Uniformity from Diversity: Vast-Range Light Sensing in a Single Neuron Type

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The brightness of our visual environment varies tremendously from day to night. In this issue of *Cell*, Milner and Do describe how the population of retinal neurons responsible for entrainment of the brain's circadian clock cooperate to encode irradiance across a wide range of ambient-light intensities.

Typically, when we talk about the impact of light on the brain, we think about "sight," or the conscious perception of the visual environment. Light information encoded by the retina also, however, drives non-image-forming brain functions. For example, light present at particular times of day serves to match our endogenous circadian rhythms to the external day-night cycle, control pupil reflexes, drive hormone secretion, and regulate mood and appetite. In considering the tremendous range of effects that light has, a conundrum emerges: as ambient light varies throughout the day, how does the nervous system deal with these variations and deliver coherent visual signals to the brain? In this issue of *Cell*, Milner and Do (2017) describe new findings that examine the neural mechanisms by which a specific category of retinal neurons work as a population to cover the full range of environmental irradiances available across the circadian day-night cycle.

The "classic" visual pathway involves retinal rods and cones converting photons into electrical signals. These signals are filtered by retinal interneurons and passed on to the retinal ganglion cells (RGCs) that communicate light information, as action potentials or "spikes," to the brain (Dhande et al., 2015). Until ~15 years ago, rods and cones were thought to be the only photoreceptors, but the identification of intrinsically photosensitive RGCs (ipRGCs) that express their own photopigment called melanopsin, vastly changed our model of how vision works. ipRGCs directly phototransduce light and control non-image-forming functions (Berson et al., 2002, Hattar et al., 2002, Güler et al., 2008). Previous studies showed that ipRGCs include at least five subtypes of RGCs (M1–5) (Schmidt et al., 2011). M1 ipRGCs are the most well-characterized subtype and project heavily to the suprachiasmatic nucleus (SCN) to control circadian photoentrainment and to the olivary pretectal nucleus (OPN) to control pupil reflexes (Chen et al., 2011).

Previous work showed that ipRGCs respond in direct proportion (monotonically) to irradiance, spiking more action potentials as they are exposed to brighter light. M1s also have very slow phototransduction (Do et al., 2009, Emanuel and Do, 2015) and wide "net-like" dendritic arbors. These properties make M1s optimal for signaling information about overall ambient light levels. However, a crucial issue regarding M1 function remains unresolved—as the world turns on its axis each 24 hr cycle, how do individual M1s process light of different irradiances? In essence, the M1 population needs to encode and signal the average light intensity at specific times—a task that requires enormous dynamic range: up to 10 log units. What ensures that range? Milner and Do (2017) begin to unveil the answer by showing that different individual M1 RGCs encode disparate levels of irradiance but that as a population, they cover the enormous range of light intensities (Figure 1A–1F).

To assay the light-driven electrical properties of M1 ipRGCs, the authors carry out patch clamp recordings from retina taken from mice expressing the fluorescent protein tdTomato, driven by the melanopsin promoter. Thus, the cell body, dendrites, and axons of M1s all glow red in this mouse line. Instead of targeting the M1 cell bodies for recordings, the authors record directly from the axons at a location away from the cell body. This allows them to assay M1 spikes with high fidelity while also reducing unwanted illumination due to microscopy.

The first discovery Milner and Do (2017) make is that M1s are actually comprised of cells with one of two firing patterns. While some M1 cells fire monotonically up to a point of saturation, the majority of M1 cells display unimodal tuning: firing increases over a limited range of increasing intensity and then sharply decreases at a certain point. Moreover, different unimodally tuned M1s cover different aspects of the irradiance scale (Figure 1G). Thus, M1s as a population respond to a broad range of irradiance levels, but most individual cells are highly tuned to specific, narrower bands of brightness. Interestingly, unimodal cells are more sensitive cells and can operate starting from very dim light, while monotonic cells first activate at twilight conditions and are less sensitive. Using pharmacological antagonists of synaptic transmission, they also show that irradiance tuning remains unchanged and thus represents properties inherent to M1 ipRGCs and melanopsin phototransduction. The authors then go on to characterize a number of interesting physiological aspects of M1 ipRGCs. They use phosphorylated S6 ribosomal subunit (pS6) immunofluorescence as a readout

