Light Affects Mood and Learning through Distinct Retina-Brain Pathways

Graphical Abstract

Highlights

- Distinct ipRGC subpopulations drive the effects of light on learning and mood
- SCN-projecting ipRGCs affect learning without disrupting the central pacemaker
- The ipRGC-PHb pathway drives the light-mediated mood alterations
- Thalamic PHb is integrated in a distinctive circuitry with mood-regulating centers

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In Brief

The effects of light on learning and mood via intrinsically photosensitive retinal ganglion cells involve a pacemaker-independent role for the suprachiasmatic nucleus as well as distinct circuitry in a region of the thalamus called the perihabenular nucleus.
Light Affects Mood and Learning through Distinct Retina-Brain Pathways

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https://doi.org/10.1016/j.cell.2018.08.004

SUMMARY

Light exerts a range of powerful biological effects beyond image vision, including mood and learning regulation. While the source of photic information affecting mood and cognitive functions is well established, viz. intrinsically photosensitive retinal ganglion cells (ipRGCs), the central mediators are unknown. Here, we reveal that the direct effects of light on learning and mood utilize distinct ipRGC output streams. ipRGCs that project to the suprachiasmatic nucleus (SCN) mediate the effects of light on learning, independently of the SCN’s pacemaker function. Mood regulation by light, on the other hand, requires an SCN-independent pathway linking ipRGCs to a previously unrecognized thalamic region, termed perihabenular nucleus (PHb). The PHb is integrated in a distinctive circuitry with mood-regulating centers and is both necessary and sufficient for driving the effects of light on affective behavior. Together, these results provide new insights into the neural basis required for light to influence mood and learning.

INTRODUCTION

The constancy of the solar cycle as a signal for the regulation of mammalian behavior is manifested, among other things, in the powerful effects that light exerts on mood and cognition. These effects have been documented in laboratory animals (Bedrosian et al., 2011; LeGates et al., 2012), as well as in humans (Vandewalle et al., 2010). Depressive symptoms and cognitive dysfunction linked to light can be brought on by natural conditions or by people’s own agency across time zones and experiencing jet lag or in shift-work (Jaeger et al., 2006; Kurlansik and Ibey, 2012; Roh et al., 2016; Scott et al., 1997). The magnitude of these mood effects is large as ~20% of the general population is affected (Melrose, 2015) with symptoms being fairly common, including anhedonia and feelings of helplessness (Russo and Nestler, 2013).

In mammals, light detection occurs exclusively in the retina (Hattar et al., 2003; Wässle, 2004). In addition to classical rod and cone photoreceptors, a subpopulation of retinal ganglion cells (RGCs) that express the photopigment melanopsin (Opn4), making them intrinsically photosensitive (ip)RGCs, constitute the third type of photoreceptors (Berson et al., 2002; Hattar et al., 2002; Provencio et al., 2000). At present, 5 subtypes (M1–M5) of ipRGCs have been described (Ecker et al., 2010; Schmidt et al., 2011). Their central projections differ amply, reflecting in this variety the multiform nature of the non-image-forming visual functions they participate in such as circadian photoentrainment and sleep regulation (Altimus et al., 2008; Hattar et al., 2006). A major ipRGC target is the hypothalamic suprachiasmatic nucleus (SCN), which houses a central pacemaker that orchestrates peripheral clocks to drive circadian rhythmic coherence (Morin, 2013). Genetic ablation of ipRGCs completely abrogates light reception at the SCN level, suggesting that ipRGCs constitute the only conduit for light input to modulate circadian brain functions (Güler et al., 2008).

Notably, the regulation of mood and learning by light is also absent in mice where ipRGCs are ablated (LeGates et al., 2012, 2014), indicating that ipRGCs are the main sensory channel driving these behavioral effects. The ipRGC central targets mediating these effects, however, remain unknown. Here, we show that a subset of ipRGCs, that are defined by the lack of Brn3b expression and project to the SCN, are sufficient to drive light-mediated cognitive deficits without disrupting the SCN clockwork machinery. An SCN-independent pathway mediates light-induced mood changes through ipRGC input to the perihabenular nucleus (PHb) of the dorsal thalamus. PHb neurons project to well-characterized mood-regulating centers. Furthermore, the PHb is both necessary and sufficient for driving the effects of light on affective behavior. These findings reveal two distinct retinal-brain pathways that mediate the direct effects of light on mood and cognition.
RESULTS

The SCN-Projecting ipRGCs Drive the Effects of Light on Learning

The use of an ultradian light cycle (T7 cycle; alternating 3.5-hr periods of light and darkness) allowed us to determine the direct effects of light on mood and learning (LeGates et al., 2012). To investigate whether light input to the SCN is sufficient to drive the behavioral alterations induced by the T7 cycle, we used a previously described mouse line, Opn4Cre/+;Brn3bDTA/+, in which Brn3b(−) ipRGCs that innervate the SCN survive, whereas Brn3b(+) ipRGCs projecting outside the SCN are substantially ablated (Figures 1A–1C and S1A–S1C). Opn4Cre/+;Brn3bDTA/+, mice photoentrained under a normal light/dark cycle (T24 cycle), and their circadian periods were lengthened when exposed to the T7 cycle (Figures 1D and 1E). Thus, the general circadian responses are intact in these animals.

