

## Review

## A Comparative Perspective on Extra-retinal Photoreception

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**Ubiquitous in non-mammalian vertebrates, extra-retinal photoreceptors (ERPs) have been linked to an array of physiological, metabolic, behavioral, and morphological changes. However, the mechanisms and functional roles of ERPs remain one of the enduring questions of modern biology. In this review article, we use a comparative framework to identify conserved roles and distributions of ERPs, highlighting knowledge gaps. We conclude that ERP research can be divided into two largely unconnected categories: (i) identification and localization of photoreceptors and (ii) linkage of non-retinal light reception to behavioral and physiological processes, particularly endocrine systems. However, the emergence of novel gene editing and silencing techniques is enabling the unification of ERP research by allowing the bridging of this divide.**

**Light Detection by Evolutionary Conserved ERPs**

In the century following the identification of **ERPs** (see [Glossary](#)) by von Frisch [1], their mechanisms and functional roles have remained one of the enduring questions of modern biology. ERPs have been identified in the vast majority of extant organisms, likely representing the most basal form of light reception [2]. ERPs are particularly common among non-mammalian vertebrates: fish, amphibians, reptiles, and birds (Table S1 in the supplemental information online). Although the precise mechanistic roles of ERPs are generally not well described, current evidence shows that these non-image-forming photoreceptors are critical, for example, in the integration of light information for movement: photokinesis in zebrafish larvae [3] and patterns of locomotor activity (*Xenopus* tadpoles [4], eels [5], lizards [6], ruin lizards (*Podarcis sicula*) [7]). The functionality of ERPs is most well characterized in relation to daily rhythms [8] and the seasonal regulation of avian reproduction [9–11].

In this review article, we synthesize our existing knowledge of ERPs across non-mammalian vertebrates, beginning with a brief background on the major types of ERPs identified and a comparison of their neuroanatomical localization across taxa. We then highlight the functional role of ERPs for the regulation of physiology, behavior, and biological rhythms including endocrine and metabolic processes. Our review article focuses on fish and birds, as the role of ERPs is best characterized in these taxa. We demonstrate that ERP research to date largely represents two distinct areas: the identification/localization of ERPs and linkage of light to behavioral/physiological outputs. However, recent developments in long-term RNAi technologies and genome editing tools are finally providing the exciting opportunity to determine the functional roles of ERPs across a diverse range of species.

**Identification and Updated Classification of ERPs**

The first extra-retinal **opsin** was isolated by Vigh-Teichmann and colleagues using immunohistochemistry on thornback ray (*Raja clavata*) pineal glands [12], using non-specific whole

**Highlights**

Light entrains rhythmic processes from daily patterns of hormone secretion and locomotor activity to annual neuroendocrine, metabolic, and behavioral cycles.

Extra-retinal photoreception, through photosensitive opsin molecules, is a widespread phenomenon in non-mammalian vertebrates.

Nearly a century of research has led to the identification of many different extra-retinal opsins.

Extra-retinal photoreceptors are a key component in numerous physiological, metabolic, behavioral, and morphological changes in response to light stimulation, but the precise underlying mechanisms governing physiological and metabolic oscillations are largely unknown.

Emerging techniques in gene editing and silencing are providing the necessary tools to finally establish the functional roles and mechanistic connections of extra-retinal opsins.

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sheep anti-cattle opsin antiserum. Subsequent molecular characterization of pinopsin, isolated from chicken **pineal gland** [13], set the stage for the characterization of the range of known non-image-forming opsins. This identification and localization of opsins, first via nonspecific antibodies and subsequently with modern molecular techniques (e.g., *in situ* hybridization and RT-PCR), represents the first major focus of ERP research. The application of modern sequencing and bioinformatics techniques has further expanded the characterization of putative opsin sequences across a wide range of taxa.

A recent phylogenetic analysis of annotated opsin sequences has identified five major ‘super’ families into which all known opsins can be placed: OPN1, OPN3, OPN4, OPN5, and retinal G protein-coupled receptor (RGR) opsin (Table 1; [14]). This reductionist re-categorization of opsins replaces the current nomenclature in the literature with a robust convention based consistently on phylogenetic relationships. This approach has appropriately contextualized the evolutionary conserved neuroanatomy and functional roles of ERPs.

In general, ERPs are comprised of a photosensitive opsin protein and a chromophore (often a 11-*cis*-retinal, although all-*trans*-retinal chromophores also exist [15]) that signal through G protein-coupled transduction pathways (e.g., [16]). Unlike most visual opsins, the majority of non-visual opsins form bi-stable pigments that transition between light and dark states solely via light exposure, rather than requiring external input to return to the dark state [17]. Variation in opsin structure has given rise to a wide range of **absorption spectra** (Figure 1 and Table S2 in the supplemental information online). This variation likely reflects evolution of opsins to meet the demands of species-specific photic environments. For example, in aquatic environments blue and green wavelengths penetrate far deeper in the water column. Thus, a fish living in deep water would be under evolutionary pressure to maintain and utilize receptors sensitive to these wavelengths, while species living in shallow water would be under no such evolutionary pressure. Similarly, the mediums through which light must pass to reach the receptors, that is, water, scales, and skull for a fish versus feathers and skull in a bird, are expected to play a role in the wavelengths of light reaching the ERPs, thus altering the optimal wavelength sensitivity for ERP function.

### ERP Localization

In this section, we review the current literature on ERP distributional patterns, focusing on neuroanatomical localization (for a detailed review of dermal opsins, see [18]). To synthesize this information, we have constructed a comparative brain atlas of ERP distribution (Figure 2 and Table S1 in the supplemental information online). Localization of ERP expression has been of particular interest, given the well-established function of discrete brain nuclei in controlling physiological processes, potentially allowing linkage of opsins and physiological outputs. The identification of conserved neuroanatomy is important for establishing hypothesis-driven experiments into the direct and indirect role of ERPs in controlling physiological processes (e.g., reproduction). However, care needs to be taken in assigning function to an ERP based solely on localization, as ERPs, if expressed in neurons, can easily communicate with distant brain nuclei, as demonstrated by entrainment of the mammalian central clock, in the supra-chiasmatic nucleus (SCN), by melanopsin expressed in retinal ganglion cells [2]. Conversely, expression of ERP in other neural cell types (e.g., tanycytes or astrocytes) may limit the distance over which detected photic information can be directly transmitted.

