was administered as a post-operative analgesic.

Food intake and body mass

Mean daily food intake (FI) was calculated for FRx and Control animals by weighing food placed atop the animal's cage every 3 – 5 days and dividing by the number of days between measures. To reduce inter-individual variability in the measures of FI and body mass (BM), these values were converted to % baseline for each animal. Baseline values were recorded over a two week interval immediately prior to surgery (FI) or at surgery (BM).

Statistical Analyses

Effects of wheel access on c-Fos expression in the LHb and MHb were determined using one-way ANOVA. Effects of surgery over time on BM, FI and activity (WR and GLA; daily means, % activity in the second half of the night, and center of gravity) were assessed using

repeated measures ANOVA. Differences in the baseline BM, baseline FI, τ , number of novel wheel (NW) revolutions, and magnitude of NW phase shifts of Control and FRx animals were determined using two-tailed, unpaired t-Tests. The magnitude of NW phase shifts was also analyzed using ANCOVA with the number of NW revolutions included as a covariate. Data were analyzed using Statview 5.0.1 (SAS Institute Inc., Cary, NC). Where appropriate, post-hoc comparisons were made using the Fisher's PLSD test, except in Figure 6, for which the Scheffe's test was used for the large number of comparisons. Nonetheless, results in Figure 6 were identical whether using the Scheffe's test or no post-hoc correction. Significance was assumed if $P < 0.05$.

Results

Novel wheel running and c-Fos expression in the habenula

Tavakoli-Nezhad and Schwartz (2006) have previously reported a rhythm of LHb immunoreactive c-Fos expression in both a light:dark cycle and DD, with high levels during the dark phase and subjective night, respectively. This rhythm was present whether or not hamsters were provided with a running wheel. Elevated LHb c-Fos expression could be a feature of nighttime circadian phase or instead due to the heightened arousal and locomotion normally associated with this phase. To determine whether activity *per se* triggers c-Fos expression in the LHb, we induced Syrian hamsters to run in the dark during their normally inactive phase (daytime) by the provision of a novel running wheel, which is known to trigger WR in hamsters at this time of day (Bobrzynska & Mrosovsky, 1998). We compared postmortem c-Fos immunoreactivity in the LHb and MHb of these hamsters (Unlocked Wheel – Day) to control groups either placed in a locked wheel that prevented WR (Locked Wheel) or left in their home cage without access to a wheel (No Wheel).

Behavioral observations revealed that Unlocked Wheel – Day hamsters generally ran throughout their time in the running wheel, whereas Locked Wheel hamsters tended to sleep in the wheel after an initial interval of investigating the wheel (~15 – 30 min); No Wheel hamsters generally spent this entire interval sleeping. Examples of c-Fos immunoreactivity in the LHb and MHb are illustrated in Figure 1A–D. Unlocked Wheel – Day hamsters had increased numbers of c-Fos-immunoreactive (c-Fos-ir) cells in the LHb compared to Locked Wheel and No Wheel hamsters (Figure 1E; ANOVA $F(2,21) = 4.5$, $P = 0.02$; Fisher's PLSD, P values < 0.05); the number of c-Fos-ir cells in Locked Wheel and No Wheel hamsters did not differ (Fisher's PLSD, $P = 0.46$). The same pattern of significance among these groups was also true for c-Fos immunoreactivity in the MHb (Figure 1F; ANOVA $F(2,21) = 9.2$, $P < 0.01$; followed by Fisher's PLSD).

WR and c-Fos immunoreactivity of Unlocked Wheel – Day hamsters were compared to a separate cohort of hamsters from a separate experiment (see Materials and Methods) provided with free access to a running wheel in their home cage and sacrificed in the early night (Unlocked Wheel – Night). Despite similar levels of wheel running before sacrifice (Figure 1G), Unlocked Wheel – Night hamsters exhibited more than twice the number of LHb c-Fos-ir cells than Unlocked Wheel – Day hamsters (Figure 1E); in the MHb, however, the number of c-Fos-ir cells was minimal and similar to that of No Wheel hamsters (Figure 1F). c-Fos immunoreactivity in the LHb of both Unlocked Wheel – Day and Unlocked

Wheel – Night hamsters was largely restricted to the medial division of the LHb (Figure 2). Slight differences in the spatial distribution of c-Fos-ir cells may exist between these two groups, sp., a slightly more ventral pattern in the Unlocked Wheel – Day hamsters, but the bulk of c-Fos immunoreactivity overlaps. In the MHb of Unlocked Wheel – Day hamsters, c-Fos-ir cells were mostly found in the dorsal third of the nucleus and along the LHb – MHb border (Figure 2A).

Transecting the fasciculus retroflexus

Results of experiment 1 demonstrate that daytime wheel running induces c-Fos expression in both the LHb and MHb. These findings suggest that increased activity/arousal contributes to the day-night rhythm of c-Fos expression in the LHb and link habenula activity with motor activity. While several studies have demonstrated altered behavioral activity (hyperactivity) after habenula or FR lesions and others have hinted at a connection to the circadian system, no study has tested whether the habenula regulates the temporal organization of motor activity. Previous studies demonstrating FR lesion-induced hyperactivity used short-duration (3 – 24 min) exploratory tests conducted during the light phase that are not sufficient to assess the temporal profile of spontaneous motor activity (Nielson & McIver, 1966; Lee & Huang, 1988; Thornton *et al.*, 1994; Murphy *et al.*, 1996). Toward this end, we continuously monitored two forms of spontaneous motor activity, WR and GLA, for several weeks in hamsters receiving FRx or Control surgeries.