Under the T24 cycle, Opn4Cre/+ and Opn4Cre/+;Bm3bDTA/+ mice showed similar learning and cognitive functions (Figures 1F–1H; novel object recognition [NOR] test: p = 0.57; Morris water maze [MWM], test trial: p = 0.5, by Tukey’s test), indicating that the ablation of Brn3b(+) ipRGCs did not affect the cognitive functions of these animals. When exposed to the T7 cycle for
2 weeks, Opn4<sup>Cre/+</sup>;Bm3b<sup>DTA/+</sup> animals displayed cognitive deficits, similar to those observed in T7 cycle-housed control (Opn4<sup>Cre/+</sup>) mice (Figures 1F–1H). Specifically, T7 cycle-housed mice spent significantly less time exploring the novel object than mice housed under the T24 cycle (Figures 1F and S1D). In the MWM, T7 cycle-housed mice exhibited significant deficits during training, test, and reverse phases, relative to mice housed under the T24 cycle (Figures 1G, 1H, S1E, and S1F). For both behavioral tests, no significant differences were found in the results obtained from Opn4<sup>Cre/+</sup> and Opn4<sup>Cre/+;Brn3b<sup>DTA/+</sup></sup> mice exposed to the T7 cycle (Figures 1F–1H; NOR: p = 0.45; MWM, test trial: p = 0.86; by Tukey’s test).

In line with the behavioral studies, hippocampal long-term potentiation (LTP) was significantly attenuated in both Opn4<sup>Cre/+</sup> (control) and Opn4<sup>Cre/+;Bm3b<sup>DTA/+</sup></sup> mice when housed under the T7 cycle, relative to T24-housed groups (Figures 1I and S1G). The LTP alterations were specific to activity-dependent potentiation by the theta-burst stimulus, because other aspects of transmission were normal (Figures S1H and S1I). Together, these results highlight the sufficiency of the Bm3b<sup>-/-</sup> ipRGCs in mediating light-induced LTP and cognitive deficits.

### The T7 Cycle Alters the Photoresponsiveness of the SCN

We previously found that the rhythmic expression of the clock gene period2 (PER2) in the SCN was unperturbed by the T7 cycle (LeGates et al., 2012). This raises a conundrum: if the SCN remains responsive to light under the T7 cycle, why are PER2 levels unaffected under a recurring light activation? To address this question, we first tested whether the photic responsiveness of SCN neurons is affected under the T7 cycle, by evaluating the phosphorylation of CREB (cAMP response element-binding protein), one of the first steps in gene induction in response to light (Chawla, 2002). Light-induced pCREB levels were similar in the SCN from animals maintained under T24 and T7 cycles (Figures 2A–2D), indicating that the SCN is still responsive to light in T7 cycle-housed mice. Interestingly, it was recently shown that the SCN can act not only as a circadian pacemaker, but also as a relay for light input to peripheral oscillators (Izumo et al., 2014). To explore the role of the SCN as a light relay versus a circadian pacemaker, we analyzed the effects of T7 cycle on Period1 (PER1), a clock gene that shows both a robust rhythmic expression and a fast response to light (Travnickova-Bendova et al., 2002). We found that under T7 cycle, the expression levels of PER1 were rhythmic in the SCN (Figures 2E and 2F), whereas the acute effects of light on PER1 induction were abrogated (Figures 2G and 2H). Combined, the effects of light on pCREB levels and PER1 induction indicate that the photic responsiveness of the SCN is distinctively altered by the T7 cycle.

To further investigate the overall changes in SCN photic responsiveness, we performed RNA-sequencing (RNA-seq) on isolated SCN samples (Figure 2A). We found that 29 genes were differentially regulated between T24 and T7 cycle groups (false discovery rate [FDR]-adjusted p < 0.01, Figure S2A) including a striking depletion of classical immediate-early genes in T7 cycle-housed animals (Figures 2I and 2J). Gene ontology analysis of differentially regulated transcripts revealed a depletion of genes involved in transcription as well as Ca<sup>2+</sup> and cAMP responses, further suggesting that the T7 cycle dramatically alters the photic responsiveness of the SCN (Figure S2B).

In support of the RNA-seq data, we found a significant decrease in light-mediated c-Fos induction in SCN neurons under the T7 cycle, compared with T24-housed mice (Figures S3A and S3B), c-Fos induction in the SCN requires the phosphorylation of both CREB and ELK-1, leading to the recruitment of CREB-binding protein, resulting in gene transcription through the acetylation and phosphorylation of histones, including histone-3 (H3) (Chawla, 2002; Kako and Ishida, 1998). In contrast to pCREB, the light-induced pELK-1 and pH3 levels were significantly reduced in SCN neurons from animals housed under the T7 cycle (Figures S3A, S3C, and S3D). Combined, these data show that the SCN becomes refractory to light-induced transcriptional changes that would otherwise impinge on the clockwork machinery under the T7 cycle.