The ERP brain atlas reveals that the diversity of opsin types expressed and the scope of their distribution are far higher in fish and birds than in reptiles and amphibians (Figures 2 and 3), suggesting possible taxonomic difference in use of light cues. However, it is currently far from

### Glossary

**Absorption spectrum:** the degree to which a material, in this case an opsin, absorbs electromagnetic radiation over a range of frequencies. Maximal absorption occurs at the wavelength at which the greatest fraction of energy is absorbed.

**ERPs:** extra-retinal photoreceptors; generally, refers to any photoreceptive molecule located outside of the retina of the eye. When located in deep-brain regions, they are also referred to as deep-brain photoreceptors. Consist of a coupled opsin protein and chromophore, which typically signal through G coupled signaling pathways. Major studied opsins include vertebrate ancient opsin (VA or OPN1), OPN3, melanopsin (OPN4), neuropsin (OPN5), teleost multiple tissue opsin (TMT), and retinal G protein-coupled receptors (RGRs).

**Hypothalamic pituitary gonad (HPG):** the reproductive endocrine axis. Consists of the hypothalamus that releases gonadotrophin-releasing hormone that acts on the anterior pituitary to stimulate release of gonadotrophins (luteinizing and follicle-stimulating hormone); these then act on the gonads directly to stimulate development, gametogenesis, and sex steroid production.

**Opsin:** light-sensing protein.

**Photoperiod:** period of day during which light exposure occurs. Synonymous with day length.

**Photorefractoriness:** physiological phenomenon related to light-induced activation of the HPG axis in seasonal breeders, where the animal spontaneously becomes nonresponsive to further light-based stimulation of the axis. Typically leads to termination of breeding and regression of gonads regardless of photoperiod. Classically thought to be all-or-none process, a spectrum from relative to absolutely refractory species has been described with relative refractoriness requiring a loss (decrease for long-day breeders) of stimulatory photoperiod and absolute becoming refractory regardless of conditions.

**Pineal gland:** a small endocrine gland in vertebrates, primarily

clear whether this pattern represents true taxonomic differences in ERP expression and diversity or whether it is a consequence of research focus being heavily biased toward commercially important taxa (i.e., poultry and fish). Further studies are needed to determine whether amphibians and reptiles truly express fewer types of opsins in a narrower range of neural sites that do other taxa.

One consistent observation across species is the high abundance of ERPs in the pineal gland. Pineal ERPs consistently include members of the OPN1 family, with OPN4 (melanopsin) found in fish and birds [3,19] and RGR opsins found in fish alone [20]. In addition, uncategorized opsin expression determined by nonspecific antibodies has been reported in the pineal of fish, amphibians, and reptiles [21–24]. Whether these represent novel opsin expression or previously identified opsins cannot be determined from the present data. However, it is clear, based on the conserved and consistent expression of opsins within the pineal complex, that extra-retinal photoreception must play a critical role in regulation of pineal activity and melatonin production and thus be critical for regulation of circadian rhythms.

Another major site of conserved opsin expression is the hypothalamus, consistent with its established role as an endocrine system mediator and critical neural node for the seasonal regulation of reproduction [25,26]. As with the pineal, OPN1 expression appears to be a key feature of hypothalamic opsins in all taxa examined except fish, where it is only found in the thalamus and habenula (Figure 2 and Table 1). In birds, hypothalamic OPN1 has been identified specifically as vertebrate ancient (VA)-opsin and is expressed widely throughout the hypothalamus including the preoptic area, paraventricular nucleus, bed nucleus of the stria terminalis, nucleus magnocellularis preopticus par ventralis, anterior medial hypothalamus, median eminence (ME), and nucleus supraopticus pars ventralis [9,27].

OPN4 has also been identified in the hypothalamus of amphibians (medial preoptic nucleus and SCN; [28]), birds (lateral septal organ [29], premamillary nucleus (PMM) [30,31,32]), and fish (SCN and lateral tuberal nucleus [33]). Although identified, reptilian OPN4 has only been isolated from joint telencephalon and diencephalon tissue preparations, preventing further localization [34]. OPN5 (neuropsin) represents the final major hypothalamic opsin; mRNA expression has been identified in the medial lateral septal organ [35,36] and in the paraventricular organ (PVO) [37,38]. OPN5 expression has been identified in cerebrospinal fluid (CSF)-contacting neurons of the PVO that project to the ME in birds [38] and the PVO of fish via *in situ* hybridization [39]. Finally, OPN5 protein has also been detected in the periventricular area of the hypothalamus in *Xenopus* tadpoles [4]. OPN5 orthologs have been identified in reptilian sequence data [14], but they have yet to be localized. The shared hypothalamic distribution of these three opsins places them in close proximity to neuroendocrine cells that mediate major endocrine axes, suggesting a role in their regulation. This is supported by the colocalization of OPN1 with arginine vasotocin and gonadotropin-releasing hormone (GnRH)-expressing neurons in the median eminence of birds [9].

The non-conserved regions of ERP expression, particularly those representing brain regions that have been assumed to be non-photosensitive, are also of interest. For instance, the olfactory bulbs of fish contain OPN1 [40] and OPN3 [41], but this region has not been examined in any other taxa. Similarly, both OPN1 and OPN3 family opsins have been isolated in the hindbrain region of fish, an area that was linked to the photomotor response [41,42], and an unknown opsin was linked to the amphibian brainstem [4]. Other regions of unique expression include the fish optic tectum [39,41] and the avian cerebellum [43]. A lack of data from other species leaves open whether these patterns are unique. Furthermore, it is unclear whether these brain regions (e.g., brainstem, cerebellum) are themselves detecting and integrating photoperiodic information locally. One possibility is that these

responsible for the production of melatonin.

**Third ventricle (3V):** one of the multiple ventricular, fluid-filled spaces, located along the midline of the brain in the diencephalon and is filled with CSF. Cells lining the ventricle have been implicated as critical for the induction of the HPG axis.

