Fear of the Light or Need for Action: The IGL Will Judge

Ludovic S. Mure[1] and Satchidananda Panda[1,*]
[1]Regulatory Biology Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
*Correspondence: satchin@salk.edu
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Temporal adaptation of behaviors is of crucial importance for every organism. In this issue of *Neuron*, while elegantly establishing the developmental program of the subcortical visual shell (SVS), a group of retinorecipient nuclei, Delogu et al. (2012) also implicate one of its structures, the IGL, as a potential important player in the regulation of daily activity pattern.

Consolidation and timing of activity and rest to diurnal rhythms are of crucial importance for an organism's survival. This temporal regulation is under the control of at least three overlapping mechanisms—homeostatic drive for sleep, circadian clock, and light modulation of activity. Homeostatic drive for sleep modulates sleep periods as a response to accumulating sleep debt from activity and arousal. Consolidation of sleep and its timing to the day or night by the circadian oscillator temporally assigns an ecological niche for nocturnal or diurnal species. Lastly, light modulates the activity-sleep cycle by changing the phase of the circadian oscillator in a time-of-the-day-specific manner as well as by acutely modulating arousal or sleep. In general, light promotes arousal in diurnal animals and suppresses or masks activity in nocturnal species. In mammals including humans, chronic disruption of this activity-rest cycle predisposes to chronic diseases and/or is a hallmark symptom of several diseases. Identifying the molecules, cells, and circuits underlying diurnal rhythms will help toward managing these diseases.

Circadian rhythm in activity is generated and sustained by a master pacemaker resident in ~20,000 neurons of the suprachiasmatic nucleus (SCN). In natural conditions of light:dark cycle and associated environmental changes, the phase of the SCN oscillator is adjusted by both photic and nonphotic cues. The SCN receives direct monosynaptic innervation from intrinsically photosensitive and melanopsin-expressing retinal ganglion cells (ipRGCs or mRGCs) as part of the retinohypothalamic tract (RHT). The molecules and cells of the RHT-SCN axis underlying a light-entrainable circadian clock are conserved in both diurnal and nocturnal mammals (Hatori and Panda, 2010; Welsh et al., 2010). The timing of diurnal rhythms in molecular clock components as well as the timing of SCN firing rhythms are similar between nocturnal and diurnal species (Challet, 2007). This implies that the temporal elaboration of activity/sleep determining diurnal/nocturnal behavior is determined by cellular networks outside the RHT-SCN axis.

In diurnal animals, light is known to promote arousal and suppress sleep. In nocturnal rodents light acutely suppresses activity by a phenomenon called masking. Light masking of activity during the day and the absence of it during the night can drive diurnal activity rhythms in nocturnal rodents lacking a functional clock. Masking persists even after acute ablation of the SCN in rodents, but disappears upon acute ablation of the ipRGCs (Hatori et al., 2008), thus suggesting that extra-SCN targets of the ipRGCs mediate the masking phenomenon (Mrosovsky, 2003).

The ipRGCs send collaterals beyond the SCN and innervate several parts of the subcortical visual shell (SVS). The SVS has been defined as a group of up to a dozen retinorecipient nuclei in the diencephalon (Morin and Blanchard, 1998). In the diencephalon the ipRGCs innervate the lateral hypothalamus, lateral geniculate nucleus (LGN), olivary pretectal nucleus (OPN), lateral habenula, and superior colliculus (Hatori and Panda, 2010). Among these targets, the intergeniculate leaflet (IGL) constituting a thin stripe of cells between the ventral and dorsal lateral geniculate receives dense innervation from the ipRGCs. NPY-expressing cells of the rodent IGL project directly to the SCN constituting the geniculohypothalamic tract (GHT), which has been implicated in resetting the SCN clock (Rusak et al., 1989). In addition to the SCN and ipRGCs, the IGL is extensively connected to several brain centers including those mediating stress, sleep, arousal, and novel object recognition (Morin and Blanchard, 2005). Hence, the IGL is thought to integrate multiple inputs and fine-tune the diurnal activity pattern. However, the current knowledge on IGL mediation of activity-rest largely stems from pharmacological or ablation studies in which specificity is often inconclusive. This partly stems from the paucity of understanding the ontogeny, molecular markers, circuitry and function of the IGL. The ontogeny of the predominantly GABAergic SVS that arises within the diencephalon is also unclear.