### The Effects of Light on Mood Are Independent of the SCN

To investigate whether Brn3b<sup>-/-</sup> SCN-projecting ipRGCs also affect mood-related behaviors, mice were housed under the T24 or T7 cycles, and the sucrose preference test (SPT), tail suspension test (TST), and forced-swim test (FST) were performed. Under normal light conditions, Opn4<sup>Cre/+</sup> (control) and Opn4<sup>Cre/+;Bm3b<sup>DTA/+</sup></sup> mice showed no significant differences in their behavioral responses (Figure 3; SPT, p = 0.600; TST, p = 0.921; FST, p = 0.859; by Tukey’s test). Remarkably, ablating ipRGC projections outside the SCN abolished mood alterations induced by the T7 cycle. Specifically, Opn4<sup>Cre/+;Bm3b<sup>DTA/+</sup></sup> mice exposed to the T7 cycle showed no significant differences in the sucrose preference index (Figure 3A), immobility time during the TST (Figure 3B) and FST (Figure 3C), compared to mice housed under the T24 cycle. In addition, under the T7 cycle, results obtained from Opn4<sup>Cre/+;Brn3b<sup>DTA/+</sup></sup> mice were significantly different compared with Opn4<sup>Cre/+</sup> mice (Figure 3; SPT, p < 0.001; TST, p < 0.05; FST, p < 0.001; by Tukey’s test). Thus, ipRGC pathways, distinct from the SCN, must be responsible for driving light regulation of mood.

### PHb Neurons Receive Direct Input from ipRGCs

Among the ipRGC targets that might mediate the perturbation of affective behavior by unnatural light cycles, a region of the dorsal thalamus, adjacent to the epithalamic lateral habenula (LHb), is of particular interest. This region, which was previously termed perihabenular region (PHb), has been shown to receive retinal innervation (Hattar et al., 2006; Morin and Studholme, 2014). We first evaluated the light-responsiveness of the PHb. Wild-type (WT) mice showed a robust light-mediated c-Fos induction throughout the PHb (Figures 4A and 4C), whereas mice lacking ipRGC innervation to the PHb (Opn4<sup>Cre/+;Bm3b<sup>DTA/+</sup></sup>) showed no induction of c-Fos (Figure S3E). We found that T7-housed WT mice showed a normal c-Fos induction in the PHb after a light pulse exposure (Figures 4B and 4C), suggesting that, contrary to the SCN, the PHb photoresponsiveness is unaltered under T7 cycle.

PHb neurons show a robust rhythmic expression of the clock gene PER2 under normal light conditions (Figure 4D). The c-Fos induction observed in PHb neurons raises the possibility that...
Figure 2. Photic Responsiveness and Gene Expression in the SCN

(A) T24- or T7-housed mice were kept in darkness for 1 day, exposed to a light pulse, and immediately perfused for assess pCREB levels (green star), perfused after 90 min for PER1 levels (red star), or after 60 min for SCN dissections (blue star).

(B) Scheme showing a coronal brain section at SCN level.

(C and D) A significant increase in pCREB levels were observed in both light pulse (LP)-treated groups, versus no light pulse (noLP) controls. Data are mean ± SEM. **p<0.01; by Tukey’s test (n = 5–6 mice).

(E and F) No significant differences in the rhythmicity of PER1 levels were observed between groups (F). Data are mean ± SEM; by Student’s t test (n = 4–5 mice).

(G and H) The exposure of mice to the T7 cycle affected the light-mediated induction of PER1 in the SCN. Data are mean ± SEM. **p<0.01; by Tukey’s test (n = 5–6 mice).

(I) Heatmap of relative gene expression levels for selected immediate-early genes.

(J) Fold change in expression level of immediate-early genes in T7 versus T24 samples (T24: n = 3, T7: n = 4 replicates; SCN tissue was pooled from 3 mice per replicate, FDR-adjusted p < 0.01 for all samples).

3v, third ventricle; ox, optic chiasm; DD, constant darkness; ZT, zeitgeber time. Scale bars, 100 μm.

See also Figures S2 and S3.
under the T7 cycle, neurons may be abnormally (and chronically) activated by the appearance of light every 3.5 hr, which would lead to alterations in PER2 rhythmic expression. Indeed, when WT mice were housed under the T7 cycle, PER2 rhythmicity was abolished in the PHb, maintaining high PER2 levels throughout (Figure 4D). These results suggest that light input has a direct impact over clock genes expression, as revealed by alterations in PER2, known to show a slow response to light (Travnickova-Bendova et al., 2002; Wilsbacher et al., 2002). To further evaluate whether ipRGCs are involved in this process, we tested mice in which ipRGCs are ablated by the expression of the attenuated diphtheria toxin (Opn4aDTA/Brn3bDTA). We previously found a drastic reduction of ipRGC innervation in these animals at 6 months of age (Ecker et al., 2010; Güler et al., 2008; LeGates et al., 2012). Specifically, only sparse retinal fibers were observed in the most caudal PHb region in Opn4aDTA/Brn3bDTA mice (Figures S4A and S4B). Importantly, in the absence of the ipRGC projections, PER2 rhythmicity in the PHb was maintained in Opn4aDTA/Brn3bDTA mice housed under the T7 cycle (Figures 4E, S4C, and S4D). These findings reveal that irregular light/dark cycles perturb the circadian clock machinery selectively in the PHb through an ipRGC-dependent circuit.

