Quantitative Analysis of Thalamic Regions in Response to Blue Light in the Leopard Frog (*Rana pipiens*)

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Blue light has powerful effects on human health. During the day, blue light exposure elevates mood, alertness and cognition. Excessive exposure at night is linked to sleep disorders, depression, cancer, and heart disease. The neural mechanisms responsible for mediating these effects are unclear. Frogs have a unique response to blue light – they are attracted to it more so than any other colored light. Elucidation of the neural mechanisms responsible for guiding the frog's recognition of blue light may help describe the influence of blue light on vertebrate nervous systems in general. In frogs, a distinctive response to blue light is seen in a retinorecipient area of the anterior thalamus known as the NCZ (neuropil of Bellonci/Corpus Geniculatum Zone). The NCZ is reciprocally connected to an area of the posterior medial dorsal thalamus (PMDT). In order to assess the PMDT's physiological relationship with NCZ and its role in mediating responses to blue light, a Fourier transform is used to analyze extracellularly recorded multicellular receptive fields in the NCZ and PMDT. Results indicate: (i) the NCZ is characterized by a main tonic oscillatory response to blue light; (ii) blue light elicits tonic oscillatory electrical responses in the PMDT quite similar to the responses to blue light in the NCZ. This analysis suggests a reciprocal connection between NCZ and PMDT. PMDT has no other connections besides the NCZ, therefore, the visual activity recorded in PMDT is derived from activity originating in the NCZ. Primary visual activity in the NCZ may be modulated by cells in the PMDT, creating a feedback loop that guides the frog's responses to blue light. The discovery of a novel, blue light-driven thalamic circuit in the frog suggests similar circuits may be present in other vertebrates, and may be a potential target for therapies.

Abbreviations: Discrete Fourier transform – DFT; Inverse Discrete Fourier Transform – IDFT; Neuropil of Bellonci/Corpus Geniculatum Zone – NCZ; Posterior Medial Dorsal Thalamus – PMDT;

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Introduction

The relatively simple and tractable visual system of the frog can be used to help understand the fundamental operating principles of vertebrate vision (Lettvin, 1959; Ingle, 1973). Leopard frogs (*Rana pipiens*) are highly visual animals. They rely on vision to recognize behaviorally relevant aspects of their environment and respond appropriately. Vision enables the frog to navigate around obstacles, localize prey, evade predators, and escape to hiding places.

Previous investigators have shown that the frog's visual system processes moving and stationary stimuli in two independent visual modules. Removal of the optic tectum renders a frog unresponsive to moving prey and looming stimuli. However, atectal frogs are still able to avoid stationary obstacles and jump cleanly

through holes (Ingle, 1973; Saltzman et al., 2004). The visual system of frogs—and vertebrates in general—is not a unitary system. This finding demonstrates that the visual system of frogs is divided into several components, as every visual function is not terminated by the removal of one aspect. Vertebrate vision is subdivided into separate modules, each responsible for processing different aspects of vision (Schneider, 1969). In frogs, there are at least two independent visual systems: one system for processing moving visual stimuli, which is mediated by the midbrain optic tectum and another for processing stationary visual stimuli. Evidence suggests that thalamic visual areas are responsible for mediating responses to stationary stimuli (Kicliter, 1973).

In frogs, the retina projects to several regions of the thalamus. Two areas of the anterior lateral thalamus receive bilateral retinal projections. These areas are designated as the nucleus of Bellonci (NB) and the corpus geniculatum thalamicum (CG) (Roth et al., 2003). Electrical recording and disconnection studies have shown that the NB and CG process visual information about light and color and mediate frogs' responses to light sources, including their robust, preferential attraction to blue colored light (Muntz, 1962).

Recktenwald (2017) showed that the anterior lateral thalamus contains a physiologically defined area called the "NCZ" that includes both the superficial NB and CG retinal projections and also extends medially to include adjacent neuropil and cellular areas. Electrical recordings in the NCZ have revealed that it responds in a characteristic phasic manner to the white light and in a characteristic tonic oscillatory manner to the blue light. The NCZ has strong, bilateral, reciprocal connections with an area in the PMDT. Anatomical studies (Recktenwald, 2017) suggest the PMDT is not connected to any other part of the brain and therefore functions as a satellite of the NCZ; and initial physiological results suggest cells in the PMDT respond preferentially to blue light in a manner similar to responses in the NCZ. These reciprocal connections between the NCZ and its satellite – the PMDT – likely function as a feedback circuit responsible for mediating frogs' responses to the stationary visual world and specifically in

guiding the frog's attraction to blue light. To test the hypothesis that the NCZ and PMDT display similar responses to the onset of blue light, this paper analyzes the frequency spectrum of multiunit electrical activity recorded extracellularly in both the NCZ and the PMDT in response to a blue light stimulus. Since the PMDT does not receive retinal input, and is connected only to the NCZ, similar oscillatory activity in the PMDT and the NCZ will suggest that PMDT is involved in generating the unique responses to blue light in the retinorecipient NCZ through a sensory feedback system that selectively amplifies signals generated by blue light stimuli.