Table 1. Consolidation of Existing Opsins as Used in This Work Using Framework of Beaudry *et al.* [14]

Family name	Opsins consolidated	Signaling pathway
OPN1	Vertebrate ancient (VA) opsins Pinopsins Parietopsins Parapinopsins	G protein-coupled cyclic nucleotide signaling
OPN3	Teleost multiple tissue opsins (e.g., TMT1, TMT2, TMT3) Encephalopsins	G <sub>i/o</sub> protein-coupled cyclic nucleotide signaling
OPN4	Melanopsins	G <sub>q</sub> protein-coupled phosphoinositol signaling
OPN5	Neuropsin (OPN5) OPN6 group OPN7 group OPN8 group OPN9 group	G <sub>i</sub> protein-coupled cyclic nucleotide signaling
RGR	Retinal G protein-coupled receptors & Peropsins	All- <i>trans</i> -retinal [16]

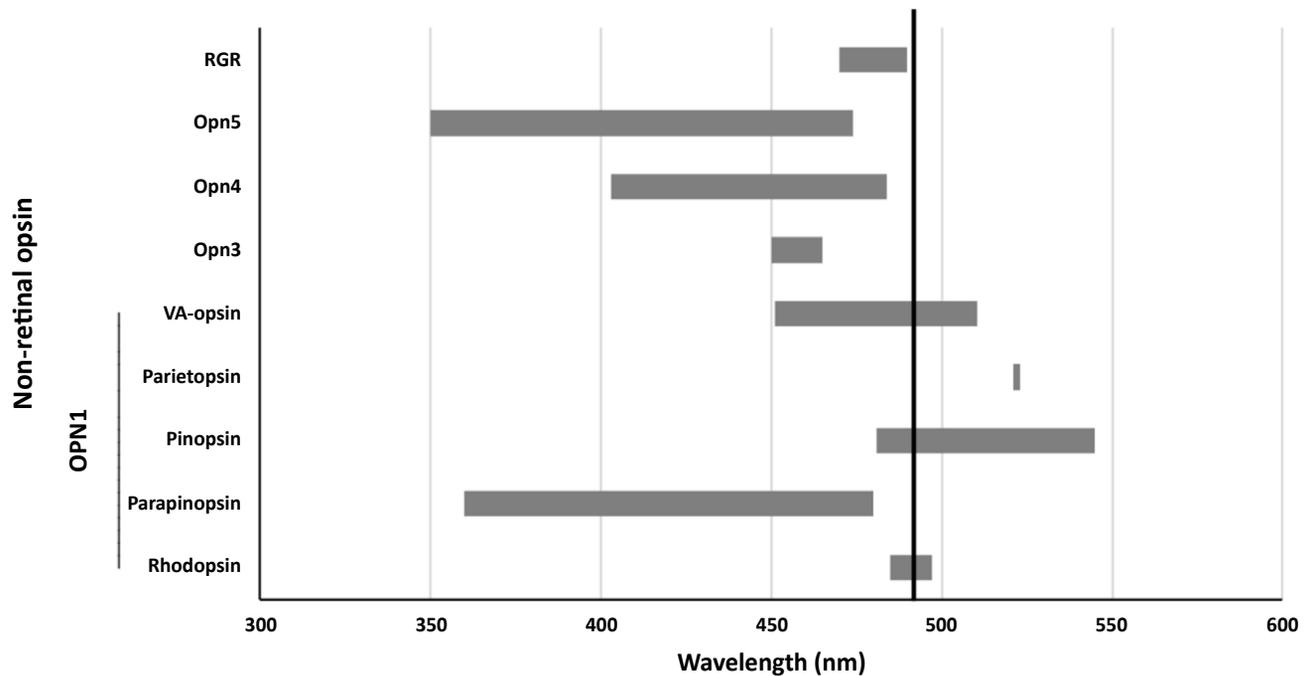
opsins have classical light detection functions, but act to signal distant brain regions (e.g., SCN, pineal gland, or hypothalamus) similar to transmission of light information from mammalian OPN4 in retinal ganglion cells [2]. Alternately, it is possible that these opsins have been co-opted into physiological roles independent of light detection, similar to the involvement of visual rhodopsins in the mechanotransduction of sound in the auditory pathway of *Drosophila* [44]. Future research should focus on determining whether these patterns of expression are in fact conserved and establish the functional role of opsins, light sensing or otherwise, in these presumptively non-photosensitive brain regions.

### Functional Roles of ERPs

In this section, we outline the second major focus of ERP research: the linking of extra-retinal light detection to the control of a myriad of physiological and behavioral phenomena (Figure 4, Key Figure) from movement behavior to seasonal and circadian rhythms (Box 1; [45]). We highlight studies that have experimentally linked a specific opsin to its output, bridging the classic divide between ERP localization and regulatory roles, such as the use of mutant zebrafish lacking OPN4 expression in behavioral studies or the ablation of a specific population of opsin-containing cells.

### ERPs in Color Change

The adaptation of body color change to the coloration of the surrounding environment was the first phenomenon to be linked to ERP photoreception. von Frisch [1] demonstrated that light-induced skin pigmentation change in European minnows (*Phoxinus phoxinus*) was dependent upon deep-brain photoreception. He observed that minnows lacking eyes were still able to vary their skin color, but not those whose diencephalon was damaged [1]. The identity of these ERPs remains unresolved as OPN1, OPN3, OPN4, and OPN5 have all been identified in the diencephalon of fish (Figure 2 and Table S1 in the supplemental information online). The neural pathways and signaling mediators by which photic information detected by deep-brain ERPs is transduced into color



