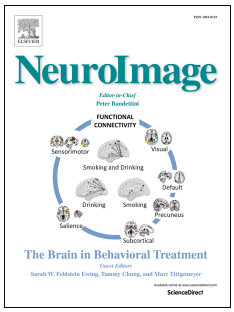
Accepted Manuscript



The human habenula is responsive to changes in luminance and circadian rhythm

Christian Kaiser, Christian Kaufmann, Tobias Leutritz, Yan Luis Arnold, Oliver Speck, Markus Ullsperger

PII: S1053-8119(19)30070-9

DOI: https://doi.org/10.1016/j.neuroimage.2019.01.064

Reference: YNIMG 15589

To appear in: *NeuroImage*

Received Date: 4 October 2018

Revised Date: 24 December 2018

Accepted Date: 25 January 2019

Please cite this article as: Kaiser, C., Kaufmann, C., Leutritz, T., Arnold, Y.L., Speck, O., Ullsperger, M., The human habenula is responsive to changes in luminance and circadian rhythm, *NeuroImage* (2019), doi: https://doi.org/10.1016/j.neuroimage.2019.01.064.

The human habenula is responsive to changes in luminance and circadian rhythm

Christian Kaiser[a], Christian Kaufmann[a,b], Tobias Leutritz[c,d], Yan Luis Arnold[c], Oliver Speck[c,e,f,g], Markus Ullsperger[a,g]

a. Department for Neuropsychology, Institute for Psychology, Otto-von-Guericke University, D-39106 Magdeburg, Germany
b. Department of Clinical Psychology, Institute for Psychology, Humboldt-Universität zu Berlin, D-10099 Berlin, Germany
c. Department for Biomedical Magnetic Resonance, Institute for Physics, Otto-von-Guericke University, D-39106 Magdeburg, Germany
d. Department for Neurophysics, Max Planck Institute for Human Cognitive and Brain Sciences, D-04103 Leipzig, Germany
e. Leibniz Institute for Neurobiology, D-39120 Magdeburg, Germany
f. German Center for Neurodegenerative Diseases (DZNE), D-39120 Magdeburg, Germany,
g. Center for Behavioral Brain Sciences (CBBS), D-39106 Magdeburg, Germany

Corresponding author:
Christian Kaiser
Otto-von-Guericke-University Magdeburg, Universitätsplatz 2, D-39106 Magdeburg
christian.kaiser@ovgu.de

Pages: 33 Figures: 2

Abstract

The habenula is a pivotal structure in the neural network that implements various forms of cognitive and motivational functions and behaviors. Moreover, it has been suggested to be part of the brain's circadian system, not at least because habenular neurons are responsive to retinal illumination and exhibit circadian modulations of their firing patterns in animal research. However, no study has directly investigated the human habenula in this regard. We developed a paradigm in which alternating phases of high and low luminance are used to study human habenular functioning. In two experiments with independent samples, fMRI data of 24 healthy participants were acquired at a field strength of 7T, and of 21 healthy participants at 3T. Region of interest analyses revealed that the human habenula is responsive to light as well, resulting in a decrease in activation when a change in luminance occurs. Although this pattern is not predicted by animal research, we were able to replicate this finding in a second independent data set. Furthermore, we demonstrate that the strength of decrease in activation is modulated in a circadian fashion, being more strongly deactivated in morning than in afternoon sessions. Taken together, these findings provide strong evidence that changes in illumination elicit changes in habenular activation and that these changes appear to be more pronounced in the morning than in the afternoon.

Keywords

human habenula, luminance, circadian rhythm, fMRI

Highlights

- Changes in luminance result in a deactivation of the human habenula in fMRI data
- This signal difference is presumably modulated in a circadian fashion
- Deviating from animal research, the importance of human basic research is underlined

1.1 Introduction

The habenula is a small but complex epithalamic structure located at the medial-dorsal end of the thalamus (Andres et al., 1999). Habenular activity is predominantly associated with the inhibition of the release of dopamine and serotonin to the forebrain (Herkenham & Nauta, 1977, 1979; Hikosaka, 2010; Lecourtier & Kelly, 2007; Matsumoto & Hikosaka, 2007; Jhou et al., 2009; Hong et al., 2011, Brown et al., 2017). Therefore, the habenula is thought to contribute to a broad variety of cognitive and motivational functions and behaviors including reinforcement learning, decision making, motor suppression and stress evasion (e.g., Matsumoto & Hikosaka, 2007; Salas et al., 2010; Ullsperger & von Cramon, 2003; Wirtshafter et al., 1994; see Hikosaka, 2010 for a review). Moreover, an increasing number of studies points to the habenula's involvement in pathogenesis of mental disorders such as depression (Kumar et al., 2018; Li et al., 2011; Sartorius & Henn, 2007; Winter et al., 2011; Yang et al., 2008), schizophrenia (Bernstein et al., 2016; Sandyk, 1992; Zhang et al., 2017) and addiction (Carlson et al., 2001; Ellison, 1992; Velasquez et al., 2014). However, little is known about non-pathological functioning of the human habenula.

It has been suggested that the habenula is part of the brain network that drives circadian rhythms. In rodents, the habenula receives direct input from retinal ganglion

cells (Qu et al., 1996; Reuss & Decker, 1997) as well as from brain structures that encode changes in light–dark cycles and drive circadian rhythms, e.g. the suprachiasmatic nucleus (Buijs, 1978; de Vries et al., 1981) and the pineal gland (Guglielmotti & Cristino, 2006; Rønnekleiv et al., 1980). It is thus conceivable that habenular activity is modulated by light, e.g. via a direct retinal input, or by circadian rhythms, e.g. on the basis of an endogenous circadian activity pattern of the habenula itself or due to its interconnection to external circadian oscillators. The pineal gland, which is also part of the epithalamus and coevolves with the habenula, is one of these interconnected structures that plays a significant role in the control of circadian rhythm (Bell-Pedersen et al., 2005; Fukada & Okano, 2002). In the mammalian brain, this eminent function is based on the production and secretion of melatonin, one of the most important hormones involved in the entrainment of circadian rhythms. With regard to the habenula's reactivity to light, Zhao and Rusak (2005) demonstrated in a series of in vivo experiments that firing rates of a subpopulation of habenular neurons in rats are modulated in response to changing lighting conditions. The firing rates of the majority of these photoresponsive cells were increased by a phasic retinal illumination. In addition, the authors found higher mean firing rates of habenular neurons during the day than at night under constant illumination conditions, indicating their activity is modulated in a circadian fashion. Guilding et al. (2010) demonstrated that a subpopulation of lateral habenular neurons expresses core clock genes and proteins that drive circadian timekeeping in cells. Moreover, circadian rhythms in the lateral habenula persist in the presence of the sodium channel blocker tetrodotoxin, indicating that circadian oscillations arise intrinsically (Guilding et al., 2010). Taken together, these findings suggest that, at least in rodents, the habenula is part of the circadian clock network that it is

responsive to light, and that it is functionally influenced by circadian rhythms or influences these rhythms itself.