Transecting the fasciculus retroflexus: Histology and general measures of health

Histological examination of postmortem tissue revealed that 14 of 15 FRx hamsters sustained bilateral damage to the FR as assessed by 1) GFAP staining, demarcating the knife cut, intersecting the projected location of the FR and 2) decreased MBP staining of the FR in sections ventral and posterior to the center of the cut (Figure 3). One hamster received only unilateral damage and was therefore excluded from all further analyses. In all but one of these 14 successful surgeries, MBP staining of the FR was negligible bilaterally, suggesting complete degradation of the pathway. In the remaining hamster, the loss of FR MBP staining was complete on the right, but partial on the left. This hamster was included in all analyses. Damage from the knife cut in FRx hamsters extended rostro-caudally from the anterior habenula (corresponding to plates 24–26 in Morin & Wood, 2001) to the posterior commissure (plates 32–33), with the center of the knife cut positioned at the level of the posterior habenula (plates 29–30). At the center, the cut transected tissue immediately ventral to the habenula where the FR buds off; damage extended from just lateral to the LHb to nearly the midline. No damage to the FR was detected in the Control surgeries. In Control hamsters with the knife turned laterally, GFAP staining surrounding the knife cut was observed in the posterior dorsal thalamus (Figure 3).

All hamsters, FRx and Control, recovered well from surgery and appeared healthy throughout the study. Both FRx and Control hamsters gained weight following surgery (repeated measures ANOVA main effects of time WR: $F(2,20) = 194.5$, $P < 0.01$; GLA: $F(3,27) = 86.5$, $P < 0.01$), with FRx hamsters gaining more weight than Controls (main effect of surgery) whether provided with a running wheel (Figure 4A; $F(1,10) = 16.3$, $P < 0.01$) or not (Figure 4B; $F(1,9) = 7.3$, $P = 0.02$); pre-surgical BM did not differ between

groups (mean pre-surgical BM $\pm$ SEM = 121.5 $\pm$ 2.9g [FRx] and 116.3 $\pm$ 8.9g [Control], t-Test, $t(10) = 0.77$, $P = 0.46$, for hamsters with a wheel, and 122.3 $\pm$ 5.7g [FRx] and 121.1 $\pm$ 6.8g [Control], t-Test, $t(9) = 0.13$, $P = 0.90$, for hamsters without a wheel). At least part of the increased weight gain in FRx hamsters was likely due to increased FI, as FRx hamsters without a running wheel increased their post-surgical FI to a greater degree than Control hamsters (Figure 4C; repeated measures ANOVA main effect of surgery, $F(1,9) = 7.8$, $P = 0.02$; FI measures not recorded for hamsters provided with a running wheel). Here too, pre-surgical measures did not differ between groups (mean pre-surgical FI $\pm$ SEM = 7.9 $\pm$ 0.5g [FRx] and 8.1 $\pm$ 0.4g [Control], t-Test, $t(9) = 0.29$, $P = 0.78$).

Transecting the fasciculus retroflexus: Wheel running behavior and general locomotor activity

Inspection of activity records revealed striking changes in WR and GLA after FRx (Figure 5A-L). For hamsters provided with a running wheel, FRx substantially decreased the overall amount of WR. In an extreme case, one hamster displayed little to no WR for the first 2 wk after surgery and subsequently stopped running in the wheel altogether. Despite decreases in overall levels, the duration of nighttime WR was expanded after FRx: while WR of Control hamsters was largely restricted to the first half of the night, WR of FRx hamsters occurred almost homogeneously throughout the nighttime hours. For hamsters not provided a running wheel, GLA increased rather than decreased after FRx, but the temporal organization of nighttime GLA resembled that of WR. The majority of nighttime GLA of Control hamsters occurred in a continuous activity bout during the first half of the night, which was followed by periodic activity bouts in the second half of the night. In FRx hamsters, this periodic activity was absent, and as seen for WR, GLA occurred more homogeneously across the night.

Quantitative analyses confirmed these qualitative assessments of activity records. FRx decreased mean daily WR by 79% compared to Controls, but increased mean daily GLA by 63% (Figure 5M&P; repeated measures ANOVA for WR: main effect of surgery $F(1,9) = 6.0$, $P = 0.04$; main effect of time $F(1,9) = 23.7$, $P < 0.01$; interaction $F(1,9) = 5.7$, $P = 0.04$; for GLA: main effect of surgery $F(1,9) = 2.6$, $P = 0.14$; main effect of time $F(1,9) = 10.5$, $P = 0.01$; interaction $F(1,9) = 10.8$, $P = 0.01$; mean daily WR and GLA after surgery, FRx versus their respective controls, Fisher's PLSD, P values < 0.05). Because even Control hamsters do not run in the wheel during the day, decreased WR in FRx hamsters was exclusively the result of decreased nighttime WR, which also decreased by 79% (t-Test, $t(9) = 6.0$, $P < 0.01$). Increased GLA of FRx hamsters, however, was due to increases in both daytime (75%; t-Test, $t(9) = 2.5$, $P = 0.03$) and nighttime (56%; t-Test, $t(9) = 2.4$, $P = 0.04$) GLA. The increase in nighttime GLA was due, in part, to a decrease in periods of inactivity. Figure 6 illustrates the number of 15 min nighttime GLA bins with increasing amounts of activity (# of infrared deflections) that occurred during the 15 min interval. The number of inactive bins (infrared deflections = 0) was high in both Control and FRx hamsters before surgery, and there were no differences between groups (Scheffe's post hoc correction, $P > 0.05$ for all comparisons). After surgery, however, the number of these inactive bins dropped dramatically in FRx hamsters (Scheffe's post hoc correction, $P < 0.05$ for bins with 0 and 1–10 infrared deflections, $P > 0.05$ for bins of all other activity levels).