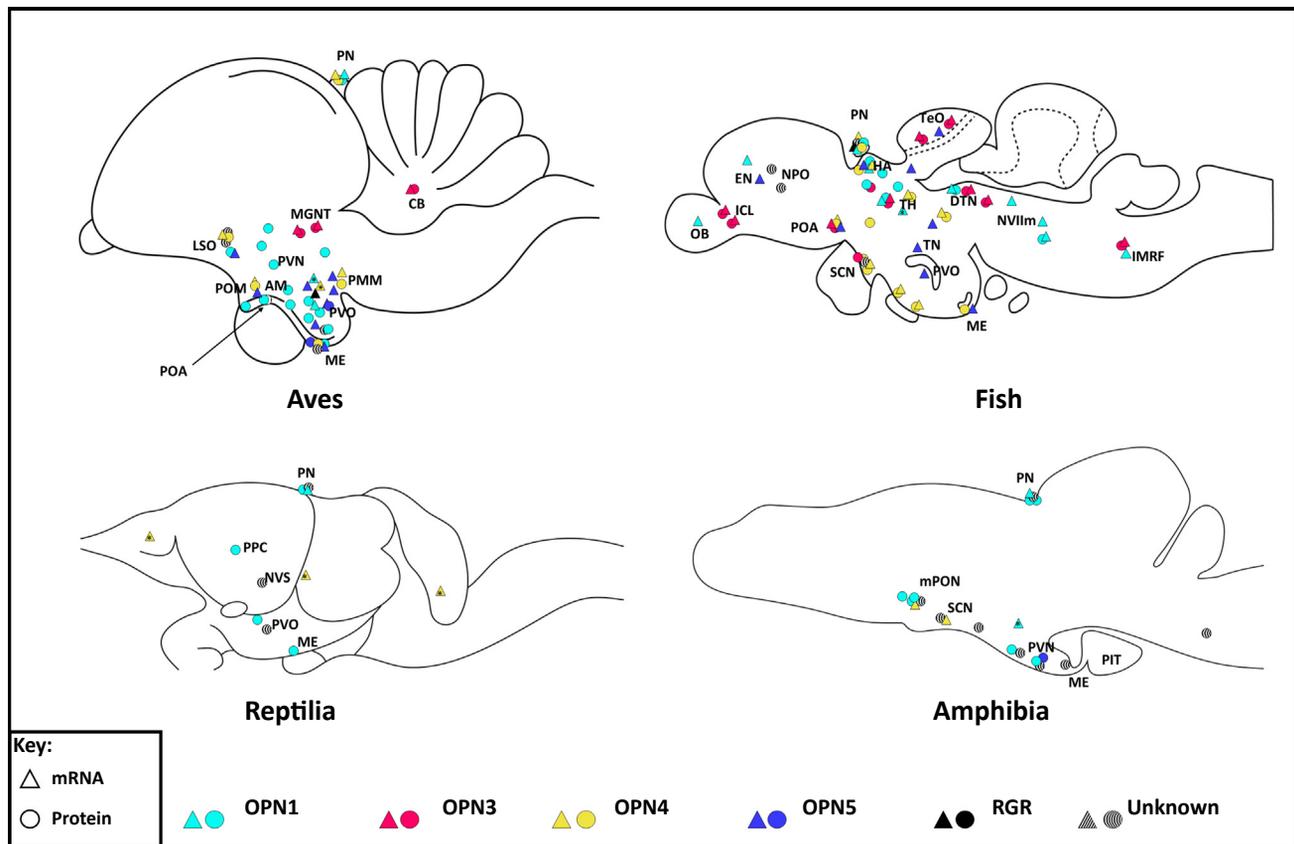
Trends in Endocrinology &amp; Metabolism

Figure 1. Spectrum Maxima for the Five Main 'Super' Families of Opsins as Reported in the Literature for Extra-retinal Photoreceptors. Note each family may be comprised of multiple receptors. Bars represent the full range of values reported in the current literature under a variety of test conditions (for all references, see Table S2 in the supplemental information online). Solid black vertical line at 492 nm indicates the maxima for avian reproduction determined by Foster and Follett [78] for reference. OPN, opsin; RGR, retinal G protein-coupled receptor; VA, vertebrate ancient opsin.

change at the skin level remain poorly described. However, control of color change is not limited to brain-based opsins. Blinded chameleons (species unspecified) have been shown to retain their ability to change body color in response to dermal photostimulation, implying a direct detection of light in the skin [46]. Similarly, body color change has also been linked to dermal ERPs in *Xenopus*, wherein changes in light exposure trigger changes in dermal melanosome aggregation [47]. RT-PCR and nested PCR studies of tadpole tails have also identified expression of OPN1 and OPN4 mRNA in the dermal melanophores, suggesting localized control of color change [47]. RT-PCR studies have identified both OPN1 and visual cone type opsins in the skin of the neon tetra (*Paracheirodon innesi*) as potential regulators of color change [48]. Gene expression profiling in zebrafish has identified more than 25 opsin gene variants expressed in the skin [18]. The mechanisms and molecular pathways linking skin and neural opsin photoreception to color change remain an active and open area of robust research (for review, see [18]).

#### Phototaxis and Locomotive and Feeding Behaviors

Another major functional role of ERPs is control of phototaxis and movement related behaviors. Currie and colleagues [4] found that preparations of tadpole neural tissue exposed to light produced rhythmic patterns of locomotive neural output activity *in vitro*, supporting ERP involvement. They further identified that peak responsivity occurred at wavelengths between 390 and 410 nm and was isolated to preparations containing the caudal diencephalon, where strong OPN5 expression was also identified [4]. Excision of the regions expressing OPN5 rendered the tissues light insensitive, providing a tight linkage between OPN5 in the caudal diencephalon and locomotive activity.