As the habenula complex is considered to be phylogenetically highly conserved in vertebrates (Aizawa, 2013; Fakhoury, 2018), it is reasonable to assume these findings from rodent literature might be translatable into human habenular functioning. Thus, activity patterns of the human habenula might also be receptive to changes in retinal illumination and modulated in a circadian fashion. However, to our knowledge, no study has directly addressed these potential influences in humans. Hence, we designed a paradigm in which the luminance a large visual area is systematically manipulated in order to achieve high and low levels of retinal illumination in human participants. This large area serves as background for a simple counting task that participants were asked to perform. For the counting task, participants fixated a centrally presented small dot and counted how often it changed into an annulus. This paradigm was tested in morning as well as afternoon fMRI sessions. We hypothesized the human habenula responds to phasic changes in luminance as well as changes in the phase of circadian rhythmicity. Specifically, following the results of Zhao and Rusak (2005), we predicted that strong retinal illumination should lead to an increased habenular activation as compared to low retinal illumination. This prediction is based on the assumption that like in rats, the majority of human habenular neurons that are photoresponsive belong to the lateral part of the habenula, and that the larger proportion of these neurons shows increased activation in response to retinal illumination. We also tested the hypothesis that the human habenula might not be sensitive to changes in luminance rather than luminance per se. Furthermore, we aimed to investigate whether the habenula's presumed sensitivity to differences or changes in luminance underlies a circadian rhythm.

2.1 Materials and Methods

2.1.1 Experiment 1: Habenular Responses to Differences and Changes in Luminance at 7T

2.1.1.1 Participants

30 participants with no self-reported history of neurological or psychiatric diagnosis took part in this study (mean age 25.8 years, *SD* = 4.8 years, 15 females, 27 right-handed). All participants had normal or corrected-to-normal vision. Each participant provided written informed consent to participate, the experiment was approved by the local ethics committee. Participants were paid 30 €. Data for 3 participants were lost due to scanner failure, 1 participant discontinued his participation early, and 2 were excluded from the analysis due to excessive movement inside the scanner, leaving 24 participants (mean age = 25.5 years, *SD* = 4.6 years, 11 females, 22 right-handed) in the analysis.

2.1.1.2 Stimuli

The basic composition of the visual stimulus displays consisted of a small, centrally presented grey dot that was superimposed on a large background area (see Fig. 1d). The grey dot had an effective stimulus size of 1 × 1 degrees of visual angle (1.75 × 1.75 cm^2 onscreen size), and an annulus of the same shade of grey with identical dimensions for its outer circle and 0.6 × 0.6 degrees of visual angle for its inner circle (1.0 × 1.0 cm^2 onscreen). The background had an effective stimulus size of 15.4 × 11.7 degrees of visual angle (27.0 × 20.5 cm^2 onscreen), which represents the maximum stimulus size achievable with the projection system. The background consisted of a monochrome gray area with 4 different levels of luminance (1 level with a luminance of 3 cd/m^2, labeled as “low” level, and 3 “high” levels with 600,

1200, and 1800 cd/m^2, respectively). Luminance values were obtained with a luminance meter. Participants viewed stimuli via a mirror mounted at the head coil at a viewing distance of 100 cm. Stimuli were presented with a projector (JVC DLA-G 150 CLE) at a resolution of 1280 × 1024 pixels and with a refresh rate of 60 Hz. Stimulus presentation was controlled using Presentation 18.1 (NeuroBehavioral Systems, http://www.neurobs.com/).

Our rationale on choosing 3 different levels of high luminance was based on findings by Zhao and Rusak (2005). For their examination of the rat's habenular responses to retinal illuminations, the authors deployed 3 different light intensities (140, 600, and 1000 lux). By inspecting the corresponding peristimulus time histograms reported in the author's paper, one can easily infer that the level of light intensity shapes the firing patterns of the recorded photoresponsive habenular neurons in a way that is relevant for a translation into a human fMRI study. For example, the degree of activity increase in light-activated cells as well as the degree of activity decrease in light-suppressed cells varies across conditions, with 600 lux yielding the most reliable results according to the authors. Moreover, light intensity seems to influence the firing behavior of habenular neurons immediately after the offset of retinal illumination even stronger. There is a pronounced difference in the amount of time that is needed for these neurons to reach their pre-illumination baseline: After 30 s of retinal illumination with 140 lux, the firing rate returns to baseline firing quickly (approx. 10 s), whereas after 30 s of retinal illumination with 1000 lux, it takes substantially longer (approx. 90 s). It appears that luminance related "overstimulation" causes habenula neurons to preserve their activity in the absence of a light-stimulus much longer, therefore not allowing for a clear separation of activity patterns. Assuming that human habenular neurons operate similarly to those of rats, these observations suggest at least two interrelated conclusions for a

human fMRI study that wants to utilize a contrast of high vs. low luminance to investigate habenular activation changes. Firstly, one needs to choose a luminance level that is high enough to evoke differences in the habenula's activation that can be detected in the BOLD signal. Secondly, however, the luminance level must not exceed a certain threshold that "overstimulates" habenular neurons, or they would otherwise possibly remain in a state of elevated (or reduced) activity in phases of low luminance. When deciding on specific luminance values for our experiments, a luminance meter was used to measure the capabilities of our projection system, reaching a maximum luminance of 2000 cd/m^2 for pure white and a minimum luminance of 3 cd/m^2 for pure black at the in-bore projection screen. In a small pilot (N = 3), a luminance of 1800 cd/m^2 was identified as the just tolerable maximum intensity that was not unpleasant to look at for the desired duration of 21 s. We decided to test within the entire range from 3 to 1800 cd/m^2 and to include 3 equidistant levels of high luminance, resulting in target luminance levels of 1800, 1200, and 600 cd/m^2 (high luminance) to be contrasted against the minimum of 3 cd/m^2 (low luminance).

2.1.1.3 Procedure

Participants were instructed to fixate a grey dot in the center of the display inside the scanner. From time to time, this dot changed into an annulus of the same size and color for 2 s (see Fig. 1d). Participants were asked to count how often an annulus appeared throughout a run and report that number verbally to the experimenter after each run. This change occurred on average every 20 s, with a stimulus onset asynchrony jittered between 5 and 35 s, resulting in an average number of 20 appearances per run (range 15 to 25). After the report, the correct number of annulus appearances was presented on the screen as a feedback of the participant's performance. This counting task was used in order to keep participants awake and

alert, and to ensure participants would not close their eyes. Additionally and more importantly, while participants fixated the dot, the luminance of the background alternated between high luminance conditions (either 600, 1200 or 1800 cd/m^2, respectively, with a duration of 21 s) and a low luminance condition (3 cd/m^2, with a duration of 42 s; see Fig. 1b). This alternation of background luminance occurred independently of the annulus' appearance and represented the manipulation of interest for the fMRI analysis. Participants were explicitly and truthfully instructed that changes in background luminance do not have any influence on the counting task. Each run lasted for 6 min 48 s and consisted of 6 high-luminance and 6 low-luminance phases (see Fig. 1a), resulting in 11 changes of luminance. Each participant completed 4 runs.