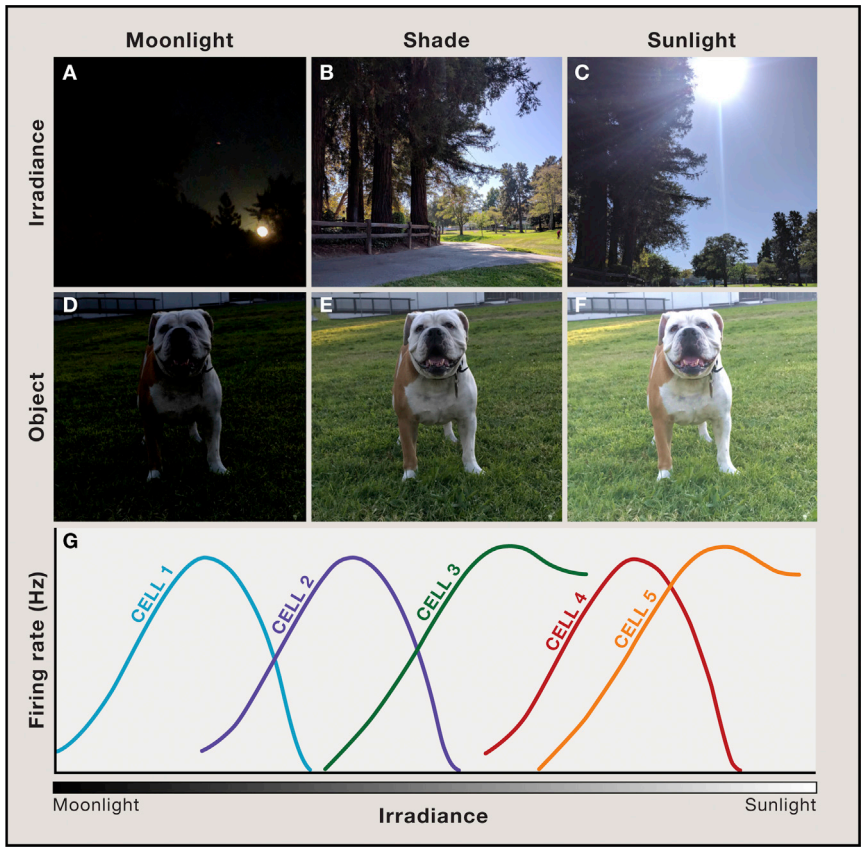

Figure 1. M1 ipRGCs Fire Selectively at Specific Irradiances
M1 ipRGCs fire selectively at specific irradiances.
(A–C) Images showing moonlight (A), partial sunlight (B), and bright sunlight (C).
(D–F) Objects (Costello Huberman the bulldog) seen under different irradiances.
(G) Irradiance-firing relations for five different cells show unimodal and monotonic tuning. Cells are activated at different irradiances, peak at saturation, and then either descend in unimodal or plateau in monotonically tuned cells.

of neuronal activity and find it to be consistent with unimodal tuning, suggesting that M1 ipRGCs are unimodally and differentially tuned to irradiance *in vivo*. Next, Milner and Do (2017) explore how M1 cells respond to light adaptation. They find that cells shift their activation range such that highly sensitive cells fire at lower irradiances, while less-sensitive cells shift to activate at brighter irradiances, thus together covering a broader irradiance range.

Regardless of where on the continuum of light intensity a given M1 responds best, it remains tuned to a particular band of intensities. By and large, cells maintain either unimodal or monotonic tuning, indicating these are committed fates. The authors also examine the biophysical mechanisms underlying M1 tuning. They find that the availability of the voltage-gated sodium channels is critical in determining the firing rate. Phototransduction depolarizes the cell, which first activates voltage-gated sodium channels, initiating spiking, and then as irradiance increases, sodium channel availability slowly decreases, marking the descent of the irradiance-firing relation. In other words, depolarization of the membrane potential, i.e., depolarization block, which is generally considered aberrant, in fact acts as an upper threshold for firing to build normal responses.

In addition to solving a fundamental set of issues related to M1 ipRGC physiology and the retina's ability to encode a broad range of irradiances, the findings of Milner and Do (2017) raise a large number of exciting new directions for future studies to address. First, an important question to explore are the factors that determine whether a given M1 cell responds unimodally or monotonically and when developmentally this fate is determined? These answers could shed light on whether intrinsic firing patterns of neurons could be altered and whether it would result in functional and behavioral consequences. Second, it will be crucial to understand whether the central brain targets of M1s, such as the SCN and OPN, receive pooled input from M1s or whether M1s with different response properties segregate to distinct targets, sub-regions, or specific cell types. Both the SCN and the OPN harbor functional subdivisions (Baver et al., 2008). Given that M1s of both response types are physically interspersed (Milner and Do, 2017), a pooling of axonal inputs from both M1 response types in individual targets seems likely. By receiving multiple inputs, the SCN may be able to record average light intensities throughout the day and set a functional baseline in response to activities. The OPN, on the other hand, operates under shorter timescales to control pupil reflexes and may benefit from having immediately restricted and functionally segregated inputs.

The results of Milner and Do (2017) provide an elegant example of how biophysical mechanisms relate to population dynamics in a single, functionally heterogeneous cell type. The retinal connectivity of M1, and now also their detailed physiology under different light conditions, has been elucidated. Once the fine-scale axonal connectivity of M1s to the SCN is resolved, the present findings can be leveraged toward making specific predictions about the roles of M1s for circadian clock setting *in vivo*. The results of Milner and Do (2017) thus generally serve as an elegant template for linking multiple levels of organization in the mammalian CNS.