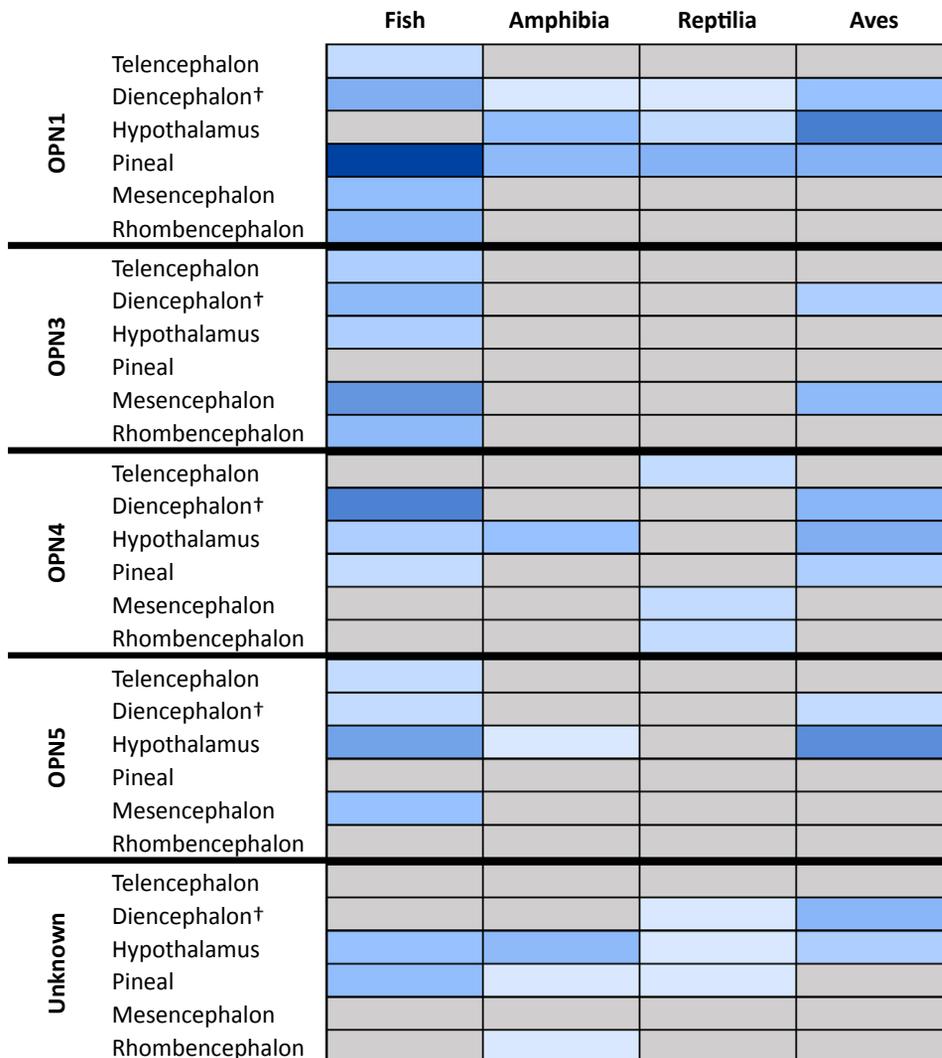


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**Figure 2. Neural Distribution of Opsins in Non-mammalian Vertebrates (Amphibians, Reptiles, Birds, and Fish) Displayed on Generalized Sagittal Sections Based on Current Literature.** Protein (circle) and mRNA (triangle) localizations are indicated independently. An asterisk (\*) indicates that the tagged marker represents expression of that opsin was characterized in a broad brain region (e.g., hypothalamus, telencephalon, mesencephalon, etc.) not at the specific location of the marker. See Table S1 in the supplemental information online for full descriptive list of studies included in the figure and associated references. AM, nucleus anterior medialis hypothalami; CB, cerebellum; DTN, dorsal tegmental nucleus; EN, entopeduncular nucleus; HA, habenula; ICL, inner cellular layer of olfactory bulb; IMRF, intermediate reticular formation; LSO, lateral septal organ; ME, median eminence; MGNT, medial geniculate nuclei thalamus; NPO, nucleus preopticus magnocellularis; NVS, nucleus ventromedialis of the septum; NVllm, facial motor nucleus; OB, olfactory bulb; PH, posterior hypothalamus; PMM, premammillary nucleus; PN, pituitary; POA, preoptic area; POM, medial preoptic nucleus; PPC, posterior pallial commissure; PVN, periventricular nucleus; PVO, paraventricular organ; SCN, suprachiasmatic nucleus; TeO, optic tectum; TH, thalamus; TN, tuberal nucleus.

Photoperiodic entrainment of daily locomotor rhythms, in multiple species of lizard, does not require the lateral eyes, pineal or parietal eye, supporting control by brain-based ERPs [6,49]. Administration of antisense mRNA to inactivate rhodopsin (OPN1; cone-opsin Ps-RH2) directly to the **third ventricle (3V)** of pinealectomized and retinalectomized ruin lizards caused a free-running cycle of locomotive activity [7]. Inactivation of opsins in the free-running ruin lizards was verified by immunohistochemical analysis of brain sections using CERN874 and CERN901 antisera designed against chicken rod and cone opsins [7]. These data provide clear causal linkage between opsin expression and daily rhythms of movement behavior.

Similarly, photic cues have been linked to locomotive and phototactic behavior in several fish species. Blinded and pinealectomized eels also have disrupted circadian patterns of locomotor activity that is dependent upon the ability of light to reach the deep brain regions [5]. Light-seeking behavior in zebrafish has been experimentally narrowed to the preoptic area, by a



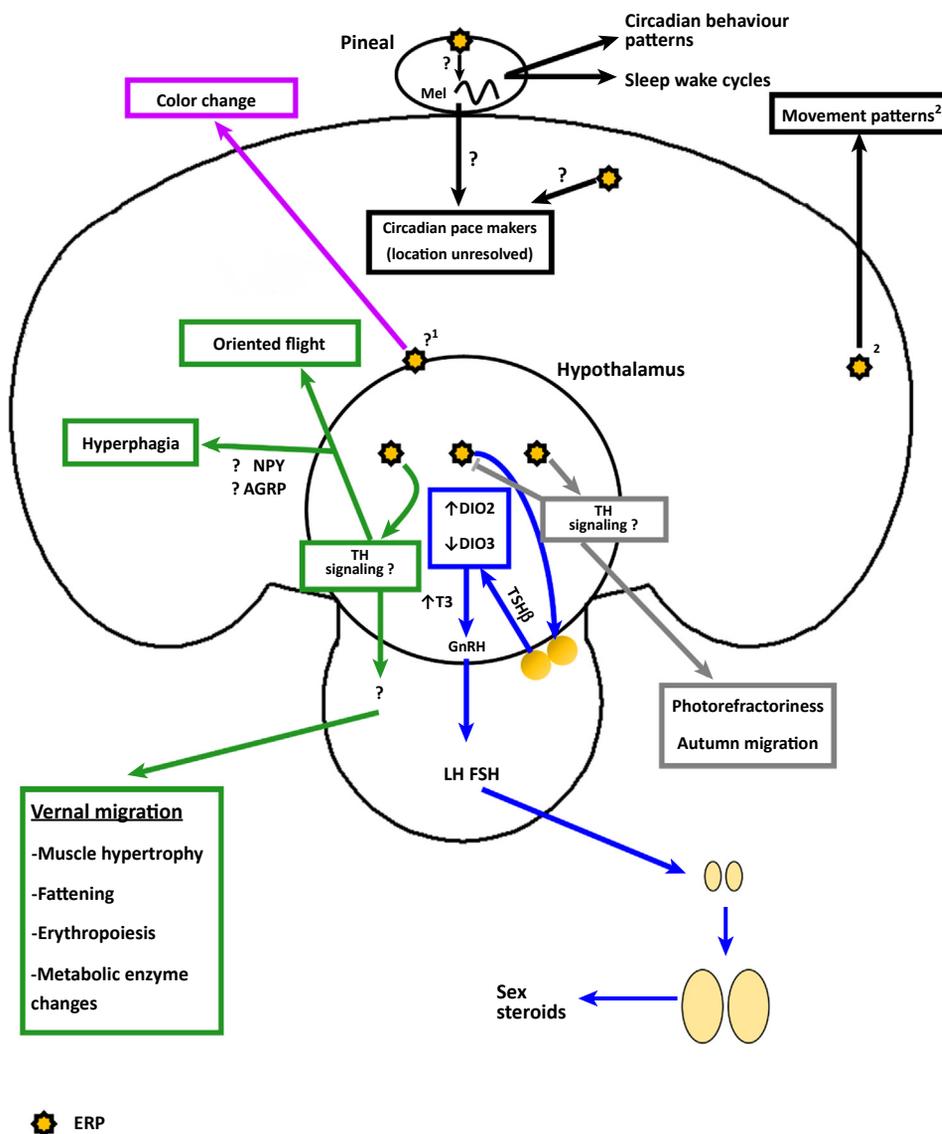
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Figure 3. Heatmap Summarizing Evidence for Expression of Each Opsin Family by Brain Region and Taxa. Degree of evidentiary support is denoted on a color scale from light to dark blue (least evidence to greatest) based on degree of support in the literature. Values were derived from Table S1 in supplemental information by summing the column labelled 'Evidence Level'. In brief, studies were assigned a value of 1, protein level data only; 2, mRNA localization only; or 3, both protein and mRNA. Gray coloration indicates an absence of evidence in the literature for any expression of the opsin in that region and taxa. Retinal G protein-coupled receptor has been excluded as it has only been reported in the fish pineal and avian hypothalamus. Dagger (†) indicates diencephalon expression excludes any hypothalamic expression, which has been subdivided into an independent hypothalamic category presented independently. 'Unknown' opsins as a category represent localization of opsins by nonspecific antibodies that prevent categorization into one of the five major families.