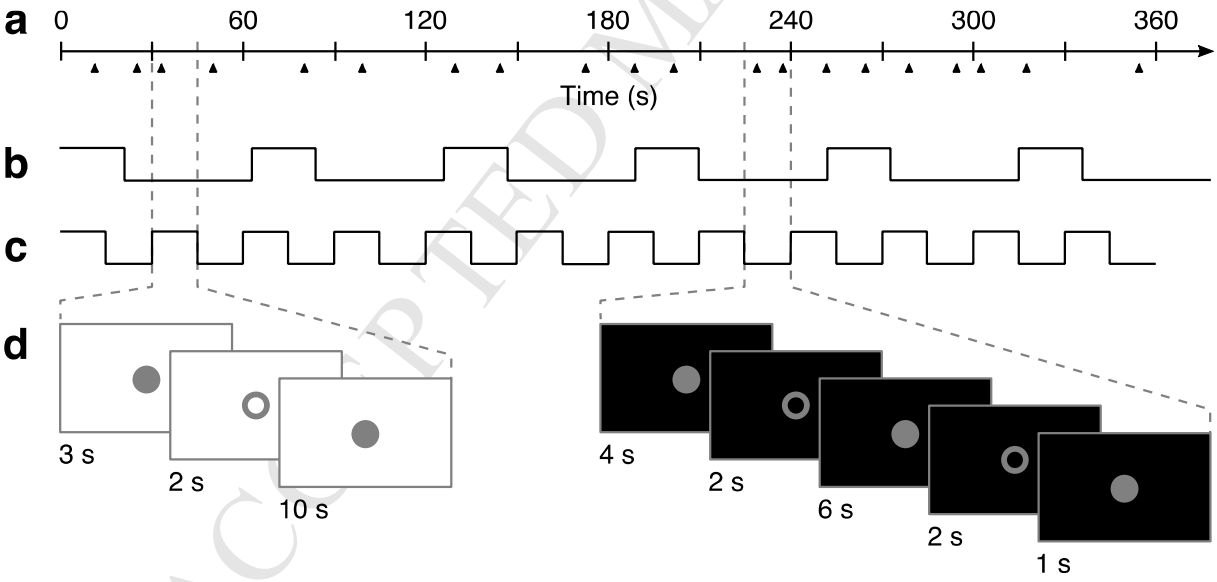


Figure 1. Scheme of time structure, luminance conditions, and microstructure of one exemplary run. Panel (**a**) shows a time scale in seconds (s). Each triangle represents the onset of an annulus over the course of a run. For the run depicted, the number of annulus appearances is 20, thus the correct answer to the counting task is 20. Panel (**b**) and (**c**) illustrate the luminance-timing scheme for Experiment 1 (**b**) and Experiment 2 (**c**). Upward deflections of the lines indicate phases of high background

luminance (either 600, 1200 or 1800 cd/m^2, duration of 21 s in Experiment 1 and 15 s in Experiment 2), downward deflections represent phases of low background luminance (3 cd/m^2, duration of 42 s in Experiment 1 and 15 s in Experiment 2). Panel (**d**) represents the exemplary microstructure of a phase with high (left) and low (right) background luminance, and the appearance of 1 annulus (left) or 2 annuli (right). The on- and offset of a 15-seconds phase is indicated by dashed lines. The duration of each of the presented displays is given below.

2.1.1.4 Image Acquisition and Preprocessing

Structural and functional MRI data were acquired with a Magnetom 7T MRI scanner (Siemens Healthineers), using a 32-channel head coil (Nova Medical Head Coil TxRx, Siemens Healthineers).

For each participant, two high-resolution T1-weighted structural images were obtained using an MPRAGE and a GE sequence, respectively (acquisition voxel size 0.8 mm isotropic with a FOV of 256 × 256 mm^2, an imaging matrix of 320 × 320 and 256 transverse slices with a thickness of 0.8 mm, TE 2.73 ms, TR_{MPRAGE} 2.30 s and TR_{GE} 1.34 s). High-resolution functional images were obtained using a gradient EPI sequence (1.0 mm isotropic, FOV 200 × 200 mm^2, matrix size 200 × 200, partial Fourier 6/8, GRAPPA factor 4, 48 transverse slices with a thickness of 1.0 mm, manually aligned to the AC-PC line and centered to the habenula, interleaved slice order, TE 22.00 ms, TR 3.00 s). In order to allow for T1 equilibration, 3 initial dummy volumes were discarded. Additionally, pulse and respiration data were acquired for the functional images using a pulse oximeter (NONIN Pulse Oximeter 8600FO) and a pneumatic belt. At the end of the experiment, 3 additional resting state EPI volumes with 100 transverse slices (same parameters as reported before, except TR 6.24 s) were acquired. Online motion correction and PSF distortion correction (In & Speck, 2012; Oh et al., 2012) was applied.

All image files were converted to NifTi format using the dcm2niix converter included in MRIcron (version 1.8.8, https://www.nitrc.org/projects/mricron) and preprocessed using SPM 12 (version 6225, Wellcome Trust, http://www.fil.ion.ucl.ac.uk/spm/software/spm12). Since heterogeneities in the local B0 field are especially prominent at high field strengths, signal intensity and contrast inhomogeneities in the MPRAGE image were corrected using the GE image as proposed by van de Moortele et al. (2009). Functional scans were coregistered to the contrast-corrected MPRAGE in a two-step procedure: first, the mean functional EPI covering 100 slices (i.e., data of the whole cerebrum) was coregistered to the MPRAGE, and second, the mean EPI of each run was coregistered to the mean 100-slices EPI of its respective run, resulting in a more precise coregistration. After coregistration, functional images were slice-time corrected and smoothed with a 2 mm Gaussian kernel. The corrected MPRAGE was used for segmentation and creation of individual flow fields using SPM's DARTEL algorithm.

Pulse and respiration data were processed using the PhysIO toolbox (R2016.1, Kasper et al., 2017), resulting in nuisance regressors (RETROICOR + HRV + RVT) for the GLMs.

2.1.1.5 Data Analysis

We tested 3 different models, from here on denoted as Differential Contrast hypothesis (600 vs. 3, 1200 vs. 3, 1800 vs. 3 cd/m^2), Simple Contrast hypothesis (high vs. low, e.g. 600 + 1200 + 1800 vs. 3 cd/m^2) and Change hypothesis (change in luminance vs. baseline). Separate GLMs were run for each participant and each hypothesis. For the Differential Contrast hypothesis, each GLM contained regressors for each high luminance level and their respective darkness periods. For the Simple Contrast hypothesis, each GLM contained one regressor for high luminance periods (collapsed across all high luminance levels) and one regressor for low luminance

periods. For both Contrast models, box car regressors were convolved with a canonical hemodynamic response function. For the Change hypothesis, each GLM contained one regressor for the appearance of a change in luminance. Additionally, all models (both Contrast and Change) contained one regressor for the appearance of an annulus as well as nuisance regressors for the six motion parameters estimated during realignment and nuisance regressors for physiological noise. In all GLMs, time and dispersion derivatives for all regressors were included.

For each second level analysis, individual contrast images were spatially normalized to MNI space using each participant's individual flow field. All statistical analyses were performed on the normalized contrast images. For region of interest (ROI) analysis of the habenula region, a ROI consisting of two spheres surrounding the left and the right habenula was constructed using the MarsBaR region of interest toolbox (version 0.44, Brett et al., 2002) and used as a binary mask in second level GLMs. The MNI coordinates of the center of the left habenula (x = –2.8, y = –24.4, z = 2.3) and right habenula (x = 4.8, y = –24.1, z = 2.2, see Lawson et al. 2013) served as midpoint for the first and second sphere, respectively, with both spheres having a radius of 5 mm. ROIs were manually inspected to ensure coverage of the habenulae for each participant.