Consistent with a post-surgical switch to a more homogeneous temporal organization of activity, the center of gravity of nighttime WR and GLA in FRx hamsters shifted toward the midpoint of the night (repeated measures ANOVA for WR: main effect of surgery $F(1,9) = 3.4$, $P = 0.10$; main effect of time $F(1,9) = 10.9$, $P = 0.01$; interaction $F(1,9) = 9.5$, $P = 0.01$; for GLA: main effect of surgery $F(1,9) = 11.9$, $P < 0.01$; main effect of time $F(1,9) = 5.7$, $P = 0.04$; interaction $F(1,9) = 5.9$, $P = 0.04$). The center of gravity of nighttime WR was 3.3 h into the 10 h night for Control hamsters both before and after surgery. In FRx animals, however, the nighttime WR center of gravity shifted from 3.0 h to 4.3 h into the night (Figure 5N). Similarly, Control values for nighttime GLA center of gravity were maintained around 4.1 h before and after surgery, but FRx shifted the center of gravity from 4.0 to 4.6 h into the night (Figure 5Q). Consistent with this shift in center of gravity, there was an increase in the percent of activity occurring in the second half of the night (from 12 to 36% for WR and from 35 to 43% for GLA; Figure 5O&R; repeated measures ANOVA for WR: main effect of surgery $F(1,9) = 9.3$, $P = 0.01$; main effect of time $F(1,9) = 14.6$, $P < 0.01$; interaction $F(1,9) = 15.3$, $P < 0.01$; for GLA: main effect of surgery $F(1,9) = 7.3$, $P = 0.02$; main effect of time $F(1,9) = 8.8$, $P = 0.02$; interaction $F(1,9) = 5.6$, $P = 0.04$).

Transecting the fasciculus retroflexus: Circadian timing

To assess the status of the circadian pacemaker, hamsters were placed in DD. Robust WR and GLA rhythms were observed in both Control and FRx hamsters, but the period of these rhythms (τ) was longer in FRx hamsters (WR mean $\pm$ SEM $\tau = 24.23 \pm 0.03$ h versus 24.16 ± 0.16 h for FRx and Control, respectively; GLA mean $\pm$ SEM $\tau = 24.24 \pm 0.05$ h versus 24.10 ± 0.04 h for FRx and Control, respectively). This difference was significant for GLA (t-Test, $t(9) = 2.4$, $P = 0.04$), but not for WR (t-Test, $t(9)$, $P = 0.49$), because one Control hamster in the WR experiment displayed an abnormally long τ (24.47 h).

Transecting the fasciculus retroflexus: Novel wheel running-induced phase advances

The daily light:dark cycle is the predominant environmental time cue that synchronizes an animal's endogenous free-running rhythm to the 24-h day, a process known as entrainment (Morin & Allen, 2006). Nonetheless, certain non-photoc manipulations that increase arousal (e.g. social interactions, cage changes, saline injections, and novel wheel running), can also influence the circadian pacemaker (Mrosovsky, 1988; 1996; Mistlberger & Skene, 2004; Paul & Schwartz, 2007). These findings are thought to reflect behavioral feedback mechanisms on the SCN by which non-photoc environmental factors fine-tune circadian entrainment to the day-night cycle (Janik *et al.*, 1994; Sinclair & Mistlberger, 1997; Mistlberger & Holmes, 2000). Daytime novel wheel running phase advances locomotor activity rhythms in Syrian hamsters (Bobrzynska & Mrosovsky, 1998). Given the postulated role of the habenula in the regulation of arousal, as well as our present findings of novel wheel-induced c-Fos expression in the MHb and LHb and decreased wheel running after FRx, we tested whether or not FRx would disrupt the behavioral feedback of novel wheel running or its downstream neuronal pathways that influence the SCN.

Hamsters from exp. 2b were re-entrained to the original 14:10 h light:dark cycle for 2 weeks, then transferred back to DD, and placed in a novel running wheel from projected ZT4 – ZT7 on the second or third day of DD. Subsequent phase shifts of GLA in DD were

measured for each hamster. Control hamsters displayed the expected phase advances in GLA, but FRx hamsters did not (Figure 7). Control hamsters ran more vigorously than FRx hamsters in the novel wheel, although this difference fell short of significance (Figure 7B; t-Test, $t(9) = 1.9$, $P = 0.9$). When the number of wheel revolutions was entered as a covariate, the difference in phase shifts between Control and FRx hamsters was no longer significant (effect of surgery ANCOVA $F(1,7) = 0.04$, $P = 0.84$).

Discussion

The habenula has been implicated in the regulation of motivation and locomotion. In addition, it contains an endogenous circadian oscillator with ~24 h rhythms in electrical activity (Zhao & Rusak, 2005) and gene expression (Tavakoli-Nezhad & Schwartz, 2006; Guilding *et al.*, 2010). Our results link the cellular/molecular rhythms with behavioral rhythms and implicate a role for the habenula in the regulation of locomotor activity cycles. We show that running in a wheel during the day induces c-Fos activation in both the LHb and MHb and that interruption of the FR alters the daily amount and reduces the nighttime temporal organization of WR and GLA, as well as lengthens the period of the circadian rest-activity rhythm.

The results of the our first experiment suggest that locomotor activity/arousal contributes to the previously reported (Tavakoli-Nezhad & Schwartz, 2006) day-night rhythm of c-Fos expression in the medial division of the LHb of the hamster, a night-active rodent. Hamsters induced to run in a novel running wheel during the day displayed increased numbers of c-Fos-ir cells in the medial division of the LHb, as compared to animals in a locked wheel or no wheel at all. Whether such activation reflects locomotion per se or heightened arousal cannot be determined from the current experiments. Exposure to one of several stressors, including restraint, also induces c-Fos immunoreactivity in the medial division of the LHb (Chastrette *et al.*, 1991; Wirtshafter *et al.*, 1994), which is consistent with the notion that changes in arousal contribute to novel wheel running-induced c-Fos activation.