Retinal innervation to the PHb was precisely mapped using intravitreal injections of the tracer cholera toxin β-subunit (CTb). Retinal axons labeled a wedge-shaped zone near the boundary between the dorsal thalamus and the LHb, especially caudally, in regions typically included in the central lateral (CL) or the lateral posterior (LP) thalamic nuclei (Figures 4F and S4A). A few retinal fibers penetrate into the most lateral edge of the LHb (Figure S4A). The retina-PHb circuit was further characterized by mapping the presynaptic terminals of ipRGC axons by using Opn4CreERT2;ROSA26Sorptophycin-tdTomato mice (Figure S4E). Labeled puncta, marking ipRGC output synapses, were densely distributed throughout the PHb (Figures 4G and S4F). To determine the identity of the RGCs that project to this area, we injected the tracer CTb into the PHb in WT mice (Figure 4H). In the contralateral retina, all retrolabeled RGCs (70.6 ± 5.2 cells/retina, n = 4 mice) were melanopsin immunopositive (Figures 4I and 4J). Thus, the PHb receives its retinal input exclusively from ipRGCs.

Next, we sought functional evidence for retinal influence on PHb neurons. For that, we expressed channelrhodopsin-2 (ChR2) in retinal axons by intravitreal injections of an AAV2/ChR2-EYFP (Figures 4K, S4G, and S4H). PHb cells recorded in current clamp exhibited bursting responses when relatively hyperpolarized (−70 mV), whereas a tonic mode of firing was observed when cells were relatively depolarized (−60 mV) (Figures S4I and S4J), similar to previous descriptions of thalamic neurons (Kim et al., 2001; Sherman, 2001), and a subpopulation of LHb neurons (Wagner et al., 2017). Activation of local ChR2-positive retinal axons by flashes of blue light (2 ms) evoked cationic excitatory postsynaptic currents (EPSCs) in a subset (9.5%, n = 63 cells) of patch-recorded PHb neurons (Figures 4L, 4M, and S4K–S4M). Together, these data indicate that ipRGCs provide excitatory synaptic input to PHb neurons.

PHb Is a Distinct Thalamic Region that Projects to Mood-Regulating Centers

The location of the PHb was delineated using markers of gene expression exclusively associated with thalamic or epithalamic areas. For that, the PHb area was outlined based on the light-mediated c-Fos expression. We found that most c-Fos(+) cells were co-labeled with GRID2IP (Figure 5A), a marker for dorsal thalamic nuclei (Nagalski et al., 2016). Only a minor fraction of total PHb c-Fos(+) cells co-localized with Bm3a (also known as POU4F1, Figure 5B), a marker for the epithalamic LHb (Quina et al., 2015). Additionally, we observed that most ipRGC terminals were in close apposition with thalamic neurons immunopositive for GRID2IP or PKCd (Figures S5A and S5B), another marker used for delineating the dorsal thalamus (Nagalski et al., 2016). In sum, these results assign the PHb as part of the dorsal thalamus.

We then investigated the PHb downstream circuitry using CTb injections to uncover targets potentially implicated in mood regulation. We found that the most prominently labeled region was the ventral medial prefrontal cortex (vmPFC) (Figure S5C).
Figure 4. Characterization of Retinal Input to the PHb

(A–C) A significant increase in the number of light-induced c-Fos(+) cells in PHb were observed in both groups, versus no light pulse controls. Data are mean ± SEM. **p<0.01; by Tukey's test (n = 5 mice).

(D and E) Immunohistochemical evidence for the rhythmic PER2 expression in the PHb (white arrows) in mice housed under the T24 or T7 cycles. In WT mice, PER2 levels exhibited circadian rhythmicity in the PHb under the T24 cycle, but the T7 cycle exposure significantly raised PER2 expression levels (D). In mice lacking ipRGC projections (Opn4aDTA/aDTA), PER2 rhythms in the PHb (E) became impervious to the effects of the T7 cycle. Data are mean ± SEM. *p < 0.05, **p < 0.01, by Student’s t test (n = 4–5 mice).

(F) Retinal projections to the PHb traced by CTb.

(G) ipRGC afferents to PHb contain a synaptophysin fusion protein (Opn4CreERT;ROSaSyn-tdTomato).

(H–J) CTb was stereotaxically injected in the PHb (H), and all retrolabeled RGCs (I) were melanopsin(+) (J). From a total of 49 ipRGCs analyzed, 46 cells were identified as M1 and 3 were M3 (n = 8 mice).

(legend continued on next page)
In particular, there were retrogradely labeled somas in layers V and VI of the infralimbic (IL) and prelimbic (PL) cortices bilaterally with respect to injection site and anterograde-labeled fibers in layers I and III-IV of the IL ipsilaterally. This result pointed to the existence of a thalamocortical loop between the PHb and vmPFC that is distinct from the CL or the LP nuclei, which are

(K–M) Optogenetic evidence for functional connections between ipRGCs and PHb neurons (K). Current response in a representative PHb neuron is shown (L). A subpopulation of PHb neurons showed a ChR2-induced response to light pulses (M) (n = 63 cells).

See also Figures S3 and S4.