2.1.2 Experiment 2: Habenular Responses to Changes in Luminance at 3T (Confirmation) and Their Dependence on Circadian Rhythmicity

2.1.2.1 Participants

A separate sample of 25 participants with no self-reported history of neurological or psychiatric diagnosis took part in this study (mean age 24.8 years, *SD* = 4.4 years, 11 females, 20 right-handed). All participants reported regular sleep-wake cycles

(wake onset on average at 7:56 am, *SD* = 69 min, sleep onset on average at 11:29 pm, *SD* = 46 min). All participants reported normal or corrected-to-normal vision. Each participant provided written informed consent to participate. The experiment was approved by the local ethics committee. Participants were paid 30 €. Data were lost for 2 participants due to technical failure, and 2 were removed from the analysis due to excessive movement inside the scanner, leaving 21 participants (mean age = 25.1 years, *SD* = 4.6 years, 10 females, 17 right-handed) in the analysis.

2.1.2.2 Stimuli and Procedure

Stimuli and procedure were identical to Experiment 1, with the following exceptions: Using a different projection system (Siemens HN-3460S, resolution of 1280 × 1024 pixels, refresh rate 60 Hz), the viewing distance between the mirror mounted at the head coil and the screen was 63 cm. The onscreen sizes of the dot and background stimuli differed from Experiment 1 (6.6 × 6.6 mm^2 and 10.2 × 7.7 cm^2, respectively) but chosen to obtain an identically effective stimulus size as compared to Experiment 1 (1 × 1 and 15.4 × 11.7 degrees of visual angle, respectively). In consequence of the results of Experiment 1 (see section 3.1.2 *Responsiveness to Changes in Luminance (Experiment 1 at 7T)*), we modified the timing of the task. Instead of 21 s for high and 42 s for low luminance phases, we now used a duration of 15 s for both bright and dark phases, preserving the overall structure of comparatively long blocks with the same background luminance while simultaneously allowing for more frequent luminance changes (every 15 s, leading to 23 luminance changes per run, see Fig. 1c, as compared to one luminance change on average every 31.5 s in the 7T version, resulting in 11 luminance changes per run, see Fig. 1b). Again, each participant completed 4 runs in each session with an individual run time of 6 min 30 s.

In order to address the question whether circadian rhythmicity influences light-dependent habenular activation, we used a within-subject design and asked participants to complete two separate MRI sessions, one in the morning (between 8 and 11 am) and one in the afternoon (between 3 and 6 pm). However, participants differ in respect to their endogenous circadian rhythm: Sleep and wake times are distributed in a nearly-Gaussian fashion (Roenneberg et al., 2007). In order to account for these interindividual differences in circadian rhythmicity, participants were asked to fill out the German version of the morningness–eveningness questionnaire (D-MEQ; Griefahn et al., 2001) prior to scheduling MRI appointments. The D-MEQ score provides a measure for endogenous circadian rhythmicity in a way that high(er) D-MEQ scores (range 16 – 86) indicate morning types while low(er) scores indicate evening types. Participants with higher D-MEQ scores were assigned to early appointments (8 am and 3 pm), participants with lower D-MEQ scores to late appointments (9.30 am and 4.30 pm). Potential session effects were counterbalanced: Half of the participants started with the morning session and completed the afternoon session at the same day, while the other half started with the afternoon session and completed the morning session the following day. After completing the first session, participants were asked to fill out the D-MEQ for a second time. The second score was used to ensure no significant differences in chronotype assessment have emerged between the first score used to schedule early and late appointments and the actual MR data collection (ranging from 2 to 22 days).

2.1.2.3 Image Acquisition and Preprocessing

Structural and functional MRI data were acquired with a Magnetom Skyra 3T MRI scanner (Siemens Healthineers), using a 32-channel head coil (Nova Medical Head Coil 1Tx/32RX, Siemens Healthineers).

For each participant, a high-resolution T1-weighted structural image was obtained using a MPRAGE sequence (0.8 mm isotropic resolution, FOV 256 × 256 mm^2, matrix size 320 × 320, 256 transverse slices with a thickness of 0.8 mm, TE 3.10 ms, TR 2.54 s). High-resolution functional images were obtained with a multiband gradient EPI sequence (1.0 mm isotropic, FOV 170 × 170 mm^2, matrix size 170 × 170, partial Fourier 6/8, GRAPPA factor 2, 60 transverse slices with a thickness of 1.0 mm, manually aligned to the AC-PC line and centered to the habenula, multiband factor 2, interleaved slice order, TE 34.20 ms, TR 3.00 s). In order to allow for T1 equilibration, 3 initial dummy volumes were discarded.

As in Experiment 1, all image files were converted to NifTi format using the dcm2niix converter included in MRIcron and preprocessed using SPM 12. Functional MRI data of each participant were slice time-corrected, realigned to the first image, and coregistered to their individual anatomical image. Functional images were smoothed using a 2 mm Gaussian kernel. The anatomical image was used for segmentation and creation of individual flow fields using SPM's DARTEL algorithm.

2.1.2.4 Data Analysis

In order to replicate the results of Experiment 1, we again tested the Change hypothesis (see *Data Analysis* for Experiment 1 within this section). Separate GLMs were run for each participant and each session. All GLMs contained one regressor for the appearance of a change in luminance and for the appearance of an annulus, respectively, and regressors of no interest for the six motion parameters estimated during realignment, as well as time and dispersion derivatives for all regressors. For the purpose of investigating possible influences of circadian rhythm onto the effect of altered habenula activation in response to changes in luminance, images of the Change contrast acquired in morning and afternoon sessions were tested against each other in a repeated measurements design.

For each second level analysis, individual contrast images were spatially normalized to MNI space using SPM's DARTEL algorithm. All statistical analyses were performed on the normalized contrast images. Same habenula ROIs were used as in Experiment 1.

3.1 Results

3.1.1 Behavioral Results of the Counting Task (Experiments 1 and 2)

Participants were asked to count how often the fixated dot in the middle of the screen changed into an annulus of the same size throughout a run and to report that number verbally. The purpose of this simple counting task was to keep participants awake and alert, but it was not a factor of interest in the experimental design. Overall mean accuracy of the reported counting result was very high with 97.9% (*SD* = 3.4%) correct responses across all experiments and sessions. The lowest individual accuracy for a single participant was still comparatively high with 91%, hence we did not exclude any participant based on his or her behavioral performance. There were no significant differences between the session of Experiment 1 (97.4%, *SD* = 3.6%), the morning session of Experiment 2 (97.9%, *SD* = 3.7%) and the afternoon session of Experiment 2 (98.5%, *SD* = 2.9%), between subjects one-way ANOVA, $F(2,63) = 0.590$, $p = .557$.

3.1.2 Responsiveness to Changes in Luminance (Experiment 1 at 7T)

3.1.2.1 High vs. Low Luminance: Differential Contrast and Simple Contrast Hypothesis

For the Differential Contrast hypothesis, we aimed to investigate whether phases of high luminance (either 600, 1200, or 1800 cd/m^2) would lead to elevated levels of habenular activation compared to phases of low luminance (3 cd/m^2). In a subsequent step, we intended to identify the level of luminance intensity that yields the greatest activation for this contrast. However, contrary to our expectations, we did not find any significant activation differences in our ROI analyses for any contrast (600 vs. 3, 1200 vs. 3, 1800 vs. 3 cd/m^2), all *p*s > .05 (uncorrected), indicating that the habenula is not differentially activated by high and low retinal illumination.