REFERENCES

Baver, S.B., Pickard, G.E., Sollars, P.J., and Pickard, G.E. (2008). Eur. J. Neurosci. *27*, 1763–1770.

Berson, D.M., Dunn, F.A., and Takao, M. (2002). Science *295*, 1070–1073.

Chen, S.K., Badea, T.C., and Hattar, S. (2011). Nature *476*, 92–95.

Dhande, O.S., Stafford, B.K., Lim, J.A., and Huberman, A.D. (2015). Annu Rev Vis Sci *1*, 291–328.

Do, M.T., Kang, S.H., Xue, T., Zhong, H., Liao, H.W., Bergles, D.E., and Yau, K.W. (2009). Nature *457*, 281–287.

Emanuel, A.J., and Do, M.T. (2015). Neuron *85*, 1043–1055.

Güler, A.D., Ecker, J.L., Lall, G.S., Haq, S., Altimus, C.M., Liao, H.W., Barnard, A.R., Cahill, H., Badea, T.C., Zhao, H., et al. (2008). Nature *453*, 102–105.

Hattar, S., Liao, H.W., Takao, M., Berson, D.M., and Yau, K.W. (2002). Science *295*, 1065–1070.

Milner, E.S., and Do, M.T.H. (2017). Cell *171*, this issue, 865–876.

Schmidt, T.M., Chen, S.K., and Hattar, S. (2011). Trends Neurosci. *34*, 572–580.

Cancer Evolution during Immunotherapy

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Immune checkpoint blockade has revolutionized cancer treatment. In this issue of *Cell*, insights from a longitudinal multi-omics analysis of the largest yet-reported cohort of melanoma patients reveal how tumor and immunity co-evolve during anti-PD-1 therapy.

Cancer immunotherapy has firmly moved from the realm of the boutique to the routine, and the dramatic and durable responses it may produce continue to impress. The use of monoclonal antibodies to block immune regulatory checkpoints demonstrates benefit across multiple cancer types; however, limitations exist as only a fraction of patients respond to therapy, and optimal predictors of response are lagging behind clinical development.

An effective anti-tumor immune response requires recognition of tumor cells by appropriate effector immune elements and a tumor microenvironment (TME) that is permissive to cytolytic T cell activity. Tumor cell recognition is predicated on the concept that cellular immune responses can develop against cancer-specific antigens that arise from cancer-specific mutations. Accordingly, genomic analyses of several tumor types exposed to CTLA-4 or PD-1 blocking agents confirm that mutational burden predicts response (Hugo et al., 2016; Snyder et al., 2014) and that tumor-specific mutant antigens are the key target (Gubin et al., 2014). By extension, studies of NSCLC samples have shown that due to neoantigen heterogeneity, if clonal neoantigens can be effectively targeted, better anti-tumor responses can result (McGranahan et al., 2016). Copy number alterations provide additional information, with a higher burden of copy number losses, commonly affecting immune-related genes, predicting poor response to both CTLA-4 and PD-1 blockade (Roh et al., 2017).

But knowledge of a tumor's mutational status provides only one part of one side of the story. Expression of immunosuppressive molecules and the presence of immunoregulatory cellular elements adaptively thwart the anti-tumor immune response and are driven by the presence and action of CD8+ T cells (Spranger et al., 2013). Expression of immune checkpoints themselves (Taube et al., 2014) or evidence of an inflamed TME particularly in early-on-treatment samples (Chen et al., 2016) are arguably better—and simpler—predictors of PD-1 inhibitor response than mutational load across multiple cancer types. While the inhibitory effects of CTLA-4 and PD-1 can be overcome by immunotherapy agents targeting these molecules, therapeutic failure frequently involves upregulation of numerous other, less well-studied, immune inhibitory checkpoint molecules (Koyama et al., 2016).

In this issue of *Cell*, new analyses presented by Riaz et al., provide an integrated perspective of how the genomic landscape evolves for both players in the cancer immunity playoff (Riaz et al., 2017). In this new work, they study the largest yet-reported cohort (n = 68) of genomically interrogated tumor samples from patients receiving the PD-1 blocking agent nivolumab, examining whole-exome, transcriptome, and T cell receptor (TCR) sequencing. Importantly, this cohort includes patients who were previously treated with CTLA-4 blocking antibodies, as well as those who were naive to CTLA-4 blockade, with response rates not being significantly different between these groups (23% versus 32%, respectively). However, differences are noted when genomic profiling is taken into consideration, with a positive association of mutational load and treatment response only becoming clearly evident in patients who were naive to CTLA-4 blockade. This is provocative and further studies are warranted studying the role of mutational load in the setting of