In this issue of *Neuron*, Delogu et al. (2012) have taken a multitude of approaches to address the ontogeny and function of one of the major cell types of the rodent IGL. The sequential expression of a series of transcription factors leading up to the expression of Dlx1/2 or Sox14 is part of the GABAergic neurogenesis program, so they suspected Dlx1/2 or Sox14 participate in SVS differentiation. Surprisingly, they found the GABAergic nuclei of the SVS develop from two distinct groups of cells marked by the mutually exclusive expression of Dlx1/2 and Sox14. In the LGN region, the Sox14-positive cells are born in the rostral thalamus, the future IGL. Some

of these cells undergo two waves of migration—one rostrally to form the nucleus posterior limitans (PLi) and the lateral habenula (LHa) and the other ventrally into the ventral LGN which is largely populated by Dlx-expressing cells. The expression of Dlx in the vLGN offers a repulsive signal to the invading Sox14 cells, thus ensuring only a few Sox14 cells populate the vLGN. In the absence of Dlx1/2 this repulsive signal is abolished leading to mass migration of Sox14 cells into the vLGN turning it into an IGL phenotype territory (Figure 1A).

The Sox14-deficient mice in which the Sox14 coding sequence is replaced by GFP show no significant changes in the overall organization of the SVS except for the redistribution of cell populations in the IGL/vLGN region. The presumptive Sox14 cells fail to migrate ventrally to partially populate the vLGN and remain in the IGL region (Figure 1A), increasing their density. Sox14 appears to be dispensable for expression of Gad1 and NPY suggesting the Sox14-deficient cells are still neurotransmitter competent. An increased density of Sox14-GFP cells in the IGL also correlated with increased immunoreactivity for NPY. Taken together, the combined function of Sox14 and Dlx define the spatial distribution of Sox14-expressing cells in the IGL and vLGN region of the SVS. The loss of Sox14 leads to changes in the regional distribution of Sox14 cells and consequently potential changes in the pattern and strength of connectivity of IGL with target regions.

To assess the functional consequence of the loss of Sox14 expression, Delogu et al. (2012) used a suite of behavioral tests. Intact pupillary light reflex and light induction of c-Fos in the SCN implied normal ontogeny and projection of the ipRGCs in these mice. The circadian activity rhythm under constant darkness also showed normal periodicity, thus indicating no gross defect in the endogenous SCN clock. However, the Sox14 knockout mice showed profound alteration in activity-rest pattern.

Several studies have demonstrated the IGL participates in at least three different aspects of daily arousal-rest pattern: overall activity level, entrainment of the circadian clock, and light suppression of activity or masking. Since the loss of Sox14 affects the cellular composition of the IGL and consequently changes the circuitry, the mice show remarkable defects in all three aspects of activity regulation. Increased number of functionally active NPY-positive cells in the IGL and potentially increased NPY-mediated signaling is opposite to IGL lesion resulting in reduced activity (Redlin et al., 1999). Overall, the Sox14 knockout mice appear to exhibit increased basal activity. Light is known to enhance NPY release from the GHT at the SCN, thereby driving early morning NPY release near the SCN (Glass et al., 2010). However, direct release of glutamate from the ipRGCs at the SCN is presumed to counteract any phase perturbation by NPY (Biello et al., 1997), such that under normal 12 hr light:12 hr dark conditions rodents wake up at the dark onset. This seems to be a delicate balance as novel objects or events that trigger arousal and consequently further increase NPY release at the SCN during the daytime could overcome the glutamate counterbalance and cause a phase advance of the SCN clock (Shibata and Moore, 1993), such that the rodent progressively wakes up earlier and earlier before dark onset. In the Sox14 knockout mice, although ipRGC development and function likely remain normal, under light:dark cycle increased signaling flux through the GHT tips this fine balance in favor of NPY, such that the mice appear to undergo daily phase advance and consequently wake up almost 3 hr prior to dark onset (Figure 1B). This behavior is predictably opposite to the late evening activity onset in *NPY*$^{-/-}$ mice under certain light regimens (Kim and Harrington, 2008).

Finally, the Sox14 knockout mice exhibit profound deficiency in light suppression of activity or masking. As noted earlier, the SCN is dispensable for masking, suggesting the extra-SCN network underlies this behavioral response. However, the role of IGL in masking is far from conclusive. Masking in rodents