combination of blinding, pinealectomy, and neural ablation [3]. Fernandes and colleagues [3] were able to link dark-driven photokinesis directly to expression of OPN4 within the preoptic brain region by the use of *otpa*-deficient zebrafish, which lacking OPN4 expression, in the preoptic area. However, brain-wide lack of OPN4 expression in these mutants has not been confirmed, leaving open the possibility that other OPN4 neuron populations might contribute to light perception, with the preoptic area simply serving as a key region for downstream signal

## Key Figure

## Schematic of Attributed and Inferred Neuroendocrine and Physiological Roles of ERPs



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**Figure 4.** A condensed summary of established and hypothesized (denoted by ‘?’) processes and pathways of extra-retinal photoreceptor (ERP) action. Although numerous physiological and behavioral processes are linked to perception of light cues and/or circadian rhythms, most of the neural control mechanisms remain poorly elucidated, although thyroid signaling within the brain appears to be a common feature of transducing photic information with respect to seasonality. 1, localization of minnow color change receptors resolved to diencephalon [1]. 2, location of ERPs associated appears to vary by species and taxa. See ‘*Phototaxis and Locomotive and Feeding Behaviors*’ for details. AGRP, Agouti-related protein; DIO2, deiodinase 2; DIO3, deiodinase 3; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; Mel, melatonin; NPY, neuropeptide Y; T3, triiodothyronine; TH, thyroid hormone; TSH-β, thyroid-stimulating hormone-β.

## Box 1. Circadian Rhythms of Physiology and Endocrinology

Endogenous circadian rhythms are ubiquitous across all taxa of life. Based around a core molecular clock driven by a cyclical self-regulating transcriptional feedback loop, providing temporal information from the level of individual cells to the entire organism. Though defined by their ability to maintain rhythmic output with a periodicity of near 24 hours in the absence of external stimuli, endogenous circadian clocks *in vivo* are reset daily synchronizing their rhythm to the environment, by photic cues. Circadian rhythms are themselves a vast topic, too large to adequately address here, but excellent reviews exist elsewhere [43].

- Blood pressure
- Activity patterns
- Sleep patterns
- Feeding patterns
- Gene transcription
- Metabolism
- Melatonin
- Glucocorticoids
- Growth hormone
- Insulin
- Thyrotrophin
- Prolactin

Initially thought to be centralized based on work in mammals establishing the suprachiasmatic nucleus as the 'master clock', recent work has demonstrated the presence of peripheral clocks in nearly all tissues [103]. However, despite the dependence of the mammalian clock on input from the eyes, entrainment of daily rhythms is independent of classic visual photoreceptors (rods and cones) depending instead on melanopsin (OPN4) expressed by retinal ganglion cells. This critical role of non-visual opsins in entrainment of circadian rhythms is of particular interest in non-mammalian systems where a single 'master clock' appears to be absent, with eyes, pineal, and hypothalamus all potentially contributing to coordination of circadian rhythms [104].

Regardless of their localization circadian clocks serve as critical timekeepers, driving all manner of daily rhythms and coordinating a wide array of endocrine and metabolic cycles, as well as behavioural patterns as discussed in '*Phototaxis and Locomotive and Feeding Behaviours*'. Presently, linkage of ERPs to entrainment of these rhythms in non-mammalian vertebrates is lacking. The robust entrainment of many circadian rhythms by photic cues and the emerging ability to manipulate opsins *in vivo* make this an area primed for robust future research.

transduction. Intriguingly, the preoptic area does not appear to play a role in the earliest light-dependent behavior of zebrafish embryos, the photomotor response [50]. The use of morpholino gene knockdown (confirmed by gel imaging) found that knockdown of exo-rhodopsin and VA-opsin long A and B (all OPN1 family) all failed to inhibit the photomotor response, leaving the identity of the relevant ERP unknown [50]. A combination of brain sectioning, electrophysiology, and *in vivo* calcium imaging determined a discrete set of hindbrain neurons was necessary and sufficient for expression of the photomotor response in dark-adapted zebrafish embryos, but responsible opsins remain to be identified [50]. Thus, in zebrafish multiple light-driven locomotor behaviors appear to exist and be driven by distinct ERP populations.