This study helps to define the neural substrates for a visually guided behavior in frogs; and also helps elucidate a vertebrate neural circuit that is preferentially activated by blue light. Blue light has been shown to be useful in the treatment of depression, mood disorders, daytime sleepiness, and attention deficit disorder, however the neural mechanisms responsible for these effects are not understood (Holzman, 2010). This research may help explain the neural mechanisms responsible for the unique and powerful effects that blue light has on vertebrate animals including humans.

Materials and Methods

Specimens

Adult leopard frogs (*Rana pipiens*) 6.0- 7.5 cm snout to vent were obtained from Vermont (Hazen Company, Alburg, VT, USA). These frogs (N=15, 8 male, 7 female) were housed in a terrarium (32 cm x 76 cm x 31 cm, H × W × D) with running water, kept on a 12 hour light/dark cycle, maintained at 21 - 23 ^{0}C, and fed live crickets (*Acheta domestica*) 3 times a week.

Surgical Preparation and recording in NCZ and PMDT

The day prior to electrophysiological recording sessions, animals were anesthetized by immersion in 0.3% 2-methane-sulfonate (Sigma), and a flap of skin was cut in the region above the diencephalon. A patch of bone over

the thalamus was excised with a dental wheel to expose the thalamus. The patch of bone was replaced; a viscous solution of Xylocaine (Sigma-Aldrich, St. Louis, MO) was applied to the wound; The flap of skin was sewed back in place and the animal was allowed to recover overnight at room temperature (21ºC).

Approximately 2 hours prior to electrical recording sessions, the frog's trunk was subcutaneously injected with 0.02 ml - 0.05 ml of turbocurarine (Sigma-Aldrich). Once the animal's legs were flaccid, the patch of bone created the day before was removed and the blood vessels were deflected gently, exposing the dorsal surface of the thalamus. Extracellular recordings were performed using platinum tipped microelectrodes (Dowben and Rose, 1953) connected to a Grass P15 A/C preamplifier, which was connected to an analog to digital converter (PowerLab, ADInstruments, Colorado Springs, CO). ADInstruments Chart software was used to sample, display, and record the data at 20,000 Hz.
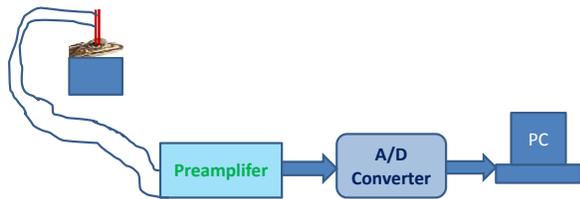

Figure 1. Overview of electrophysiological setup.

A blue laser pointer was used as a visual stimulus. The laser was held 30 cm away from the frog, and pointed toward the tip of the snout. The laser was covered with transparent paper so that a point of light (4° at 30 cm) was visible. The laser was held stationary in the receptive field and turned ON and OFF in 1-3 second intervals. The illuminance of the laser was 0.02 lx. (measured using an ORIEL LightMeter Model 70286.) For recordings in the NCZ and the PMDT the microelectrode was lowered into either the anterior lateral (NCZ) or posterior medial (PMDT) thalamus. Each location was confirmed when oscillatory responses to the blue light stimulus were observed.

Fourier transform

In order to understand the useful information and features of electrophysiological responses to blue light in the NCZ and PMDT, the Fourier transform was used to convert signals from the time domain to the frequency domain. The inverse Fourier transform was used to convert the signal from the frequency domain back to the time domain.

The Fourier transform is a powerful tool to analyze signals and construct them to and from their frequency components. If the signal is discrete in time that is sampled, the discrete Fourier transform (DFT) is used to convert the signal to the discrete frequency form. The inverse discrete Fourier transform (IDFT) is used to convert the discrete frequency form back into the discrete time form.