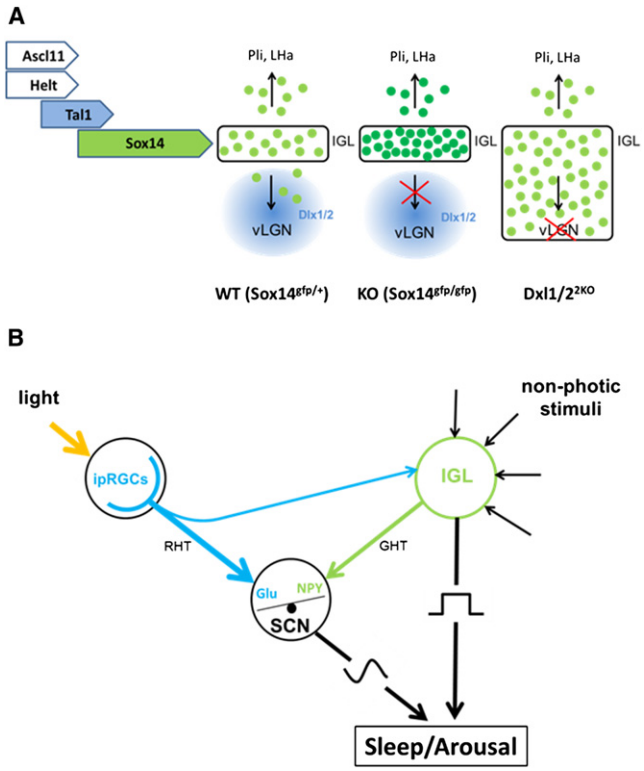

Figure 1.
(A) The sequential expression of Ascl1, Helt, Tal1, and Sox14 in the rostral thalamus gives rise to the future IGL. While tangential migration from this pool establishes other SVS nuclei, ventral migration to the vLGN is limited by the expression of Dlx. In the Sox14-deficient mice, no progenitor migrates ventrally, increasing the density of Sox14 positive cells in the IGL. In the absence of Dlx1/2, Sox14 cells invade the vLGN, giving it an IGL-like phenotype.
(B) In the wild-type mouse, the circadian clock (SCN) is entrained mainly by light information received directly from ipRGCs via the RHT and is influenced by photic and non-photic cues integrated at the level of the IGL. The IGL also participates in light modulation of activity, independent of the SCN clock. Both circadian- and light-modulation of activity determine diurnal activity pattern. In the Sox14-deficient mice, increased NPY^{+} cell density in the IGL promotes stronger inputs from the IGL on diurnal activity pattern.

is most pronounced in their home cage, while an arousal promoting environment such as access to a running wheel dampens masking (Redlin and Mrosovsky, 1999). This suggests that enhanced arousal input can override the activity suppressive effect of light. Furthermore, the IGL is known to make direct or indirect projections to the sleep promoting neurons and they also receive input from arousal system and circuitry implicated in novel object recognitions (Morin and Blanchard, 1998). Thus, the IGL serves to integrate multiple signals in determining overall activity levels. IGL ablation does not abolish light suppression of activity or masking, but enhances sensitivity to light suppression of activity, thus suggesting IGL's role in light modulation of activity is likely to counteract masking (Redlin et al., 1999). In the Sox14 knockout mice, the increased density of NPY-positive neurons in the IGL likely strengthens the counteracting role of IGL in masking such that masking is "masked" and so mice continue their normal activity even if a light pulse is administered in early night. An alternate explanation is that the changes in migration and consequently circuitry of Sox14-positive cells might alter the cellular network underlying masking. For example the cells that would otherwise populate the vLGN are sequestered in the IGL, thus potentially severing or rewiring the circuitry that would have involved the vLGN resident Sox14-positive cells. Since the vLGN also receives extensive innervation from the ipRGCs, its role in masking cannot be ruled out.

As stated earlier, the RHT and the phase of the clock in the SCN are conserved throughout evolution and across diurnal and nocturnal mammals. This is in stark contrast with SVS organization; NPYergic neuron distribution and projection in particular have undergone dramatic changes in higher/diurnal primates including humans (Chevassus-au-Louis and Cooper, 1998; Moore, 1989). Now, Delogu et al. (2012) break new ground in understanding the ontogeny and function of the SVS, specifically the IGL and vLGN and offer a framework for network regulation of the activity pattern in mammals. Importantly, they clearly demonstrate that mutually exclusive expression of Dlx- and Sox14-positive cells and their spatial distribution defines the SVS architecture. The Sox14 knockout mice illustrate how changes in their expression can reshape the underlying circuitry and profoundly change diurnal activity patterns. The 3 hr advance in activity onset in Sox14 knockout mice might be detrimental for survival since they would shift their activity into a period that would make them more vulnerable to predators. Ultimately, changes in the SVS architecture in different species and the corresponding changes to the underlying cellular networks could fine-tune adaptation to the ambient light environment. This could account for the specification of diurnal and nocturnal activity pattern or changes in seasonal behavior in different species.

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