For the Simple Contrast hypothesis, we again contrasted periods of high and low luminance, irrespective of the luminance level (600, 1200, or 1800 lux). By pooling the data for all luminance levels, the amount of data for our contrast of interest increased threefold compared to the Differential Contrast analysis, thus potentially resulting in an improved noise cancellation in the process of data averaging. Once again, we did not find significant activation changes in our ROI analysis, $p > .05$ (uncorrected), indicating activation levels of the habenula are not significantly different in phases of high retinal illumination compared to phases of low retinal illumination. A whole-brain analysis for the contrast high vs. low luminance preformed as a plausibility check revealed large clusters of activated voxels with significant differences in activation for the contrasts mentioned above, especially within visual cortices, *t*s > 5.31, $p_{FWE} < .01$ (see Fig. 2a).

3.1.2.2 Luminance Change Hypothesis

Another hypothesis was that the habenula may react differently to light in humans than it has been shown in rodents. For this reason, we also contrasted all occurrences of luminance changes irrespective of direction (high to low or low to high) against the habenula's mean activation. For this contrast, a ROI analysis revealed a significant deactivation of the right habenula in response to changes in luminance, $t(23) = 4.49$, $p_{FWE} = .034$ ($x = 4$, $y = –26$, $z = 2$; see Fig. 2b). A post-hoc analysis of the directional change (high to low vs. low to high) did not reveal significant differences, all p_{uncorr}s > .05, indicating this deactivation in response to luminance changes does not depend whether luminance changes from high to low or vice versa. Additionally, analogous to the response to changes in luminance, we performed a ROI analysis for potential effects of the annulus occurrence onto habenular activation in contrast to its mean activation, however, we did not find significant results, all p_{uncorr}s > .05.

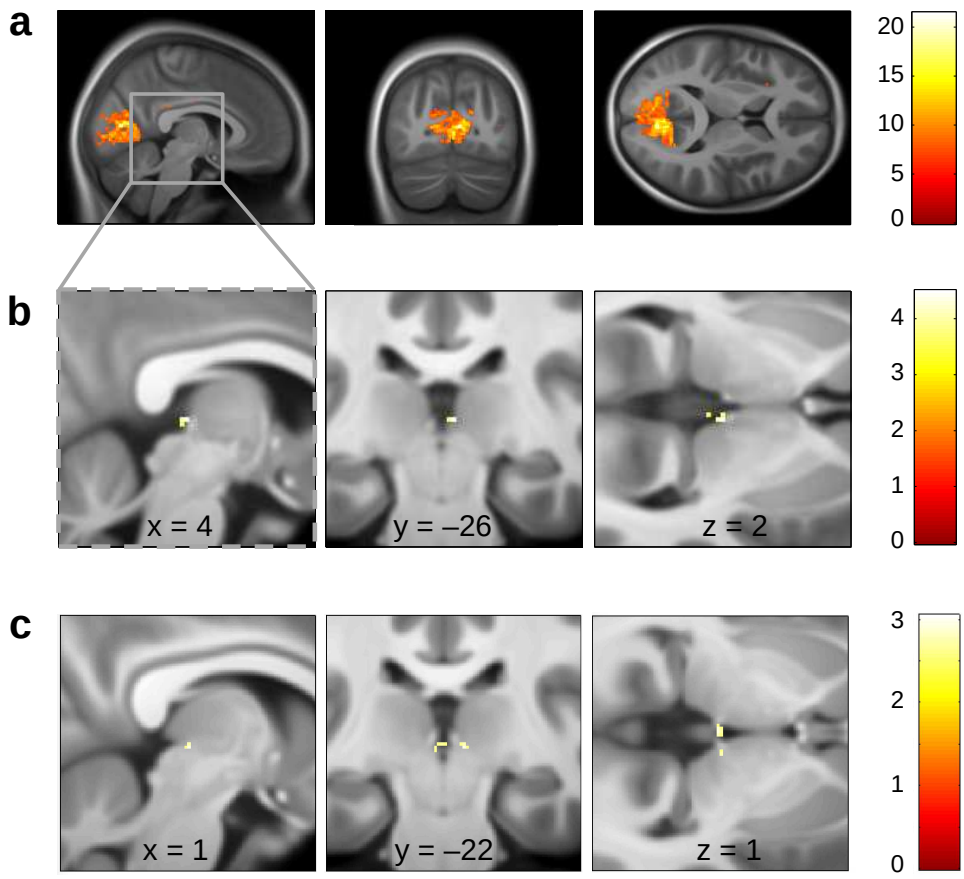


Figure 2. Results of the fMRI data analyses. Colored bars on the right side represent *t* values (**a** – **c**). Panel (**a**) depicts the results of the whole-brain analysis for the contrast high luminance > low luminance used as a plausibility check (see Results), with widespread activations across visual cortices (p_{FWE} < .01). The grey rectangle in (**a**) indicates the segment selected in (**b**) and (**c**), respective MNI coordinates of the slice are depicted at the bottom. In panel (**b**), colored voxels represent voxels with decreased activation in response to a change in luminance in Experiment 1 (luminance change < mean activation; p_{FWE} < .05). Panel (**c**) depicts voxels that are more strongly deactivated by a change in luminance in the morning than in the afternoon in Experiment 2 (morning < afternoon; p_{uncorr} < .01).

3.1.3 Replication of Luminance Change Hypothesis (Experiment 2 at 3T)

In order to replicate the results of Experiment 1, we again performed a ROI analysis for all occurrences of luminance changes against the mean activation. For both the

morning and the afternoon session, the habenula's activation was significantly decreased in response to a change in luminance, $t(20) = 4.22$, $p_{uncorr} < .001$ (peak: $x = –4$, $y = –26$, $z = 1$), and $t(20) = 3.77$, $p_{uncorr} < .001$ (peak: $x = –2$, $y = –22$, $z = 2$) , for the first and second session, respectively. By pooling the data of both sessions, we were able to observe a luminance-dependent habenular deactivation that survives a FWE correction approach, $t(20) = 4.60$, $p_{FWE} = .023$ (peak: $x = –2$, $y = –21$, $z = 2$), indicating habenular responses to changes in luminance can be successfully demonstrated in 3T data as well.

3.1.4 Circadian Influences (Experiment 2 at 3T)

3.1.4.1 Chronotype Questionnaire

D-MEQ scores at the time of recruitment were used to control for interindividual differences in circadian rhythmicity by scheduling participants with higher scores for earlier MR appointments and participants with lower cores for later MR appointments (see Methods, Experiment 2, Stimuli and Procedure). For the D-MEQ scores used for scheduling, the mean score of the Early group (54.6 points, $SD = 10.0$ points, $N = 11$) was significantly higher than the mean of the Late group (45.5 points, $SD = 6.2$ points, $N = 10$), independent samples t test, $t(19) = 2.442$, $p = .025$. Additionally, D-MEQ scores at the time of recruitment and the time of MR data collection were highly correlated, Pearson correlation coefficient, $r = .916$, $p = 5.706 \times 10^{-9}$, indicating reliable D-MEQ scores for our sample. It is therefore reasonable to assume our division of participants into 2 groups with higher and lower scores, respectively, was still preserved at the time of data collection.