Importantly, the number of c-Fos-ir LHb cells in hamsters running during the day was ~40% of that in hamsters running during the night, even though the number of wheel revolutions before sacrifice was similar in both groups; so another factor(s) (e.g., circadian phase) or differing conditions between the two experiments (e.g., home cage vs. novel environment or age of animals) must be involved. Also, daytime novel wheel running was associated with c-Fos activation in the MHb, even though this nucleus did not exhibit a day-night rhythm in gene expression. Perhaps, c-Fos activation in the MHb is responsive to unique features of the novel wheel, not associated with wheel running in general (e.g., novel environment). Thus, the relationship between habenula c-Fos, locomotion, and circadian rhythm is complex and will require further study.

To further investigate the relationship between the habenula and motor activity, we analyzed the effects of disrupting the habenula's output pathway in the FR. Hikosaka (2010) has argued that a common theme for the habenula's role in a multitude of behaviors is motor suppression. Indeed, destruction of the habenula or its outputs leads to hyperactivity in short-duration exploratory tests (Nielson & McIver, 1966; Lee & Huang, 1988; Thornton *et*

al., 1994; Murphy *et al.*, 1996) and home cage GLA (present results). On the other hand, we found that FRx resulted in hypoactivity when WR was used as the motor assay. Since WR is a motivated, self-reinforcing behavior (Sherwin, 1998), decreased WR after FRx might be an indirect consequence of altered reward properties of wheel running due to disruption of LHb connections with the mesolimbic dopamine (DA) reward system. It is believed that LHb neurons receive reward prediction error information from the border of the internal segment of the globus pallidus (Hong & Hikosaka, 2008) and that LHb neurons, in turn, inhibit DA neurons in the ventral tegmental area (VTA) and substantia nigra via output neurons that traverse the FR and synapse on inhibitory GABA neurons in the rostromedial tegmental nucleus (Christoph *et al.*, 1986; Ji & Shepard, 2007; Jhou *et al.*, 2009; Kaufling *et al.*, 2009). Thus, interruption of this pathway by FRx may diminish the rewarding properties of WR. Of note, modulation of WR by VTA DA secretion does not appear to be a simple relationship: while 6-hydroxydopamine lesion of DA neurons in the VTA (Isobe & Nishino, 2001) and FRx (present results) both decrease WR, FRx increases DA secretion in VTA and substantia nigra target tissues (Nishikawa *et al.*, 1986) (but see Lisoprawski *et al.*, 1980).

In addition to altering the amount of daily activity, FRx lengthened the period of the circadian rest-activity rhythm. This effect on τ might be due to the destruction of habenula efferents to serotonergic neurons in the raphe nuclei (Wang & Aghajanian, 1977; Nishikawa & Scatton, 1985; Kalén *et al.*, 1989), which have been implicated in both photic and non-photic regulation of the circadian system (Morin, 1999; Yannielli & Harrington, 2004). The precise role of serotonin, however, is not yet clear (Webb *et al.*, 2010). Habenula efferents to dopaminergic neurons also might play a role, as τ is lengthened after 6-hydroxydopamine lesion of the VTA (Isobe & Nishino, 2001).

Behavioral activity and vigilance state provide feedback to the SCN (Schaap & Meijer, 2001; Deboer *et al.*, 2003). Of note, τ in rodents is shortened by running in a wheel (Yamada *et al.*, 1988; Edgar *et al.*, 1991), although the magnitude of this feedback is smaller and the direction is inconsistent in Syrian hamsters (Aschoff *et al.*, 1973; Pratt & Goldman, 1986). Nonetheless, a longer τ after FRx cannot be ascribed solely to decreased WR in these animals because a longer τ was also evident in FRx hamsters housed without a wheel and exhibiting increased GLA. To further evaluate the effect of FRx on behavioral feedback to the circadian pacemaker, we tested whether FRx prevented novel wheel-induced phase shifts of the GLA rhythm. While FRx did block these phase shifts, the number of wheel revolutions induced by the novel wheel was also decreased and when included as a covariate, the difference in the magnitude of novel wheel-induced phase shifts of Control and FRx hamsters was no longer significant. Given that the magnitude of novel wheel-induced phase shifts is known to be related to the intensity of novel wheel running (Janik & Mrosovsky, 1993; Bobrzynska & Mrosovsky, 1998), the most parsimonious explanation for our result is that FRx prevented the phase shifts by decreasing motor activity/arousal. Whether the habenula is part of the neural circuitry that communicates behavioral feedback to the SCN remains to be determined. In a preliminary experiment, we asked whether habenular activation lies upstream or downstream of motor activity by measuring the number of immunoreactive c-Fos cells in the habenula of FRx and Control hamsters provided an unlocked or locked novel running wheel during the day. However, FRx alone

resulted in a massive, habenula-wide c-Fos upregulation, presumably due to injury-induced glial expression (Paul and Schwartz, unpublished findings).

Lastly, FRx affected the temporal organization of motor activity during the active phase of the rest-activity cycle. For Syrian hamsters, nighttime activity is characterized by a prolonged bout of elevated activity in the early night, which subsequently degrades into smaller sporadic bouts or ceases altogether before lights-on. FRx disrupted this pattern of motor activity, resulting in a more homogeneous, undifferentiated temporal profile extending across the night. Importantly, this effect was independent of FRx-induced changes in the overall level of motor activity, as it was observed both when the daily amount of activity was decreased (for WR) or increased (for GLA).