Figure 5. Thalamic PHb Projects to Mood-Regulating Centers

(A and B) Light induced cFos(+) cells in the PHb were colocalized with GRID2 (A) or Brn3a (B) markers. Data are mean ± SEM (n = 3 mice).

(C–F) HSV/cre was injected into the vmPFC, while AAV/DIO-synaptophysin-tdTomato was injected into the PHb. Labeled somas were exclusively found in the PHb (C). PHb neurons have three targets: the vmPFC, including the IL (D), the dorsomedial striatum (E), and the NAc (F) (n = 12 mice).

(G) These experiments revealed a thalamocortical loop, represented here diagrammatically.

cc, corpus callosum; M2, secondary motor cortex; AC, anterior cingulate cortex; Str, striatum; aco, anterior commissure; Sep, septal nuclei; Orb, orbitofrontal cortex, Hip, hippocampus. Scale bars, 50 μm (D, inset); 100 μm (A–C); 200 μm (D–F).

See also Figure S5.
Figure 6. Disynaptic Circuits Connect ipRGCs to Mood-Regulating Centers

(A–D) A three-virus retrograde transsynaptic tracing system was used to identify the retina-PHb-vmPFC pathway (A). Injection sites were confirmed in the vmPFC (B) and PHb (C). EnvA-GΔ-mCherry expresses mCherry in PHb neurons expressing both Cre-EYFP and the Cre-dependent helper AAV (starter neurons) and presynaptic inputs to these neurons. A retrolabeled RGC expressing mCherry counterstained for melanopsin is shown (D).

(E) The PHb was transfected with a fluorescent Ca²⁺ sensor by injecting a cre-dependent GCaMP6m vector in the PHb region and a retrogradely transported cre virus in the vmPFC.

(F) Average response to a 2-s light pulse delivered at zeitgeber time (ZT) 18 to a mouse kept under the T24 cycle. The Ca²⁺ transient comprises a fast-rising component plateauing for 1.1 s before a second phase supervenes to generate a higher peak 3 s after light presentation. A biphasic response could bespeak either the transfection in our experimental design of two distinct (sub)populations that respond to light with different time courses or a functional property of a single neuronal population.

(G) A map of the 14 individual trials averaged in (F) is shown.

(H) PHb activity following regularly scheduled lights-on event in T24 cycle.
not known to project to the vmPFC (Noseda et al., 2010; Voorn et al., 2004). To increase the precision of our projection tracing, we used a dual injection strategy with a retrogradely transported Cre-carrying vector injected in the IL and a Cre-dependent vector carrying synaptophysin-tdTomato injected in the PHb (Figures 5C and 5D). We found that one of the PHb targets is indeed the vmPFC (Figure 5D). To determine which vmPFC subdivisions are innervated, we used vGlut2 staining to reveal the ventral boundary of the cortex (Figure S5D). PHb terminals extended ventrally within 200 μm of the ventral cortical boundary (Figure 5D), which is assigned IL territory. Furthermore, PHb terminals revealed a compression of cortical layers characteristic of the cytoarchitecture of IL. Based on the same factors, the most dorsal extent of the PHb’s cortical innervation field may fall within the ventral PL. The second and third targets of PHb neurons are the dorsomedial striatum (Figure 5E) and the nucleus accumbens (NAc) (Figure 5F). It should be noted that the dorsomedial striatum also receives input from the same cortical areas targeted by the PHb, indicating that PHb is part of a thalamo-frontocortico-striatal loop (Figures 5G, S5E, and S5F).

Finally, we noticed that a subset of labeled PHb neurons appear to send collaterals into the lateral part of the LHB (Figure S5G). Control injections of HSV-cre in the dorsomedial prefrontal cortex did not result in any labeled cells in the PHb proper (Figure S5H). Together, these results demonstrate that the PHb is incorporated into a loop reminiscent of limbic thalamic nuclei (Vertes et al., 2015). As a cohesive neuronal cluster with distinctive connectivity, genetic, and functional properties (including being a circadian oscillator), the PHb must be properly reckoned as an independent, previously unrecognized thalamic nucleus.

**Disynaptic Circuits Connect ipRGCs to Specific Mood-Regulating Regions**

To determine whether the PHb links ipRGCs to vmPFC through a disynaptic circuit, we used a triple-virus trans-synaptic retrograde tracing strategy (Schwarz et al., 2015): a retrogradely transported AAV/Cre-YFP was injected into the vmPFC (Figures 6A and 6B). Then, presynaptic partners of these thalamocortical relay neurons were labeled transsynaptically by injecting a Cre-dependent helper virus followed by EnvA-pseudotyped gAδRabies-mCherry in the PHb (Figure 6C). Finally, labeled PHb-projecting RGCs were found, and all of them were melanopsin(+) (Figure 6D). Using this strategy, we labeled from one to seven ipRGCs per animal; the low number of labeled ipRGCs could be due to the low probability requirement for the three viruses to infect the same cells in the PHb. Therefore, we also deployed a dual-virus strategy: a retrograde helper virus was injected into the vmPFC, followed by EnvA-pseudotyped G-deleted rabies injected into the PHb (Figures S6A–S6D). Using this strategy, 25 ± 7.1 labeled ipRGCs were found per retina. In sum, these results confirm the existence of a disynaptic pathway linking ipRGCs through PHb to vmPFC.