As with general patterns of locomotive behavior, feeding behavior displays distinct circadian rhythmicity, well characterized in mammalian species (reviewed in [45,51]). Evidence from non-mammalian species also supports circadian patterns of feeding, but linkage to extra-retinal photoreception is sparse. Rhythmic feeding patterns in fish appear to be entrainable by light (e.g., [52]). However, variation in timing of food availability can also independently entrain feeding rhythms, leaving the contribution of light versus food availability unclear [52]. Thus, it remains unclear whether opsin-related mediation occurs directly through neural pathways or is mediated by entrainment of various endocrine rhythms (e.g., melatonin or glucocorticoids) that, in turn, regulate daily cycles of metabolic activity and feeding behavior (Box 1).

*ERP Regulation of Seasonal Reproduction*

**Photoperiod** as a key cue regulating seasonal rhythms of reproduction appears to be widely conserved across vertebrates [53]. Photoperiod has been suggested to play at least a permissive role in seasonal breeding in amphibians and reptiles ([54], and references therein). The identification of OPN1 (pinopsin) in CSF-contacting neurons within the anterior preoptic nucleus of toads [55] and additional expression of opsins in the hypothalamus of both amphibians and reptiles (Figure 1 and Table S1 in the supplemental information online) have strengthened support for ERP involvement in mediation of seasonal breeding, given the importance of these neural regions to regulation of the **hypothalamic pituitary gonad** (HPG) axis.

There is strong evidence that reproductive physiology is programmed by photoperiod in multiple species of fish: ayu (*Plecoglossus altivelis*) [56], Atlantic salmon (*Salmo salar*) [57], rainbow trout (*Oncorhynchus mykiss*) [58,59], Nile tilapia (*Oreochromis niloticus*) [60], and sapphire devil damselfish (*Chrysiptera cyanea*) [61,62]. Ophthalmectomy and pinealectomy of ayu demonstrated that short day-induced gonadal recrudescence and skeletal growth were independent of both the eyes and pineal complex, indicating perception of light cues occurs via ERPs [56]. Attempts to determine the optimal stimulatory wavelengths, and thus narrow opsin identity, have produced mixed results, with blue light showing the strongest effect in Nile tilapia [60] and red light showing the strongest effect in sapphire devil damselfish [63]. These differences in optimal stimulatory wavelengths are likely due to evolutionary adaptation to different photic environments, as different wavelengths can penetrate effectively to different depths [64]. The localization of OPN3, OPN4, and OPN5 (Figure 2) to the medial basal hypothalamus and preoptic area make them strong candidates for regulation of the breeding response given their proximity to the HPG axis.

ERP perception of photic cues has also been tied to multiple seasonal changes in endocrine states across birds species, including vernal migration [65], reproductive physiology [66–70], and **photorefractoriness** [69,71]. ERP control of reproductive endocrinology has been the best studied of these areas, beginning with the demonstration by Benoit and colleagues [10,72] that direct hypothalamic illumination is capable of inducing breeding during winter in ducks. Subsequently, fiber optic illumination of the ventromedial and infundibular regions of the hypothalamus of sparrows and quails [73,74] and radio-illumination [11] were also shown to induce gonadal growth under short days. Blinding and lesioning studies have further supported localization of the regulation of seasonal variation in avian reproductive physiology, metabolism, and growth to the deep brain, particularly the medial basal hypothalamus (reviewed in [9,75]), although as with fish, the precise nature and functional connections of avian breeding ERPs are not well established. To date, three major candidates, melanopsin (OPN4), neuropsin (OPN5), and VA-opsin (OPN1), have been suggested to control reproduction in birds.

OPN4-ir cells within the PMM and ME of turkeys and Pekin ducks (*Anas platyrhynchos domesticus*) provided initial correlative evidence linking hypothalamic light detection and the photoperiodic physiological response [32,76]. These data, combined with the role of melatonin in transducing circadian information, are used to support OPN4 as a candidate for the breeding photoreceptor. However, PMM OPN4 neurons are blue-light sensitive, but blue light alone is unable to elicit a gonadal response in Pekin ducks. Furthermore, the spectrum maximum ( $\lambda_{\max}$ ) for avian OPN4 is 476–484 nm [77], a range that is inconsistent with the established  $\lambda_{\max}$  range from 483 to 492 for breeding (Figure 1; [27,78]). Taken together, these findings make it likely that hypothalamic OPN4 is not the opsin responsible for the breeding photoperiodic response. An alternative role for PMM OPN4 may be the induction of reproductive involution and reduced growth rates in Pekin drakes [79].

Using *in situ* hybridization, OPN5 (neuropsin) has consistently been found in CSF-contacting neurons in the PVO with projections to the ME. As with OPN4, the presence of OPN5 in this neural node critical to control of reproductive physiology has been put forth as evidence of its involvement in said regulation. Attempts to determine the spectral maximum of OPN5 have presented contradictory results that remain to be resolved (reviewed in [9]), with reports of 360, 419–420, and 474 nm, none of which match the established 492 nm (or 483 nm) of the breeding response. OPN5 hypothalamic gene expression from red-headed buntings (*Emberiza bruniceps*) transitioned from short days to long days does not change, although OPN1

(rhodopsin), OPN4 (melanopsin), and RGR (peropsin) were all found to increase significantly [69]. OPN5 (neuropsin) was also found to remain constant across the photoinducible period in border canaries (*Serinus canaria domesticus*) during exposure to a single long day [37]. Attempts to alter hypothalamic OPN5 function via siRNA silencing resulted in an increase in thyroid-stimulating hormone  $\beta$  (TSH- $\beta$ ), a key component of the photoinduction cascade, supporting a role in the regulation of breeding. Subsequently, Nakane and colleagues [80] found that exposure to UV light induced the HPG axis and resulted in a decrease in OPN5-expressing neurons within the PVO. However, siRNA suppression of OPN5 coupled with UV photostimulation, to selectively trigger OPN5, in Japanese quail (*Coturnix coturnix japonica*) decreased TSH- $\beta$  in this instance. Thus, the linkage between OPN5 stimulation and TSH- $\beta$  remains to be definitively resolved.

OPN1 (VA-opsin) has been put forth as another major candidate for the photoinduction of breeding, again based initially on its hypothalamic distribution (Figures 1 and 3). Furthermore, immunocytochemical data indicate that OPN1 protein may be colocalized to arginine vasotocin and GnRH cells in the preoptic area of the anterior hypothalamus [9]. Light information directly regulates GnRH expression in birds [81], and the colocalization of OPN1 in this cell population could provide control over the photoperiodic regulation of neuroendocrine reproductive function in birds; however, this warrants further investigation. Furthermore, OPN1 has a spectrum maximum of approximately 490 nm in birds [9,82], making it the most consistent of the three putative breeding photoreceptors in terms of matching the target  $\lambda_{\max}$  492 nm for the seasonal regulation of avian reproduction and metabolism.