To reduce the mathematical operations used in the calculation of DFT and IDFT, the fast Fourier transform algorithm FFT and IFFT should be used, which corresponds to DFT and IDFT, respectively. The fast Fourier transform is a mathematical method for transforming a function of time into a function of frequency. It is useful for analysis of time-dependent phenomena. In the research, fft() in MATLAB is used to compute the discrete Fourier transform (DFT) of signal s.

All animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee of Temple University (July 16, 2015)

Results

Electrical recordings in PMDT and NCZ

Electrical activity in the superficial neuropil of the NCZ and PMDT was recorded in multiple experimental animals (N=15). Representative recordings are shown in Fig. 2A. A tonic oscillatory response to the onset of a blue light stimulus in both regions was found. This response continued as long as the blue light was on. A similar activity pattern is seen in both the NCZ and PMDT (Fig. 2B, 2C).
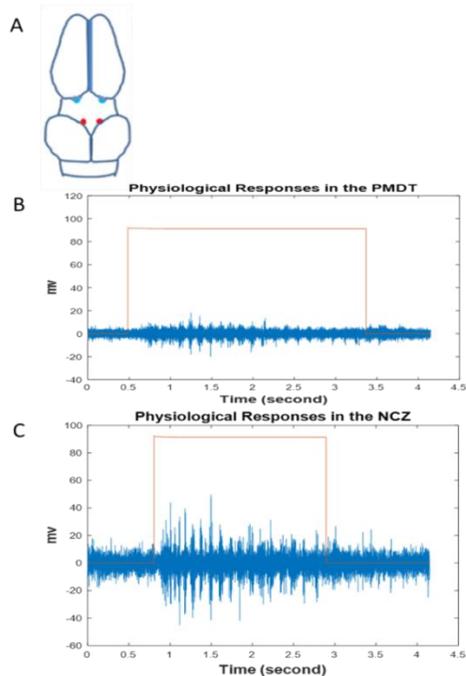
Figure 2. Electrical recordings in PMDT and neuropil of Bellonci/corpus geniculatum zone (NCZ). **A.** Dorsal view of frog brain; filled red circle, PMDT recording location; filled blue circle, NCZ recording location; top is rostral, bottom is caudal. **B.** Extracellulary recorded responses in the PMDT during baseline and blue light stimulation (orange line) **C.** Extracellulary recorded responses in the NCZ during baseline and blue light stimulation (orange line).

FFT of Electrophysiology in PMDT

I analyzed and compared electrical activity in response to blue light in the NCZ vs the PMDT in two representative recordings (Fig. 2). The NCZ receives primary input from the retina. Visual information is relayed from the NCZ to the PMDT.

The tendency of response frequencies during blue stimulation in the PMDT (posterior region of thalamus) is similar to that of baseline response frequencies, however, the amplitudes of some frequencies from 5 to 80Hz increase obviously. Several small groups' frequencies increase more than others in response to blue stimulation. Unique characteristics are seen in specific frequencies distribution. All frequencies are not distributed uniformly. Several small clusters/groups are distributed around 10Hz, 18Hz, 26Hz, 36Hz and 48Hz, etc (Fig. 3) which have larger amplitudes compared to other frequencies.
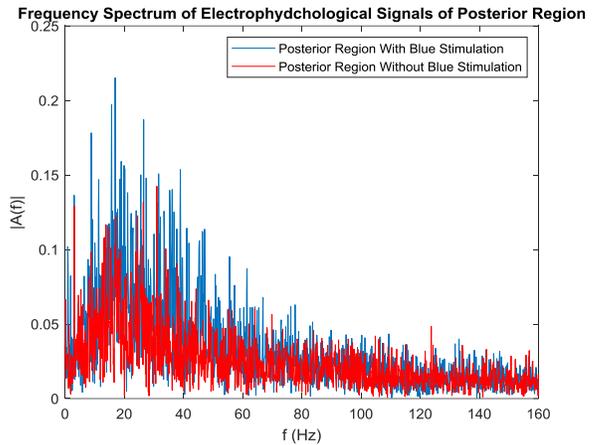