Next, we sought to characterize the physiology of the PHb in vivo. The target cell population was transfected with a fluorescent Ca2+ indicator, GCaMP6m, by injections of a cre-dependent vector in the PHb and a retrogradely transported cre-carrying vector in the vmPFC; an optical fiber was implanted above the PHb to deliver and collect light locally in awake, freely-moving mice (Figure 6E). We first confirmed that vmPFC-projecting PHb neurons responded to brief light pulses delivered in the active phase of mice (Figure 6F) with a biphasic Ca2+ transient characterized by a fast component rising 90 ± 3 ms after external light presentation and by a subsequent slower further rise peaking 3 s thereafter. Return to the pre-stimulus baseline occurs with high temporal variability and irrespective of continued environmental illumination (Figure 6G). To analyze the PHb physiology over longer time-spans, we exposed injected mice to the T24 or T7 cycles, and the PHb activity was recorded. Under both lighting conditions, we observed a fast response of PHb neurons to each dark-light transition (Figures 6H and 6I). A comparison of the magnitude of the peak fluorescence in the PHb in the course of exposure to the two light cycles revealed a significant increase of such activity under the T7 cycle (Figures 6J–6L). In sum, these results indicate that light input modulates the Ca2+ dynamics of vmPFC-projecting PHb neurons in vivo, with the T7 cycle specifically shown to increase activity.

**Chronic Activation of PHb Neurons Is Sufficient to Regulate Mood**

The PHb is well positioned to link environmental lighting to affective behavior. If the PHb is required for the effects of light on mood, then chronic activation of the PHb should induce mood disorders in animals housed in a normal light/dark cycle, whereas silencing PHb neurons should block the dysphoric effects induced by the unnatural light cycle. To achieve long-lasting activation of light-responsive PHb neurons, we used a chemogenetic strategy based on designer receptors exclusively activated by designer drugs (DREADDs) (Roth, 2016) combined with c-FosCreERT2 mice. A Cre-dependent AAV encoding an excitatory (Gq) was bilaterally injected in the PHb (Figure 7A). One week later, mice received a light pulse (circadian time [CT] 14), leading to the expression of the tamoxifen (Tam)-sensitive Cre recombinase only in light-responsive neurons. At the end of the light pulse, mice were injected with 4-OH-Tam. Injection sites were invariably restricted to PHb and immediately adjacent thalamic nuclei (Figures 7A and S6E). In AAV-injected c-FosCreERT2 mice that received 4-OH-Tam, but without light pulse exposure, only sparse mCherry(+) cells or no detectable mCherry expression was observed in the PHb (Figure S6F). As a control group, littermate Cre-negative mice received the

(!) In T7 cycle, the PHb responds to dark-to-light transitions with a biphasic Ca2+ transient analogous to the one observed in T24 cycle.
(J) Comparison of peak fluorescence in T24- versus T7-housed mice recorded over 72 hr. Peak fluorescence in T7 cycle is significantly higher (**p < 0.001; by Student’s t test).
(K and L) Time course of peak fluorescence (calculated as the difference between maximum and median for each sample recording) recorded over multiple days in T24 (K) or T7 (L) cycles. Dashed orange lines indicate light transitions.

Data are mean ± SEM (n = 3 mice). Scale bars, 100 μm (D); 200 μm (C); 500 μm (B).
See also Figure S6.
same treatment and viral injections. Beginning 4 weeks post-Tam injections, mice continuously received the designer ligand clozapine-N-oxide (CNO) in their drinking water to selectively and tonically activate neurons expressing the designer receptor (hM3D) (Figures S6 G–S6I). After 2 weeks of chronic CNO treatment, virally injected c-FosCreERT mice displayed mood alterations, spending significantly more time immobile in both the TST and FST, compared with control mice (Figures 7 B and 7C). The SPT was not evaluated in this case out of concern that CNO treatment could affect water palatability. To avoid issues with palatability, in a different set of experiments, AAV-injected c-FosCreERT and Cre-negative mice received two (intraperitoneal [i.p.]) injections of CNO (1 mg/kg) per day, during 2 weeks. We found that this CNO treatment induced a significant reduction in the sucrose preference index in c-FosCreERT+ injected mice, compared to the control group (Figures 7D and S6J).

To determine whether the PHb-vmPFC circuit is sufficient for driving mood alterations, we performed a long-lasting activation of vmPFC-projecting PHb neurons. WT mice were injected with a retrograde transported AAV/Cre (J and K) and an AAV5/DIO-hM3D-mCherry in PHb. (F and G) CNO treatment in DREADD-injected mice caused a significant increase in the immobility time in the TST (F) and FST (G) versus control mice (n = 12–15). (H and I) CNO treatment in DREADD-injected mice had no effect on cognitive functions. For the NOR test (H), the statistical analysis versus 50% (dotted lines) was: Control, p < 0.05 and DREADD, p < 0.05 by one sample t test. Using the MWM test, we found no significant deficits during the test (I) trial (n = 12–14).