Although there is strong correlational evidence for OPN1 and OPN5 to act as the photoreceptors controlling seasonal breeding [83], the experimental data remain inconsistent, contradictory, or absent, leaving the identity of the breeding opsin(s) to be conclusively established. If indeed there is a single opsin and future studies succeed in experimentally establishing its identity, the following question inevitably arises: What roles do the other 'non-breeding' opsins located within the hypothalamus play? As mentioned, there are numerous seasonal endocrine and metabolic processes other than reproduction tied to photoperiodic control that these non-breeding hypothalamic opsins might be controlling. However, clear linkage of these opsin populations to other physiological or endocrine processes (e.g., migration) remains to be established.

#### *Molt and Migration*

Seasonal changes in a constellation of neuroendocrine, endocrine, and nutritional cues mediate the development and expression of avian molt and migration. However, both vernal migration and molt life history stages have been tied to seasonal changes in photoperiod [65,84]. It is likely that distinct ERPs integrate light information at different times of the year to drive seasonally appropriate changes in the neuroendocrine and endocrine parameters that drive molt and migration (e.g., thyroid signaling). Previously, it had been suggested that all vernal events including pre-nuptial molt, vernal migration, and breeding might share the same ERP population. However, experiments using very dim green light have shown that the vernal migratory life history stage can be induced in captive white-crowned sparrows (*Zonotrichia leucophrys gambelli*) without inducing gonadal growth, supporting the neural separation of these two pathways [85]. However, as with the majority of roles attributed to ERP control, the mechanistic pathways and identity of the necessary opsins for both these stages remain unclear. One promising area of emerging research has been the linkage of thyroid hormone signaling to photoperiod-driven seasonal processes, as we discuss next.

### Thyroid Hormones as Mediators of ERP Photic Information

Thyroid hormones have begun to emerge as a key component of ERP-modulated seasonality, particularly in avian species [86]. Thyroidectomy and pharmacological suppression of thyroid hormone levels have been shown to effectively suppress the expression of molt [84,87], migratory preparation and migratory behavior [88–90], gonadal growth [84,91,92], and gonadal regression [93,94]. Replacement studies have confirmed these findings and support local thyroid signaling within the brain [84,88,91].

The central role of thyroid hormones has been best described in the context of avian breeding. Wilson and Reinert [92] found that thyroidectomy in female American tree sparrows (*Spizella arborea*) blocked onset of photorefractoriness and post-nuptial molt as long as it occurred within 1 week of photostimulation. However, gonadal growth was only blocked by thyroidectomy before photostimulation itself, supporting a temporal decoupling of breeding from refractoriness and molt [92]. Thyroid hormones have since been found to play a central role in the neural transduction of the photoperiodic signal from ERPs in the brain. The detection of increased photoperiod by ERP populations in the mediobasal hypothalamus results in the upregulation of TSH- $\beta$  expression from thyrotrophs in the pars tuberalis of the anterior pituitary [95]. TSH- $\beta$  then acts locally on ependymal cells lining the 3V, by altering the expression of deiodinase enzymes, regulators of thyroid hormone action, increasing deiodinase 2 (DIO2) and decreasing deiodinase 3 (DIO3) mRNA expression [96,97]. The down-regulation of *DIO3* may be mediated by DNA methyltransferases, as silencing of the *DIO3* promoter by methylation in response to long days has been demonstrated in Siberian hamsters (*Phodopus sungorus*) [98], but has yet to be reported outside mammals. The result of this shift in deiodinase expression is increased conversion of circulating thyroxine, the primary circulating hormone, but with low bioactivity, to the bioactive tri-iodothyronine (T3), thereby increasing thyroid hormone signaling [96,99,100]. The localized increase in T3 in turn triggers the release of GnRH [101]. This has been verified by direct intracerebroventricular injection of T3 to triggering the HPG axis in short-day birds [101,102]. Taken together, these studies support a key role for thyroid hormones in linking the detection of light information by ERPs with the release of GnRH into the peripheral circulation. The shared presence of thyroid hormone signaling across seasonal processes provides a potentially shared mechanism for transduction of photic information from opsins to downstream neuroendocrine and endocrine pathways.

### Concluding Remarks and Future Directions

Our knowledge of extra-retinal photoreception continues to expand; however, many key elements remain unresolved (see Outstanding Questions). Despite the ever-increasing array of species in which ERPs have been confirmed, the functional connections to and the role of the majority of these ERPs in key endocrine, circadian, and metabolic processes remain unclear. Similarly, several endocrine systems and behaviors have long been ostensibly linked to photoperiodic control by ERPs, but without demonstrated links to any specific opsin type (s). Aside from the handful of studies highlighted herein, evidence for involvement of a specific opsin remains correlative.

Future work must move beyond identifying novel opsins or correlating their presence/expression to sites previously established as being necessary to a given process. Researchers must seek to functionally tie specific populations of ERPs to known outputs. Circadian rhythms, with their established entrainability by photic cues, are particularly ripe for investigation [103,104]. Recent advances in molecular and gene editing technologies present new opportunities to conduct direct experimental manipulation of opsins *in vivo*. Such studies, as we have seen from

### Outstanding Questions

Which opsin(s) population regulates avian seasonal breeding?

Are apparently unique pockets of opsin expression in fact unique or instead an artefact of research focus? For example, is the expression of opsins within the olfactory bulb limited to fish? Do these opsins play a functional role with respect to light detection or are they evolutionary artefacts?

How do opsins mediate entrainment of circadian rhythms in non-mammalian vertebrates?

What physiological processes do the constellation of identified and localized opsins, particularly in the avian and fish brains, modulate/regulate?

Does thyroid hormone signaling provide a unifying mechanism neural transduction of photoperiodic information to other neuroendocrine and endocrine systems?

the few that have been conducted, provided a powerful and clear way to determine ERP involvement in a wide variety of physiological and behavioral processes. With thoughtful application and clear experimental design, the definitive linkage of specific ERPs to known processes can finally be established.

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### Supplemental Information

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