Lesions of the median raphe and paraventricular thalamus (PVT) alter the WR waveform, but such lesions do not precisely replicate the effects of FRx reported here. Selective depletion of serotonin by injection of 5,7-dihydroxytryptamine into the ventricle or median raphe lengthens the duration of the active phase by advancing activity onset and delaying activity offset (Smale *et al.*, 1990; Meyer-Bernstein & Morin, 1996); but 5,7-dihydroxytryptamine injection does not alter the daily amount of WR or lengthen τ in DD (Smale *et al.*, 1990), and FRx hamsters show no consistent advance in WR onset. Complete PVT lesion in blinded rats lengthens τ and increases activity in the late subjective night (Moga & Moore, 2000); but in sighted rodents, lesions restricted to the anterior PVT (rats and hamsters) or the mediodorsal PVT (rats) do not alter τ or the activity waveform (Ebling *et al.*, 1992; Salazar-Juárez *et al.*, 2002). With our knife cuts, damage to the neighboring PVT area was minor and, in many cases, predominantly unilateral. Whether these areas act in concert to sculpt the temporal profile of nighttime activity is an interesting area for future research.

Our experiments have focused on the timing of nighttime motor activity; but if FRx interferes with a more general temporal program that organizes the timing of multiple nighttime behaviors, then other functions should be disrupted as well. Our observation that periods of nighttime inactivity decreased by 74% after FRx (Figure 6) argues strongly that the time spent in nighttime sleep is also decreased, consistent with studies in rats showing decreased duration of sleep bouts/REM sleep and altered sleep patterns after FRx (Haun *et al.*, 1992; Valjakka *et al.*, 1998). FRx hamsters also ate more food and gained more weight than controls, a finding that might be due to more feeding during additional bouts of nighttime wakefulness.

From visual inspection of the free-running activity records of neonatally-blinded hamsters, Davis and Menaker (1980) postulated the existence of two underlying oscillators: a “window oscillator” that defines the interval during which activity can occur (i.e., the active phase) – identified as the SCN – and an unknown “bout oscillator” that regulates the pattern of activity within this window, often exhibiting more labile periodic components. We propose the habenula as a candidate site that regulates activity within the active phase, perhaps related to the aforementioned bout oscillator or part of a network for timing locomotion. It contains a self-sustained oscillator (Zhao & Rusak, 2005; Guilding *et al.*, 2010), has indirect bidirectional connections with the SCN (Kalsbeek *et al.*, 1993; Morin *et*

al., 1994; Krout *et al.*, 2002), projects to motor and arousal centers in the midbrain (Herkenham & Nauta, 1979), acts to suppress locomotor activity (Hikosaka, 2010), and FRx disrupts the temporal structure of nighttime motor activity while leaving intact a circadian window for rest and activity (present findings). Given that the LHb receives photic input (Zhao & Rusak, 2005), that melanopsin-expressing retinal ganglion cells terminate adjacent to its border (Hattar *et al.*, 2006), and, in the Syrian hamster, that the habenula expresses melatonin receptors (Weaver *et al.*, 1989), the nucleus seems ideally situated to integrate environmental input and internal time.

Acknowledgments

This work was supported by NINDS F32 NS058135 (M.J.P.), NINDS R01 NS46605 (W.J.S.), and NIGMS RO1 GM094109 (W.J.S.).

Abbreviations

BM	body mass
c-Fos-ir	c-Fos-immunoreactive
DA	dopamine
DAB	3,3'-diaminobenzidine tetrahydrochloride
DD	constant darkness
FI	food intake
FR	fasciculus retroflexus
FRx	fasciculus retroflexus transection
GFAP	glial fibrillary acidic protein
GLA	general locomotor activity
LHb	lateral habenula
MBP	myelin basic protein
MHb	medial habenula
PVT	paraventricular thalamus
SCN	suprachiasmatic nucleus of the hypothalamus
τ	tau
VTa	ventral tegmental area
WR	wheel running behavior
ZT	zeitgeber time

References

- Aschoff J, Figala J, Pöppel E. Circadian rhythms of locomotor activity in the golden hamster (*Mesocricetus auratus*) measured with two different techniques. *J Comp Physiol Psychol.* 1973; 85:20–28. [PubMed: 4744483]

