The Role of ipRGCs in Light-Dependent Modulation of Learned Fear Responses

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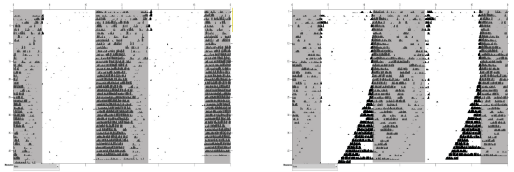
Abstract

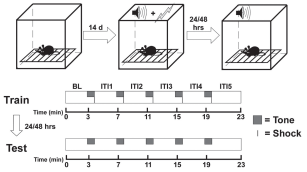
Characterizing the processes by which environmental information is perceived and conveyed to the brain to influence learned fear responses is necessary in order to better understand fear-related disorders. Previous research in mice using a tone-cued fear conditioning paradigm has established that light enhances learned fear responses. This effect is predominantly, if not exclusively, driven by rod and cone photoreceptors (4); however, the class of retinal ganglion cells conveying this photic information to central sites involved in fear responses remains unknown. Intrinsically photosensitive retinal ganglion cells (ipRGCs) have been shown to mediate light's effects on various behavioral, physiological, and higher-order cognitive processes (2, 3). Here, we demonstrate that ipRGCs are a critical component in the circuitry by which light information is conveyed to sites in the brain that control fear learning.

Methods

Opn4$^{+/+}$ Opn4$^{aDTA/aDTA}$



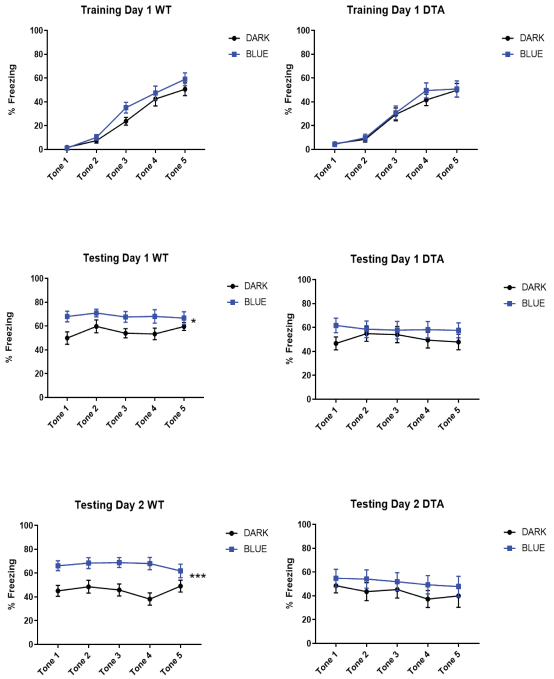
The left panel displays an actogram of a wild type (WT) mouse entrained to a 12 hour light/dark cycle. Because mice are nocturnal, the majority of its activity occurs in the dark phase. The right panel depicts an actogram of an *Opn4$^{aDTA/aDTA}$* mouse with progressive loss of ipRGCs. This mouse is homozygous for an allele where the open reading frame for attenuated diphtheria toxin A is knocked into the melanopsin locus. *Opn4$^{aDTA/aDTA}$* mice do not synchronize their running to the 12 hour light/dark cycle but instead run earlier each day irrespective of the light cycle, termed "free running" (1).



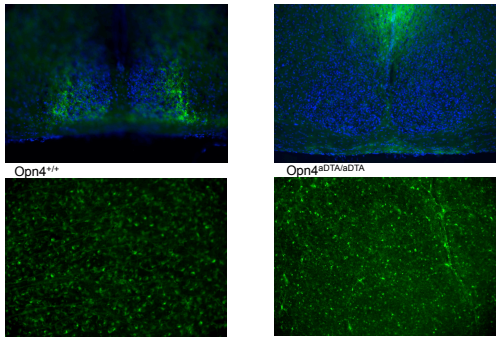
We used a tone-cued fear conditioning paradigm to test the learned fear responses of *Opn4$^{aDTA/aDTA}$* mice and their WT littermates under dark and light conditions. Image from Warthen, et. al (4).

Results

Using a two-way repeated measures ANOVA, we found that *Opn4$^{aDTA/aDTA}$* mice, which lack ipRGCs, do not exhibit a statistically significant difference in freezing levels between dark or light conditions, despite possessing a full complement of rods and cones. In contrast, their wild type littermates' freezing levels are significantly higher when tested in light conditions compared to dark. Overall, sixteen WT and sixteen *Opn4$^{aDTA/aDTA}$* mice were tested in each condition.



Verification



Because loss of ipRGCs is progressive in *Opn4$^{aDTA/aDTA}$* mice, the loss of ipRGCs was verified histologically. Cholera toxin B (CTB) conjugated to alexofluor 488 was injected into the vitreal chamber of the eye. Three days postinjection, animals were anesthetized, transcardially perfused with fixative, and brains were postfixed, cryoprotected, sectioned, and imaged. DTA-mediated loss of ipRGC projections was assessed by visualizing the hypothalamic suprachiasmatic nucleus (SCN), a primary central target of ipRGCs whose retinal innervation is exclusively from ipRGCs (1). The top left image displays this innervation. In contrast, *Opn4$^{aDTA/aDTA}$* mice fail to show CTB-positive terminals in the SCN, indicating a loss of ipRGCs as shown in the top right image. To verify the injections worked, the retinas of each were dissected and imaged for fluorescence. The lower left image and lower right image show the fluorescence of the tagged retinal ganglion cells of a WT and *Opn4$^{aDTA/aDTA}$* mouse, respectively.

Conclusion

ipRGCs function as photoreceptors and conduits of photic information initiated in the rods and cones. Our data indicate ipRGCs are indeed required to convey photic information to sites in the brain that control fear learning. Our findings have implications for the treatment of Post-Traumatic Stress Disorder (PTSD) in humans, a form of fear learning. Because light is an environmental factor that can modulate fear, light intensity of a specific wavelength may serve as a trigger for individuals with PTSD. As a result, the use of appropriate ambient lighting conditions during PTSD therapies may affect whether such treatments are ultimately successful.

References

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