Figure 3. Frequency spectrum of electrophysiological signals in posterior region of thalamus (PMDT) during blue stimulation (blue) and baseline (red).
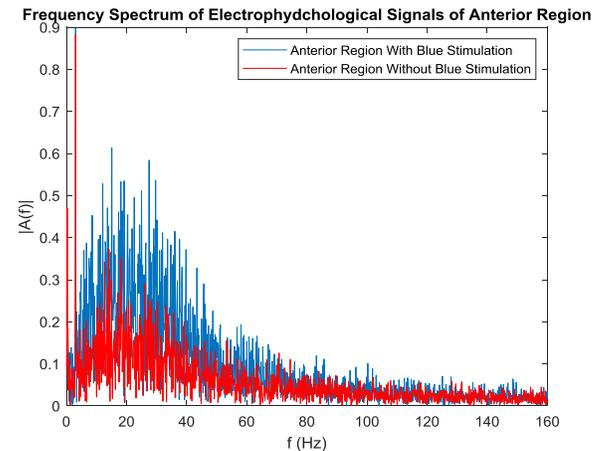

Figure 4. Frequency spectrum of electrophysiological signals in the anterior region of the thalamus (NCZ) during blue stimulation (blue), and baseline (red).

In the anterior region of the thalamus (NCZ), the tendency of response frequencies during blue stimulation has similarities to baseline responses in the frequency domain. However, the amplitudes of all frequencies from 5Hz to 80 Hz with blue stimulation are larger than those of the signals corresponding to the baseline, which implies cells responded significantly to blue stimulation. Noticeably, regardless of whether blue stimulation was added or not, there are large amplitudes at 3 Hz in the NCZ, suggesting that electrophysiological signals in the NCZ region include Delta waves (0.5 – 4.0 Hz) (Fig.

4). These specific frequencies were not found in the PMDT (Fig. 3).

The total amplitudes of response frequencies (0Hz to 80 Hz) during blue stimulation in the anterior region (NCZ) are much larger than those of response frequencies to blue stimulation in the posterior region (PMDT) (Fig.5). This suggests that the anterior region has a much stronger response to blue stimulation compared to the posterior region.
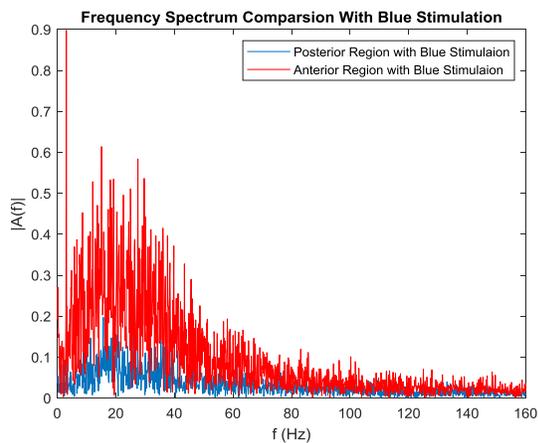

Figure 5. Frequency spectrum of electrophysiological signals during blue stimulation in both the posterior region (blue), and anterior region (red).
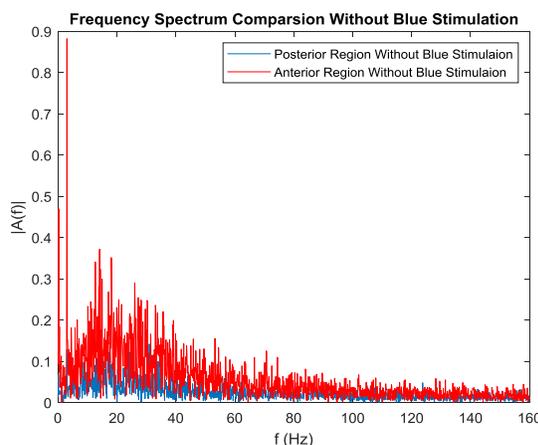

Figure 6. Baseline frequency spectrum of electrophysiological signals without blue stimulation in the posterior region (blue) and the anterior region (red) of the thalamus.

For baseline activity without any blue-light stimulus, whole amplitudes of frequencies from

0 Hz to 80 Hz in the anterior region of the thalamus (NCZ) are larger than those in the posterior region of the thalamus (PMDT), there are two response frequencies with higher amplitude/peak in the NCZ – these peaks (<5Hz) only appeared in the NCZ, and suggests that baseline electrical activity in the NCZ includes spontaneously generated Delta waves (Fig. 6). Such spontaneous oscillations in the thalamus are involved in generating mammalian Delta waves detectable by EEG recordings in mammals.

An analysis of the data shown in Fig.3-6 demonstrates: (i) The whole curve shape of PMDT is quite similar to that of NCZ; (ii) All peak values of PMDT are lower than those of NCZ; (iii) There is selective enlargement in response signals in PMDT after blue stimulation. That is, several small groups' frequencies increased more than other frequencies after blue stimulation; (iv) There are two frequencies below 5Hz with high peak values found in the NCZ. These frequencies are not seen in PMDT; (v) Response frequencies sensitive to blue stimulation are focused on the range of 0 to 80 Hz.