- Bobrzynska KJ, Mrosovsky N. Phase shifting by novelty-induced running: activity dose-response curves at different circadian times. *J Comp Physiol [A]*. 1998; 182:251–258.
- Chastrette N, Pfaff DW, Gibbs RB. Effects of daytime and nighttime stress on Fos-like immunoreactivity in the paraventricular nucleus of the hypothalamus, the habenula, and the posterior paraventricular nucleus of the thalamus. *Brain Res*. 1991; 563:339–344. [PubMed: 1786549]
- Christoph GR, Leonzio RJ, Wilcox KS. Stimulation of the lateral habenula inhibits dopamine-containing neurons in the substantia nigra and ventral tegmental area of the rat. *J Neurosci*. 1986; 6:613–619. [PubMed: 3958786]
- Davis FC, Menaker M. Hamsters through time's window: temporal structure of hamster locomotor rhythmicity. *Am J Physiol Regul Integr Comp Physiol*. 1980; 239:R149–155.
- Deboer T, Vansteensel MJ, Détári L, Meijer JH. Sleep states alter activity of suprachiasmatic nucleus neurons. *Nat Neurosci*. 2003; 6:1086–1090. [PubMed: 12958601]
- Ebling FJ, Maywood ES, Humby T, Hastings MH. Circadian and photoperiodic time measurement in male Syrian hamsters following lesions of the melatonin-binding sites of the paraventricular thalamus. *J Biol Rhythms*. 1992; 7:241–254. [PubMed: 1330085]
- Edgar DM, Martin CE, Dement WC. Activity feedback to the mammalian circadian pacemaker: influence on observed measures of rhythm period length. *J Biol Rhythms*. 1991; 6:185–199. [PubMed: 1773091]
- Gerkema MP, Daan S. Ultradian rhythms in behavior: The case of the common vole (*Microtus arvalis*). *Exp Brain Res, Suppl*. 1985; 12:11–31.
- Guilding C, Hughes AT, Piggins HD. Circadian oscillators in the epithalamus. *Neuroscience*. 2010; 169:1630–1639. [PubMed: 20547209]
- Harrington ME. The ventral lateral geniculate nucleus and the intergeniculate leaflet: interrelated structures in the visual and circadian systems. *Neurosci Biobehav Rev*. 1997; 21:705–727. [PubMed: 9353800]
- Hattar S, Kumar M, Park A, Tong P, Tung J, Yau KW, Berson DM. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J Comp Neurol*. 2006; 497:326–349. [PubMed: 16736474]
- Haun F, Eckenrode TC, Murray M. Habenula and thalamus cell transplants restore normal sleep behaviors disrupted by denervation of the interpeduncular nucleus. *J Neurosci*. 1992; 12:3282–3290. [PubMed: 1494957]
- Herkenham M, Nauta WJ. Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study, with a note on the fiber-of-passage problem. *J Comp Neurol*. 1977; 173:123–146. [PubMed: 845280]
- Herkenham M, Nauta WJ. Efferent connections of the habenular nuclei in the rat. *J Comp Neurol*. 1979; 187:19–47. [PubMed: 226566]
- Hikosaka O. The habenula: from stress evasion to value-based decision-making. *Nature Rev Neurosci*. 2010; 11:503–513. [PubMed: 20559337]
- Hikosaka O, Sesack SR, Lecourtier L, Shepard PD. Habenula: crossroad between the basal ganglia and the limbic system. *J Neurosci*. 2008; 28:11825–11829. [PubMed: 19005047]
- Hong S, Hikosaka O. The globus pallidus sends reward-related signals to the lateral habenula. *Neuron*. 2008; 60:720–729. [PubMed: 19038227]
- Isobe Y, Nishino H. Circadian rhythm of drinking and running-wheel activity in rats with 6-hydroxydopamine lesions of the ventral tegmental area. *Brain Res*. 2001; 899:187–192. [PubMed: 11311879]
- Janik D, Godfrey M, Mrosovsky N. Phase angle changes of photically entrained circadian rhythms following a single nonphotic stimulus. *Physiol Behav*. 1994; 55:103–107. [PubMed: 8140152]
- Janik D, Mrosovsky N. Non-photically induced phase shifts of circadian rhythms in the golden hamster: activity-response curves at different ambient temperatures. *Physiol Behav*. 1993; 53:431–436. [PubMed: 8451307]
- Jhou TC, Geisler S, Marinelli M, Degarmo BA, Zahm DS. The mesopontine rostromedial tegmental nucleus: A structure targeted by the lateral habenula that projects to the ventral tegmental area of Tsai and substantia nigra compacta. *J Comp Neurol*. 2009; 513:566–596. [PubMed: 19235216]

- Ji H, Shepard PD. Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABA(A) receptor-mediated mechanism. *J Neurosci.* 2007; 27:6923–6930. [PubMed: 17596440]
- Kalén P, Strecker RE, Rosengren E, Björklund A. Regulation of striatal serotonin release by the lateral habenula-dorsal raphe pathway in the rat as demonstrated by in vivo microdialysis: role of excitatory amino acids and GABA. *Brain Res.* 1989; 492:187–202. [PubMed: 2473826]
- Kalsbeek A, Teclemariam-Mesbah R, Pevét P. Efferent projections of the suprachiasmatic nucleus in the golden hamster (*Mesocricetus auratus*). *J Comp Neurol.* 1993; 332:293–314. [PubMed: 8331217]
- Kauffling J, Veinante P, Pawlowski SA, Freund-Mercier MJ, Barrot M. Afferents to the GABAergic tail of the ventral tegmental area in the rat. *J Comp Neurol.* 2009; 513:597–621. [PubMed: 19235223]
- Klemm WR. Habenular and interpeduncularis nuclei: shared components in multiple-function networks. *Med Sci Monit.* 2004; 10:RA261–273. [PubMed: 15507867]
- Kreyszig, E. *Advanced Engineering Mathematics.* Wiley; New York: 1993.
- Krout KE, Kawano J, Mettenleiter TC, Loewy AD. CNS inputs to the suprachiasmatic nucleus of the rat. *Neuroscience.* 2002; 110:73–92. [PubMed: 11882374]
- Lavie P. Ultradian rhythms: Gates of sleep and wakefulness. *Exp Brain Res, Suppl.* 1985; 12:148–164.
- Lecourtier L, Kelly PH. A conductor hidden in the orchestra? Role of the habenular complex in monoamine transmission and cognition. *Neurosci Biobehav Rev.* 2007; 31:658–672. [PubMed: 17379307]
- Lee EH, Huang SL. Role of lateral habenula in the regulation of exploratory behavior and its relationship to stress in rats. *Behav Brain Res.* 1988; 30:265–271. [PubMed: 3178997]
- Lisoprawski A, Herve D, Blanc G, Glowinski J, Tassin JP. Selective activation of the mesocortico-frontal dopaminergic neurons induced by lesion of the habenula in the rat. *Brain Res.* 1980; 183:229–234. [PubMed: 7357405]
- Meyer-Bernstein EL, Morin LP. Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. *J Neurosci.* 1996; 16:2097–2111. [PubMed: 8604054]
- Mistlberger RE, Holmes MM. Behavioral feedback regulation of circadian rhythm phase angle in light-dark entrained mice. *Am J Physiol.* 2000; 279:R813–821.
- Mistlberger RE, Skene DJ. Social influences on mammalian circadian rhythms: animal and human studies. *Biol Rev Camb Philos Soc.* 2004; 79:533–556. [PubMed: 15366762]
- Moga MM, Moore RY. Paraventricular thalamic lesions alter circadian period and activity distribution in the blinded rat. *Biol Rhythm Res.* 2000; 31:212–219.
- Morin LP. Serotonin and the regulation of mammalian circadian rhythmicity. *Ann Med.* 1999; 31:12–33. [PubMed: 10219711]
- Morin LP, Allen CN. The circadian visual system, 2005. *Brain Res Rev.* 2006; 51:1–60. [PubMed: 16337005]
- Morin LP, Goodless-Sanchez N, Smale L, Moore RY. Projections of the suprachiasmatic nuclei, subparaventricular zone and retrochiasmatic area in the golden hamster. *Neuroscience.* 1994; 61:391–410. [PubMed: 7526267]
- Morin, LP.; Wood, RI. *A Stereotaxic Atlas of the Golden Hamster Brain.* Academic Press; San Diego: 2001.
- Mrosovsky N. Phase response curves for social entrainment. *J Comp Physiol [A].* 1988; 162:35–46.
- Mrosovsky N. Locomotor activity and non-photic influences on circadian clocks. *Biol Rev Camb Philos Soc.* 1996; 71:343–372. [PubMed: 8761159]
- Murphy CA, DiCamillo AM, Haun F, Murray M. Lesion of the habenular efferent pathway produces anxiety and locomotor hyperactivity in rats: a comparison of the effects of neonatal and adult lesions. *Behav Brain Res.* 1996; 81:43–52. [PubMed: 8950000]
- Murray M, Murphy CA, Ross LL, Haun F. The role of the habenula-interpeduncular pathway in modulating levels of circulating adrenal hormones. *Restor Neurol Neurosci.* 1994; 6:301–307. [PubMed: 21551761]