J) AAV5/Cre-GFP was bilaterally injected into the PHb of mice that express tetanus toxin (tetX) in a Cre-dependent manner. GFP expression confirms injection site that included the PHb and was largely restricted to immediately adjoining thalamic nuclei.

(K–M) Suppressing PHb synaptic output eliminated any statistical difference in the TST (K), FST (L), and SPT (M) between mice housed under the T24 or T7 cycles. For the SPT, the statistical analysis compared to 50% (dotted lines, M) was: T24 p < 0.001 and T7 p < 0.001 by one sample t test (n = 14–15).

Data are mean ± SEM. *p< 0.05; ***p<0.001 by Student’s t test. Hb, habenular complex. Scale bars, 200 μm. See also Figures S6 and S7.
mPFC-projecting PHb neurons, we found that the CNO treatment has no significant effect on learning and cognitive functions (Figures 7H and 7I). In the NOR test, both control and DREADD-controlled mice spent significantly more time exploring the novel object (Figures 7H and S7C). During the MWM, both group of mice displayed similar responses during the training and test phases (Figures 7I and S7D). In sum, these results suggest that the PHb is specific for driving the effects of light on mood.

**PHb Neurons Are Necessary for the Effects of Light on Mood**

We next evaluated whether the PHb is necessary for the mood alterations induced by the unnatural light cycle by inhibiting synaptic release specifically from PHb neurons. Tetanus toxin light chain subunit (tetX) was expressed in PHb neurons by injecting an AAV/Cre-GFP in mice that express tetX in a Cre-dependent manner (R26<sup>tetX-2/EG</sup>) (Zhang et al., 2008) (Figure 7J). Injection sites were confirmed post hoc by assessing GFP expression (Figures 7J and S7E). Four weeks after AAV injections, mice were housed in either T24 or T7 cycles for 2 weeks (Figure S7F). Remarkably, expressing tetX in PHb neurons prevented the T7 cycle-induced mood alterations as tetX-AAV-injected mice housed under the T7 cycle were statistically indistinguishable from those housed under the T24 cycle on the TST, FST, and SPT (Figures 7K–7M and S7G). These results demonstrate that inhibiting PHb neurons blocks the mood alterations triggered by abnormal light cycles.

Next, we explore whether the PHb is specific in mediating the effects of light on mood or whether it is also activated by non-light-dependent treatments that induce mood changes. For that, WT mice were exposed to three different paradigms known to cause mood changes in rodents (see STAR Methods), and the induction of c-Fos was evaluated in PHb neurons, as well as in other brain regions known to be involved in mood control (Choi et al., 2013; Huang et al., 2004; Matsuda et al., 1996). For all the paradigms used, a significant increase in the number of c-Fos(+) cells were found in the LHb and prefrontal cortex, compared with control mice (Figures S7H and S7I), confirming the effectiveness of the paradigms used. However, these same stimuli did not cause a significant induction of c-Fos in PHb neurons. This is in distinct contrast to the robust c-Fos induction mediated by light (Figure S7J). Therefore, the PHb nucleus is part of a circuitry that specifically routes photic stimuli to influence mood.

**DISCUSSION**

We have previously uncovered that ipRGCs are the sole retinal conduit responsible for signaling light information to modulate mood and cognitive functions (LeGates et al., 2012). Here, we reveal that (1) distinct ipRGC subpopulations drive the effects of light on learning and mood, (2) Brn3b(-) SCN-projecting ipRGCs relay light information to influence cognitive functions without disrupting the central pacemaker, and (3) the thalamic PHb mediates the ipRGC-driven effects of light on mood. Together, these results delineate two distinct retinobrain pathways that mediate the effects of light on learning and mood.

**Light Routed through SCN-Projecting ipRGCs Affects Cognitive Functions**

The use of the Opn4<sup>Cre</sup>;<brn3bzDTA mouse line allowed us to implicate the ipRGC-SCN pathway in driving the effects of light on learning and memory, which is consistent with previous reports showing that a functional SCN is required for hippocampal learning (Fernandez et al., 2014; Ruby et al., 2008). This animal model, however, still retains minor ipRGC innervation to other brain regions, including the intergeniculate leaflet (IGL). This minor innervation is most likely arising from collaterals of the ~200 SCN-projecting ipRGCs (Fernandez et al., 2016) that remain in the Opn4<sup>Cre</sup>;<brn3bzDTA mice. It is possible that these minor innervations to the non-SCN targets could somehow contribute to the learning deficits observed in mice housed under the T7 cycle. However, based on the previous literature (Fernandez et al., 2014; Ruby et al., 2008) and the substantial innervation to the SCN, the most parsimonious explanation is that the SCN is the main driver of the effects of light on learning and memory.

Recent evidence showed that the SCN can act as a relay for light input to peripheral oscillators, even in the absence of a functional molecular clockwork machinery (Izumo et al., 2014). We found that the SCN remains responsive to light under the T7 cycle, as observed with the robust phosphorylation of CREB, solidifying the role of the SCN as a relay for light input. Additionally, we observed that SCN neurons become refractory to light-induced transcriptional changes, as suggested by the reduced levels of the immediate-early genes, including c-Fos and the light-responsive clock gene, PER1. Light-induction of c-Fos and PER1 depends on two regulatory regions: CRE and SRE, through pCREB and pELK-1 binding, respectively (Chawla, 2002; Coogan and Piggins, 2003). Our results show that under the T7 cycle, the transcription factor ELK-1 is not phosphorylated in response to light, whereas the phosphorylation of CREB was unaffected. This result suggests that CREB and ELK-1 activation pathways are differentially affected by the T7 cycle, consistent with published reports indicating that different intracellular cascades cause phosphorylation of these activators (Coogan and Piggins, 2003; Xia et al., 1996).

