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# When is a proxy not a proxy? The foibles of studying non-image forming light

Jamie M. Zeitzer[1,2,3]

[1]Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA 94305, USA

[2]Stanford Center for Sleep Sciences and Medicine, Stanford University, Stanford, CA 94305, USA

[3]Mental Illness Research, Education, and Clinical Center, VA Palo Alto Health Care System, Palo Alto, CA 94304, USA

Email: jzeitzer@stanford.edu

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Studying light-induced phase shifting responses of the circadian clock in humans is expensive, time consuming and difficult. It takes days or weeks to examine the light-induced circadian phase shifting response of a single participant. As such, other non-image forming photoreceptive behaviours have been used as proxies for light-induced circadian phase shifting responses. These include light-induced melatonin suppression, which is much faster and less expensive to study. Implicit in these studies is that by examining the impact of light on this proxy behaviour, we can induce the manner by which the circadian clock would respond to the same light stimulus. These proxy responses are, as is circadian phase shifting, mediated by the retinohypothalamic tract (RHT), formed by axons arising from the intrinsically photosensitive retinal ganglion cells (ipRGCs) (Hattar *et al.* 2002). So, the inductive reasoning goes that understanding one output of the RHT (melatonin suppression) will inform us about the output of interest (phase shifting). It must be noted, however, that there is no direct evidence that light-induced melatonin suppression is mediated by the circadian clock (suprachiasmatic nucleus, SCN). As the SCN is necessary for the production of melatonin, it has thus far been impossible to test whether the SCN is also necessary for the light-induced suppression of melatonin. Indeed, the RHT innervates many loci in the hypothalamus, including the paraventricular nucleus, which is part of the pathway leading from the SCN to the pineal, indicating that there is at least anatomical evidence that the physiological pathways leading to different aspects of non-image forming photoreception may be separable. Furthermore, the RHT does not arise from a uniform set of ipRGCs. Studies in mice have identified at least six different subtypes of ipRGC (Sexton *et al.* 2012). The impact of light on ipRGCs has both an intrinsic (melanopsin-mediated) and extrinsic (through rod–cone circuitry) component. As these different subtypes of ipRGC have different dendritic arborization patterns in retinal layers, the ipRGC subtypes could receive different rod–cone signalling, including excitatory and inhibitory colour channels, and possibly have unique integration patterns with the intrinsic melanopsin activation. Thus, the specific nature of the light stimulus (i.e. intensity, spectral and temporal distribution) might differentially impact the subtypes and, therefore, could have differential effects on downstream targets.

So, the question remains, is studying melatonin suppression a useful proxy for determining the impact of light on circadian phase shifting? To address this question, in an article in this issue of *The Journal of Physiology*, Rahman and colleagues studied 59 participants who were exposed to a variety of light stimuli ranging from continuous dim or bright light to pulses of bright light with interspersed darkness (Rahman *et al.* 2018). They report that, under these conditions, there is no significant correlation between light-induced melatonin suppression and light-induced phase shifting of the circadian clock. In some individuals exposed to long duration (6.5 h) light, robust phase shifts were accompanied by negligible suppression of melatonin (Fig. 2 in Rahman *et al.*). In some individuals exposed to an hour or less of light, robust suppression of melatonin was accompanied by negligible phase shifts (Fig. 2 in Rahman *et al.*). The data presented indicate that light induction of melatonin suppression and light induction of circadian phase shifts are separable phenomena. Previous studies using different light stimuli, including red light (Zeitzer *et al.* 1997) and millisecond flashes of light (Zeitzer *et al.* 2011), had previously reported on the poor correlation between light-induced melatonin suppression and light-induced changes in circadian phase. In these studies, the authors observed an absence of or an inconsistent melatonin suppression in response to light that induced shifts of circadian phase. In all of these studies, it appears that physiologically significant phase shifts can be induced by specific light stimuli in the absence of an accompanying robust decline in melatonin. Thus, while the overall sensitivity to light-induced melatonin suppression and light-induced circadian phase shifting is similar, these two non-image forming functions of light are not necessarily mediated by the same neural pathways and are not necessarily proxies for one another.

While light-induced suppression of melatonin is a critical modifier of the physiological representation of night length in mammals with strong seasonal behaviours (e.g. sheep, Siberian hamsters), the suppression of nocturnal melatonin in humans has limited proven physiological significance. Most studies have used this phenomenon as a proxy for the impact of light on circadian phase shifting. Light-induced changes in circadian phase is a critical physiological phenomenon that enables proper alignment of the internal circadian clock with the external light–dark cycle. Properly timed light exposure is the pre-eminent countermeasure for both intrinsic (e.g. Delayed Sleep–Wake Phase Disorder) and extrinsic (e.g. shift work, jet lag) causes of misalignment between internal circadian time and external time. Our understanding of the relationship between light and circadian phase shifting in humans has progressed tremendously in the past 40 years. Rahman and colleagues remind us that if we are truly interested in the impact of light on circadian phase shifting, that is what we should be studying – proxies may not be all they are made out to be.

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## Additional information

### Competing interests

None declared.