- Nielson HC, McIver AH. Cold stress and habenular lesion effects on rat behaviors. *J Appl Physiol.* 1966; 21:655–660. [PubMed: 4160611]
- Nishikawa T, Fage D, Scatton B. Evidence for, and nature of, the tonic inhibitory influence of habenulointerpeduncular pathways upon cerebral dopaminergic transmission in the rat. *Brain Res.* 1986; 373:324–336. [PubMed: 2424555]
- Nishikawa T, Scatton B. Inhibitory influence of GABA on central serotonergic transmission. Involvement of the habenulo-raphe pathways in the GABAergic inhibition of ascending cerebral serotonergic neurons. *Brain Res.* 1985; 331:81–90. [PubMed: 2985200]
- Omelchenko N, Bell R, Sesack SR. Lateral habenula projections to dopamine and GABA neurons in the rat ventral tegmental area. *Eur J Neurosci.* 2009; 30:1239–1250. [PubMed: 19788571]
- Paul, MJ.; Schwartz, WJ. On the chronobiology of cohabitation. In: Stillman, B.; Stewart, D.; Grodzicker, T., editors. *Cold Spring Harbor Symposia on Quantitative Biology: Clocks and Rhythms.* Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 2007. p. 615-621.
- Pratt BL, Goldman BD. Environmental influences upon circadian periodicity of Syrian hamsters. *Physiol Behav.* 1986; 36:91–95. [PubMed: 3952189]
- Salazar-Juárez A, Escobar C, Aguilar-Roblero R. Anterior paraventricular thalamus modulates light-induced phase shifts in circadian rhythmicity in rats. *Am J Physiol.* 2002; 283:R897–904.
- Schaap J, Meijer JH. Opposing effects of behavioural activity and light on neurons of the suprachiasmatic nucleus. *Eur J Neurosci.* 2001; 13:1955–1962. [PubMed: 11403689]
- Sherwin CM. Voluntary wheel running: a review and novel interpretation. *Anim Behav.* 1998; 56:11–27. [PubMed: 9710457]
- Silver R, Lesauter J. Circadian and homeostatic factors in arousal. *Ann N Y Acad Sci.* 2008; 1129:263–274. [PubMed: 18591487]
- Sinclair SV, Mistlberger RE. Scheduled activity reorganizes circadian phase of Syrian hamsters under full and skeleton photoperiods. *Behav Brain Res.* 1997; 87:127–137. [PubMed: 9331481]
- Smale L, Michels KM, Moore RY, Morin LP. Destruction of the hamster serotonergic system by 5,7-DHT: effects on circadian rhythm phase, entrainment and response to triazolam. *Brain Res.* 1990; 515:9–19. [PubMed: 2357582]
- Sutherland RJ. The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. *Neurosci Biobehav Rev.* 1982; 6:1–13. [PubMed: 7041014]
- Tavakoli-Nezhad M, Schwartz WJ. Hamsters running on time: is the lateral habenula a part of the clock? *Chronobiol Int.* 2006; 23:217–224. [PubMed: 16687295]
- Thornton EW, Murray M, Connors-Eckenrode T, Haun F. Dissociation of behavioral changes in rats resulting from lesions of the habenula versus fasciculus retroflexus and their possible anatomical substrates. *Behav Neurosci.* 1994; 108:1150–1162. [PubMed: 7893407]
- Valjakka A, Vartiainen J, Tuomisto L, Tuomisto JT, Olkkonen H, Airaksinen MM. The fasciculus retroflexus controls the integrity of REM sleep by supporting the generation of hippocampal theta rhythm and rapid eye movements in rats. *Brain Res Bull.* 1998; 47:171–184. [PubMed: 9820735]
- Wang RY, Aghajanian GK. Physiological evidence for habenula as major link between forebrain and midbrain raphe. *Science.* 1977; 197:89–91. [PubMed: 194312]
- Weaver DR, Rivkees SA, Reppert SM. Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography. *J Neurosci.* 1989; 9:2581–2590. [PubMed: 2545841]
- Webb IC, Patton DF, Landry GJ, Mistlberger RE. Circadian clock resetting by behavioral arousal: neural correlates in the midbrain raphe nuclei and locus coeruleus. *Neuroscience.* 2010; 166:739–751. [PubMed: 20079808]
- Wirtshafter D, Asin KE, Pitzer MR. Dopamine agonists and stress produce different patterns of Fos-like immunoreactivity in the lateral habenula. *Brain Res.* 1994; 633:21–26. [PubMed: 8137158]
- Yamada N, Shimoda K, Ohi K, Takahashi S, Takahashi K. Free-access to a running wheel shortens the period of free-running rhythm in blinded rats. *Physiol Behav.* 1988; 42:87–91. [PubMed: 3387483]
- Yannielli P, Harrington ME. Let there be “more” light: enhancement of light actions on the circadian system through non-photoc pathways. *Prog Neurobiol.* 2004; 74:59–76. [PubMed: 15381317]