**The Thalamic PHb Is a Relay for Light-Mediated Mood Control**

ipRGCs show widespread projections throughout the brain, including areas controlling sleep and general activity, as well as limbic regions (Hattar et al., 2006). A principal target of ipRGCs revealed in this study is an area of the dorsal thalamus, the PHb, which expresses a circadian clock and shows distinctive connectivity. Our results indicate that the PHb constitutes a distinct thalamic region, with a different pattern of projections compared with the thalamic nuclei into which it has heretofore been subsumed. Specifically, the CL is connected to premotor frontocortical regions and to the dorsolateral striatum, (Voorn et al., 2004), and the thalamic LP nucleus projects to a diverse array of sensory and motor cortices (Nosea et al., 2010). We revealed that PHb neurons send collateral projections to vmPFC, dorsal, and ventral striatum. Prefrontal cortex is fundamental to mood regulation and has been consistently implicated in major depressive disorders (MDD) by imaging studies of patients (Frodl et al., 2009; Meng et al., 2014; Shen et al., 2017) and using animal
models of mood disorders (Shrestha et al., 2015). Another target of PHb, the dorsomedial striatum, is integrated in a thalamo-frontocortical loop, which may be involved in affective-emotional processing. Reduced caudate and putamen volumes were described in MDD patients (Kerestes et al., 2014). The third target of PHb neurons is the ventral striatum, particularly the NAc, which has been extensively implicated in mood control and depression (Francis and Lobo, 2017). In sum, our results provide evidence for a previously unrecognized thalamic nucleus, the PHb, which is both necessary and sufficient for relaying the effects of light on mood.

**Perspective**

The retino-PHb pathway presented here can satisfactorily account for mood changes wrought by light and represents, therefore, a new general mechanism for mood regulation (Lazzerini Ospri et al., 2017; LeGates et al., 2014). Irregular light stimulation leads to mood alterations associated with changes in the thalamic PHb, including increased activity of PHb neurons, sustained light-mediated induction of the immediate-early gene c-Fos, and a breakdown of clock gene rhythmicity. We speculate that in humans, chronic exposure to light at night could cause similar neuronal changes leading to mood deficits, highlighting the negative impact of irregular light stimulation during the normal day/night cycle. This occurs in a context of lamentable stagnation in the development of effective treatments for mood disorders. While promising drug candidates currently undergoing trials exist, the sad truth is that no radically new class of antidepressants has reached the clinic since the introduction of selective serotonin reuptake inhibitors (SSRIs) in the 1980s (Hillhouse and Porter, 2015).

Reduced cognitive functions have been extensively reported in patients suffering from MDD (Hammar and Ardal, 2009; Jaeger et al., 2006; Roh et al., 2016). Although a direct causal link is missing, current theories suggest that neuroanatomical changes in MDD patients may be the cause of cognitive deficits (Davidson et al., 2002). Our results demonstrate that anatomically distinct neuronal circuits are involved in different light-mediated behavioral deficits and suggest that learning and mood deficits are affected independently. Understanding the neuronal basis for mood and learning regulation by light constitutes a promising step toward new treatments for neuropsychiatric disorders.

**STAR+METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **METHOD DETAILS**
  - Light-mediated gene induction/protein phosphorylation
  - PER1/2 rhythmic expression
  - Immunofluorescence
  - SCN dissection and RNA-sequencing
  - Retinal injections
  - Stereotaxic injections
  - Electrophysiology
  - Hippocampal recordings
  - Fiber Photometry

- **QUANTIFICATION AND STATISTICAL ANALYSIS**
  - Morphometric analysis
  - Statistical Analysis

- **DATA AND SOFTWARE AVAILABILITY**

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes seven figures and can be found with this article online at https://doi.org/10.1016/j.cell.2018.08.004.

**ACKNOWLEDGMENTS**

We would like to thank the Johns Hopkins Biology Mouse Tri-Lab—especially Dr. Reiji Kuruvilla—for support and discussion; the Department of Biology at the University of Maryland and Dianne and Kimberly Boghossian for assistance maintaining and genotyping mice; Dr. Antonello Bonci, Dr. Yeka Aponte, and their lab members for help addressing reviewers’ concerns; and Elaine Nguyen for help with tracer injections. We would like to thank Mio Akasako for her key assistance with the retrolabeling experiments. This work was supported by the NIH (GM076430, EY027202, EY012793, and EY017137), the generous contributions of the Alcon Research Institute (to D.M.B.), PEW Charitable Trusts (to D.C.F.), the NSF (2011110435 to P.M.F.), and the intramural research at the National Institute of Mental Health.

**AUTHOR CONTRIBUTIONS**


**DECLARATION OF INTERESTS**

The authors declare no competing interests.
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