3.1.4.2 Circadian Influences on the Habenula's Response to Changes in Luminance

In order to investigate circadian influences, we tested morning against afternoon sessions in a within-subject design. Specifically, we used the contrast images obtained for the Luminance Change hypothesis and compared morning vs. afternoon session in order to investigate any circadian modulation of the BOLD signal. We found that the habenular deactivation due to changes in luminance is more pronounced in morning sessions than in afternoon sessions, with two clusters being more deactivated in the morning, $t(20) = 3.04$, $p_{uncorr} = .003$ (peak: $x = 1$, $y = –22$, $z = 1$; see Fig. 2c), and $t(20) = 2.56$, $p_{uncorr} = .009$ (peak: $x = –4$, $y = –20$, $z = 4$). Notably, influences of session order were counterbalanced in the data acquisition protocol (half of the participants attended the morning session first and the afternoon session second, the other half vice versa).

4.1 Discussion

4.1.1 The Human Habenula is Responsive to Changes in Luminance

Based on findings from animal research, we hypothesized the human habenula would exhibit increased activation in phases of high luminance compared to phases of low luminance. However, contrary to our expectations, our data do not provide evidence for this hypothesis. Even though a considerably wide range of luminance intensities had been included in the paradigm, none of the contrasts resulted in sufficiently separable activation patterns. Thus, our data do not provide evidence for significant differences between any of the high vs. low luminance contrasts. However, our results demonstrate that the human habenula is in fact responsive to

light, but in a different way than we hypothesized based on animal research: In humans, when a change in luminance occurs, a decrease of habenular activation can be observed, irrespective of the direction of luminance change. Although this finding was not initially expected, we were able to replicate this effect it in a second, independent sample of participants. Importantly, an attention-related task did not reveal any changes in activation patterns within the same ROI, underlining the relative specificity of the light-dependent deactivation. It is therefore reasonable to conclude that an undirected change of luminance generally evokes a decrease of activation in the human habenula. Most likely, this decrease is the result of the temporal courses of activation and deactivation of the light activated and deactivated neurons that together form a net activation gap compared to the average habenular activation, which could be expressed in a temporary decline in the hemodynamic response. From a methodical perspective, we were also able to demonstrate that these differences in the habenular BOLD signal can not only be observed high field strengths (7T) but at medium field strengths (3T) as well.

Since our data do not show evidence for the activation patterns we initially hypothesized based on findings reported by Zhao and Rusak (2005), one should consider the possibility that light-dependent signals are in fact processed differently in the human and in the rat habenular complex, respectively. Taking into account that rats are primarily nocturnal animals that are most active at night whereas humans are considered to be diurnal, meaning primarily active during the daylight hours, it seems possible that there are hard-wired differences in neural processing of light signals. Based on the different results gained from animal and human research, our data exemplify the importance of human neuroscientific research in even very basic domains such as processing of exposure light stimuli, e.g. regarding chronobiological

entrainment in night shift workers (Crowley et al., 2003, Kantermann et al., 2010) and jet lag (Sack, 2010, LeGates et al., 2012).

4.1.2 Circadian Rhythmicity Presumably Modulates the Habenula's Response to Changes in Luminance

Considering the finding that photic responses of rat habenular neurons were shown to differ as a function of day time, we hypothesized that the human habenula's response to light stimuli might also be modulated in a circadian fashion. In order to test this hypothesis, we used the same paradigm as in the previous experiment and acquired fMRI data from a morning and an afternoon session. Indeed, we found that the habenular deactivation showed a tendency for modulation by the phase of the circadian rhythm: The decrease of habenular activation in response to a change in luminance trended to be greater in the morning session than in the afternoon session, indicating that the phase of the circadian rhythm should be considered as an influencing factor in the processing of light stimuli in the human habenula. This finding is in line with the results of Zhao and Rusak (2005) who reported circadian influences in the firing patterns of habenular neurons in rats.

For the interpretation of this finding, it appears important to briefly point out key aspects of our recruitment strategy: Firstly, we collected data of morning and afternoon sessions of the same participants, enabling us to use within-subject significance testing that yields a higher power due to the reduction of interindividual error variance. Secondly, we counterbalanced the order of morning and afternoon sessions across participants, hence it is unlikely that sequence effects have to be considered as an alternative explanation of the effect. Thirdly, we used an established questionnaire to account for interindividual differences in endogenous

circadian rhythmicity. There are different chronotypes (Roenneberg et al., 2003), e.g. morning types (called “larks” or “early birds”) and evening types (“owls”) whose phase of the endogenous circadian rhythm is considered to be shifted along the time-of-the-day axis. Therefore, testing one participant at the same time of the day as another participant does not necessarily mean their circadian clocks correspond. In order to achieve a more precise phase congruence of the endogenous and exogenous circadian rhythm than it would be found in a regular sample of participants, we scheduled appointments according to their chronotype: “larks” were tested earlier than “owls”, resulting in a more homogeneous phase of the participants’ circadian rhythms at the time of data acquisition. Taken together, we carefully ensured minimizing influences of other factors that potentially give rise to alternative interpretations of our results other than in a framework of circadian rhythmicity. Our observation of circadian signal modulation fits well with the suggestion that the habenula belongs to the network that is responsible for timekeeping (Guilding & Piggins, 2007).

In regard to why the deactivation of the habenula seems to be stronger in morning than in afternoon sessions, one could speculate that the amount of light exposure since waking up is one of the important factors. There might be a stronger activation of brain regions in response to retinal illumination shortly after waking up than compared to a later time of the day when there has already been a greater cumulative exposure to light. In support of this interpretation, it has been reported that humans are more sensitive to light stimuli during the biological night than in the middle of the biological day (Czeisler et al., 1989; Jewett et al., 1997). Additionally, the amount of melatonin suppression after presentation of a light stimulus has been used as a quantifiable experimental measure of responsiveness to a light stimulus (Lewy et al., 1980). Exposure to a higher intensity of light over a period of several

hours results in a significantly smaller melatonin suppression than after exposure to a smaller amount of light over the same period of time (Smith et al., 2004). Hence, it seems reasonable to assume that the cumulative amount of exposure to light after waking up has an influence on the processing of light stimuli in the paradigm we used, resulting in a more pronounced deactivation in morning sessions.

One factor that might limit the validity of a functional explanation of the circadian differences in the BOLD signal rises from the observation that there is a circadian modulation of somatic functions such as blood pressure (Curtis et al., 2007) or metabolism (Marcheva et al., 2013) that could account for differences in the BOLD signal between morning and afternoon sessions without reflecting functional differences. However, like any other fMRI study investigating circadian effects, we are not able to disentangle these physiological effects without the use of additional external measures.

4.1.3 Conclusions and Outlook

To our knowledge, we provide first evidence that the human habenula is photoresponsive: When a change in luminance occurs, a decrease in habenular activation can be observed using fMRI with a field strength of 7T and 3T. Importantly, this activation pattern could not be predicted from findings in animal research, but was replicated in a second independent sample, thus underlining the importance of basic research in human participants. We were furthermore able to show that this change in activation was stronger in morning than in afternoon hours, indicating a circadian modulation of the habenula's hemodynamic response to changes in luminance. Further research is needed in order to gain a better understanding of the contributions of the habenula to the regulation of circadian rhythms in humans and

the habenula's interplay with other structures that are related to circadian entrainment such as the pineal gland and the suprachiasmatic nucleus. Moreover, our findings allow to hypothesize about habenular contributions to clinical phenomena that are associated with disruptions in circadian rhythms, for example in depressive conditions, especially in seasonal affective disorder, or in shift work sleep disorder.