Discussion

I discovered that electrophysiological responses to blue light in the PMDT are remarkably similar to the oscillatory tonic responses to blue light in the NCZ. The magnitudes of the frequency spectrum of signals in NCZ are completely higher than the magnitudes of the frequency spectrum of signals in the PMDT (Figs. 5, 6). The NCZ receives primary visual input from the retina and likely relays this information to cells in the PMDT. Anatomical evidence also suggests the PMDT is a satellite of the NCZ since it is exclusively connected to the NCZ (Recktenwald, 2017). Therefore, it seems that blue-light driven oscillatory activity is greater in the primary visual area of the NCZ, and also present in secondary neurons of the PMDT.

I also discovered that unlike the NCZ, only several small groups of frequencies around. 10Hz, 18Hz, 26Hz, 36Hz and 48Hz, etc. in the

PMDT increase noticeably following blue-light stimulation (Fig. 3). These results suggest that the PMDT modulates activity of the NCZ by selectively amplifying certain frequency groups. The unique, powerful, oscillatory response to blue light in the NCZ may be possible through a feedback circuit with its satellite, the PMDT: Neurons in the NCZ may pass light information to the PMDT, which selectively enhances those signals corresponding to only blue colored light, creating a sensory feedback system that facilitates the recognition of light in a relatively narrow range of the visible spectrum. This feedback loop scheme is also found in other vertebrates (Gruberg, 2006), e.g., in the reciprocal connections between the thalamus and the cortex in mammals (Sherman, 2002). Sensory feedback loops allow an organism to select and attend to specific, behaviorally relevant features of the visual world.

Baseline activity in the NCZ shows two response frequencies (<5Hz) with higher amplitudes/peaks (Figs. 5, 6). This result suggests that baseline, spontaneous activity in the NCZ of the anterior lateral thalamus includes Delta waves. Delta waves are also observed in thalamic activity of humans during resting sleep (Amzica, 1998), and spontaneous thalamo-cortical oscillations are thought to be responsible for generating the Delta waves recorded by scalp EEG electrodes during sleep (Fogerson, 2016). Further research into amphibian thalamic oscillations may lead to a deeper understanding of the mechanisms of global neural oscillations and their relationships to behavioral states.

Blue light (wavelengths ranging from 450 nm to 495 nm) affects human sleep/wake cycles, mood, alertness, cognitive performance etc. (Holzman, 2010). Although these effects have been well-studied, the neural mechanisms mediating the entirety of the effects of blue light are unclear. Much attention has been focused on the role the retinohypothalmic tract plays in entraining the circadian rhythms of vertebrates (Fernandez, 2016). However, given the global effects of blue light on health and behavior, there may be additional circuits involved in mediating the effects of blue light. Frogs can be used as a model to elucidate the circuits and cellular physiology of the vertebrate nervous system.

Unlike primary visual input to the thalamus in mammals, the NCZ does not project to the telencephalon, however it is reciprocally connected to an area of the PMDT (Recktenwald, 2017). Responses to blue light in the PMDT appear to be similar to those seen in the NCZ. Here I show evidence of a feedback circuit possibly responsible for mediating the frog's recognition and response to blue light. These processes may elucidate the mechanisms used in the human brain to mediate some of the effects of blue light. The discovery of a novel, thalamic circuit involved in recognizing and amplifying neural activity driven by blue light may lead to new therapies such as targeted deep brain stimulation. Human environments are filled with unnatural light. A greater understanding of the neural circuits guiding the effects of blue light may suggest strategies for minimizing artificial light's negative effects on human health.

This study identified a physiological relationship between the retinorecipient NCZ and its satellite, the PMDT. This relationship increases our understanding of the frog's visual system responsible for recognizing and navigating the stationary visual world. The NCZ-PMDT circuit is likely responsible for recognizing areas of blue-colored light, and is the neural substrate responsible for guiding the attraction to blue light.

Future experiments will include knocking out the PMDT and recording blue light-driven activity in the NCZ. Electrical responses to blue light in NCZ are expected to be non-oscillatory and similar to responses to white light in the absence of PMDT. Future experiments will also simultaneously record responses to blue light in the NCZ and PMDT. The latency of responses in the NCZ and PMDT will be measured. If the PMDT is responsible for establishing the oscillatory response to blue light, the response should be seen in PMDT before in NCZ.

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