Zhao H, Rusak B. Circadian firing-rate rhythms and light responses of rat habenular nucleus neurons in vivo and in vitro. *Neuroscience*. 2005; 132:519–528. [PubMed: 15802202]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Wheel running induces immunoreactive c-Fos expression in the habenula

Panels A – D. Representative coronal sections demonstrating c-Fos-immunoreactive (c-Fos-ir) cells in the lateral (LHb) and medial (MHb) habenula of hamsters perfused (A) during the day without access to a wheel (No Wheel), (B) during the day with access to a locked running wheel (Locked Wheel), (C) during the day with access to an unlocked running wheel (Unlocked Wheel – Day), or (D) during the night with access to an unlocked running wheel (Unlocked Wheel – Night). Hipp, hippocampus; scale bar, 100 μ m.

Panels E – G. Mean + SEM number of c-Fos-ir cells per section in the LHb (E) and MHb (F) and number of wheel revolutions before sacrifice of Unlocked Wheel hamsters perfused during the day or night (G). *indicates significant difference from No Wheel and Locked Wheel controls, $p < 0.05$, Fisher's PLSD.

Figure 2.

Composite overlay of c-Fos-stained sections from all Unlocked Wheel – Day (A) and Unlocked Wheel – Night (B) hamsters (two consecutive sections per hamster at the rostro-caudal level of Plate 26 from Morin and Wood, 2001; one section was excluded for two Unlocked Wheel – Day hamsters due to air bubbles over the habenula). Scale bar, 100 μ m.

Figure 3. Immunohistochemistry of fasciculus retroflexus transections

Representative rostral (A, B, D, E) and caudal (C, F) coronal sections from hamsters sustaining control surgeries (Control, A – C) or fasciculus retroflexus transections (FRx, D – F) and stained for glial fibrillary acidic protein (GFAP, green) and myelin basic protein (MBP, red) by dual-label immunofluorescence (A/B and D/E are each single sections). Arrowheads indicate GFAP staining surrounding the knife cut (laterally in Control, medially in FRx), with a higher magnification image of GFAP-stained cells between arrowheads in D illustrated in the insert. Arrows indicate MBP identification of the fasciculus retroflexus in B and C, eliminated in E and F. Scale bars, 250 μ m and 50 μ m (inset).

Figure 4. Fasciculus retroflexus transections increase body mass and food intake
Mean $\pm$ SEM percent pre-surgical body mass (A, B) and food intake (C) of FRx and Control hamsters with (A) or without (B, C) free access to a running wheel in the home cage. * indicates significant difference between FRx and Control groups, $p < 0.05$, Fisher's PLSD.

Figure 5. Fasciculus retroflexus transections alter the amount and temporal distribution of wheel running behavior and general locomotor activity

Panels A – L. Pre- and post-surgical wheel running behavior (WR) and general locomotor activity (GLA) of Control and FRx hamsters, illustrated as double-plotted actograms of representative individuals (A – D) or average waveforms of each group (E – L). Values at the top of the y-axis of the waveforms indicate the upper bound of WR (E – H) and GLA (I – L). Open and closed arrows indicate day of surgery and transfer to constant darkness, respectively.

Panels M – R. Mean $\pm$ SEM daily wheel revolutions (M), nighttime center of gravity (N) and percent activity during the second half of the night (O) for WR; and daily infrared deflections (P), nighttime center of gravity (Q) and percent activity during the second half of the night (R) for GLA; in FRx and Control hamsters before and after surgery. * indicates significant difference between groups, $p < 0.05$, Fisher's PLSD.

Figure 6. Fasciculus retroflexus transections decrease periods of inactivity

The number of 15-min nighttime bins of GLA in Control and FRx hamsters before and after surgery, plotted as mean + SEM of bins at different activity levels (ranging from 0 to 290 infrared deflections per bin). The number of bins with absent nighttime activity is drastically reduced in FRx hamsters after surgery. * indicates significant difference between groups, $p < 0.05$, Scheffe's post-hoc correction.

Figure 7. Fasciculus retroflexus transections prevent novel wheel running-induced phase shifts in the circadian rhythm of general locomotor activity

Mean + SEM novel wheel (NW)-induced phase shifts in the circadian GLA rhythm (A) and number of wheel revolutions (B) of Control and FRx hamsters provided with a NW from projected ZT 4–7 on the 2nd or 3rd day of constant darkness. In (C), NW phase shifts are plotted according to the number of NW revolutions for each Control (open circles) and FRx (closed circles) hamster. Positive and negative values indicate phase advances and delays, respectively. * indicates significant difference between groups, $p < 0.05$, t-Test, $t(9) = 2.5$, $p = 0.04$.