Acknowledgements

This work was funded by the Deutsche Forschungsgemeinschaft (DFG), grant SFB 779.

References

Aizawa, H. (2013). Habenula and the asymmetric development of the vertebrate brain. Anatomical science international, 88(1), 1–9. https://doi.org/10.1007/s12565-012-0158-6

Andres, K. H., Düring, M. V., & Veh, R. W. (1999). Subnuclear organization of the rat habenular complexes. The Journal of Comparative Neurology, 407(1), 130–150.

Bell-Pedersen, D., Cassone, V. M., Earnest, D. J., Golden, S. S., Hardin, P. E., Thomas, T. L., & Zoran, M. J. (2005). Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nature Reviews Genetics, 6, 544–556. https://doi.org/10.1038/nrg1633

Bernstein, H.-G., Hildebrandt, J., Dobrowolny, H., Steiner, J., Bogerts, B., & Pahnke, J. (2016). Morphometric analysis of the cerebral expression of ATP-binding cassette transporter protein ABCB1 in chronic schizophrenia: Circumscribed deficits in the habenula. Schizophrenia research, 177(1-3), 52–58. https://doi.org/10.1016/j.schres.2016.02.036

Brett, M., Anton, J.-L., Valabregue, R., & Poline, J.-B. (June 2--6, 2002). Region of interest analysis using an SPM toolbox. [abstract] Presented at the 8th

International Conference on Functional Mapping of the Human Brain, Sendai, Japan.

Brown, P. L., Palacorolla, H., Brady, D., Riegger, K., Elmer, G. I., & Shepard, P. D. (2017). Habenula-Induced Inhibition of Midbrain Dopamine Neurons Is Diminished by Lesions of the Rostromedial Tegmental Nucleus. Journal of Neuroscience, 37(1), 217–225. https://doi.org/10.1523/JNEUROSCI.1353-16.2016

Buijs, R. M. (1978). Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. Cell and Tissue Research, 192(3). https://doi.org/10.1007/BF00212323

Carlson, J., Noguchi, K., & Ellison, G. (2001). Nicotine produces selective degeneration in the medial habenula and fasciculus retroflexus. Brain Research, 906(1-2), 127–134. https://doi.org/10.1016/S0006-8993(01)02570-7

Crowley, S. J., Lee, C.; Tseng, C. Y., Fogg, Louis F., Eastman, C. I. (2003). Combinations of Bright Light, Scheduled Dark, Sunglasses, and Melatonin to Facilitate Circadian Entrainment to Night Shift Work. *Journal of Biological Rhythms, 18*(6), 513—523. https://doi.org/10.1177/0748730403258422

Curtis A. M., Cheng Y., Kapoor S., Reilly D., Price T. S., & Fitzgerald G. A. (2007). Circadian variation of blood pressure and the vascular response to asynchronous stress. Proc Natl Acad Sci U S A, 104, 3450–3455.

Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. F., Brown, E. N., & Ronda, J. M. (1989). Bright light induction of strong (type 0) resetting of the human circadian pacemaker. Science, 244(244), 1328–1333. https://doi.org/10.1126/science.2734611

De Vries, G. J., Buijs, R. M., & Swaab, D. F. (1981). Ontogeny of the vasopressinergic neurons of the suprachiasmatic nucleus and their extrahypothalamic projections in the rat brain--presence of a sex difference in the lateral septum. Brain Research, 218(1-2), 67–78.

Ellison, G. (1992). Continuous amphetamine and cocaine have similar neurotoxic effects in lateral habenular nucleus and fasciculus retroflexus. Brain Research, 598(1-2), 353–356. https://doi.org/10.1016/0006-8993(92)90207-P

Fakhoury, M. (2018). The dorsal diencephalic conduction system in reward processing: Spotlight on the anatomy and functions of the habenular complex. Behavioural Brain Research, 348, 115–126. https://doi.org/10.1016/j.bbr.2018.04.018

Fukada, Y., & Okano, T. (2002). Circadian Clock System in the Pineal Gland. Molecular Neurobiology, 25, 19–30. https://doi.org/10.1385/MN:25:1:019

Griefahn, B., Kunemund, C., Brode, P., & Mehnert, P. (2001). Zur Validität der deutschen Übersetzung des Morningness-Eveningness-Questionnaires von Horne und Östberg. Somnologie, 5(2), 71–80. https://doi.org/10.1046/j.1439-054X.2001.01149.x

Guglielmotti, V., & Cristino, L. (2006). The interplay between the pineal complex and the habenular nuclei in lower vertebrates in the context of the evolution of cerebral asymmetry. Brain research bulletin, 69(5), 475–488. https://doi.org/10.1016/j.brainresbull.2006.03.010

Guilding, C., Hughes, A. T. L., & Piggins, H. D. (2010). Circadian oscillators in the epithalamus. Neuroscience, 169(4), 1630–1639. https://doi.org/10.1016/j.neuroscience.2010.06.015

Guilding, C., & Piggins, H. D. (2007). Challenging the omnipotence of the suprachiasmatic timekeeper: Are circadian oscillators present throughout the mammalian brain? European Journal of Neuroscience, 25(11), 3195–3216. https://doi.org/10.1111/j.1460-9568.2007.05581.x

Herkenham, M., & Nauta, W. J. H. (1977). Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study, with a note on the fiber-of-passage problem. The Journal of Comparative Neurology, 173(1), 123–145. https://doi.org/10.1002/cne.901730107

Herkenham, M., & Nauta, W. J. H. (1979). Efferent connections of the habenular nuclei in the rat. The Journal of Comparative Neurology, 187(1), 19–47. https://doi.org/10.1002/cne.901870103

Hikosaka, O. (2010). The habenula: From stress evasion to value-based decision-making. Nature Reviews Neuroscience, 11(7), 503–513. https://doi.org/10.1038/nrn2866

Hong, S., Jhou, T. C., Smith, M., Saleem, K. S., & Hikosaka, O. (2011). Negative reward signals from the lateral habenula to dopamine neurons are mediated by rostromedial tegmental nucleus in primates. Journal of Neuroscience, 31(32), 11457–11471. https://doi.org/10.1523/JNEUROSCI.1384-11.2011

In, M.-H., & Speck, O. (2012). Highly accelerated PSF-mapping for EPI distortion correction with improved fidelity. Magnetic Resonance Materials in Physics,

Biology and Medicine, 25(3), 183–192. https://doi.org/10.1007/s10334-011-0275-6

Jewett, M. E., Rimmer, D. W., Duffy, J. F., Klerman, E. B., Kronauer, R. E., & Czeisler, C. A. (1997). Human circadian pacemaker is sensitive to light throughout subjective day without evidence of transients. American Journal of Physiology, 273(5), R1800-R1809. https://doi.org/10.1152/ajpregu.1997.273.5.R1800

Jhou, T. C., Geisler, S., Marinelli, M., Degarmo, B. A., & Zahm, D. S. (2009). 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. The Journal of Comparative Neurology, 513(6), 566–596. https://doi.org/10.1002/cne.21891

Kantermann, T., Juda, M., Vetter, C., & Roenneberg, T. (2010). Shift-work research: Where do we stand, where should we go? Sleep and Biological Rhythms, 8(2), 95-105. https://doi.org/10.1111/j.1479-8425.2010.00432.x

Kasper, L., Bollmann, S., Diaconescu, A. O., Hutton, C., Heinzle, J., Iglesias, S.,. . . Stephan, K. E. (2017). The PhysIO Toolbox for Modeling Physiological Noise in fMRI Data. Journal of neuroscience methods, 276, 56–72. https://doi.org/10.1016/j.jneumeth.2016.10.019

Kumar, P., Goer, F., Murray, L., Dillon, D. G., Beltzer, M. L., Cohen, A. L.,. . . Pizzagalli, D. A. (2018). Impaired reward prediction error encoding and striatal-midbrain connectivity in depression. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. Advance online publication. https://doi.org/10.1038/s41386-018-0032-x

Lawson, R. P., Drevets, W. C., & Roiser, J. P. (2013). Defining the habenula in human neuroimaging studies. NeuroImage, 64, 722–727. https://doi.org/10.1016/j.neuroimage.2012.08.076

Lecourtier, L., & Kelly, P. H. (2007). A conductor hidden in the orchestra? Role of the habenular complex in monoamine transmission and cognition. Neuroscience & Biobehavioral Reviews, 31(5), 658–672. https://doi.org/10.1016/j.neubiorev.2007.01.004

LeGates, T. A., Altimus, C. M., Wang, H., Lee, H.-K., Yang, S., … Hattar, S. (2012). Abberant light directly impairs mood and learning through melanopsin-expressing neurons. Nature, 491, 594—598. http://dx.doi.org/10.1038/nature11673

Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A., & Markey, S. P. (1980). Light suppresses melatonin secretion in humans. Science, 210, 1267–1269. https://doi.org/10.1126/science.7434030

Li, B., Piriz, J., Mirrione, M., Chung, C., Proulx, C. D., Schulz, D.,. . . Malinow, R. (2011). Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. Nature, 470(7335), 535–539. https://doi.org/10.1038/nature09742

Matsumoto, M., & Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. Nature, 447(7148), 1111–1115. https://doi.org/10.1038/nature05860 4

Marcheva, B., Ramsey, K. M., Peek, C. B., Affinati, A., Maury, E., & Bass, J. (2013). Circadian clocks and metabolism. In A. Kramer & M. Merrow (Eds.), Circadian Clocks (pp. 127–155). Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-25950-0_6

Oh, S.-H., Chung, J.-Y., In, M.-H., Zaitsev, M., Kim, Y.-B., Speck, O., & Cho, Z.-H. (2012). Distortion correction in EPI at ultra-high-field MRI using PSF mapping with optimal combination of shift detection dimension. Magnetic Resonance in Medicine, 68(4), 1239–1246. https://doi.org/10.1002/mrm.23317

Qu, T., Dong, K., Sugioka, K., & Yamadori, T. (1996). Demonstration of direct input from the retina to the lateral habenular nucleus in the albino rat. Brain Research, 709(2), 251–258. https://doi.org/10.1016/0006-8993(95)01306-7

Reuss, S., & Decker, K. (1997). Anterograde tracing of retinohypothalamic afferents with Fluoro-Gold. Brain Research, 745(1-2), 197–204.

Roenneberg, T., Kuehnle, T., Juda, M., Kantermann, T., Allebrandt, K., Gordijn, M., & Merrow, M. (2007). Epidemiology of the human circadian clock. Sleep Medicine Reviews, 11(6), 429–438. https://doi.org/10.1016/j.smrv.2007.07.005

Roenneberg, T., Wirz-Justice, A., & Merrow, M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. Journal of Biological Rhythms, 18(1), 80–90. https://doi.org/10.1177/0748730402239679

Rønnekleiv, O. K., Kelly, M. J., & Wuttke, W. (1980). Single unit recordings in the rat pineal gland: Evidence for habenulo-pineal neural connections. Experimental Brain Research, 39(2), 187–192.

Sack, R. (2010). Jet Lag. New England Journal of Medicine, 362, 440—447. https://doi.org/10.1056/NEJMcp0909838

Salas, R., Baldwin, P., Biasi, M. de, & Montague, P. R. (2010). BOLD responses to negative rerward prediction errors in human habenula. Frontiers in human neuroscience, 4. https://doi.org/10.3389/fnhum.2010.00036

Sandyk, R. (1992). Pineal and habenula calcification in schizophrenia. The International journal of neuroscience, 67(1-4), 19–30.

Smith, K. A., Schoen, M. W., & Czeisler, C. A. (2004). Adaptation of human pineal melatonin suppression by recent photic history. Journal of Clinical Endocrinology and Metabolism, 89(7), 3610–3614. https://doi.org/10.1210/jc.2003-032100

Sartorius, A., & Henn, F. A. (2007). Deep brain stimulation of the lateral habenula in treatment resistant major depression. Medical Hypotheses, 69(6), 1305–1308. https://doi.org/10.1016/j.mehy.2007.03.021

Ullsperger, M., & von Cramon, D. Y. (2003). Error monitoring using external feedback: Specific roles of the habenular complex, the reward system, and the cingulate motor area revealed by functional magnetic resonance imaging. The Journal of neuroscience : the official journal of the Society for Neuroscience, 23(10), 4308–4314

Van de Moortele, P.-F., Auerbach, E. J., Olman, C., Yacoub, E., Ugurbil, K., & Moeller, S. (2009). T1 weighted brain images at 7 Tesla unbiased for Proton Density, T2* contrast and RF coil receive B1 sensitivity with simultaneous vessel visualization. NeuroImage, 46(2), 432–446. https://doi.org/10.1016/j.neuroimage.2009.02.009

Velasquez, K. M., Molfese, D. L., & Salas, R. (2014). The role of the habenula in drug addiction. Frontiers in Human Neuroscience, 8, 174. https://doi.org/10.3389/fnhum.2014.00174

Winter, C., Vollmayr, B., Djodari-Irani, A., Klein, J., & Sartorius, A. (2011). Pharmacological inhibition of the lateral habenula improves depressive-like behavior in an animal model of treatment resistant depression. Behavioural Brain Research, 216(1), 463–465. https://doi.org/10.1016/j.bbr.2010.07.034

Wirtshafter, D., Asin, K. E., & Pitzer, M. R. (1994). Dopamine agonists and stress produce different patterns of Fos-like immunoreactivity in the lateral habenula. Brain Research, 633(1-2), 21–26. https://doi.org/10.1016/0006-8993(94)91517-2

Yang, L.-M., Hu, B., Xia, Y.-H., Zhang, B.-L., & Zhao, H. (2008). Lateral habenula lesions improve the behavioral response in depressed rats via increasing the

serotonin level in dorsal raphe nucleus. Behavioural Brain Research, 188(1), 84–90. https://doi.org/10.1016/j.bbr.2007.10.022

Zhang, L., Wang, H., Luan, S., Yang, S., Wang, Z., Wang, J., & Zhao, H. (2017). Altered Volume and Functional Connectivity of the Habenula in Schizophrenia. Frontiers in human neuroscience, 11, 636. https://doi.org/10.3389/fnhum.2017.00636

Zhao, H., & Rusak, B. (2005). Circadian firing-rate rhythms and light responses of rat habenular nucleus neurons in vivo and in vitro. Neuroscience, 132(2), 519–528. https://doi.org/10.1016/j.neuroscience.2005.01.012