Tectal activity underlying phototactic preferences in the *Xenopus laevis* tadpole

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Summary statement: *Xenopus laevis* tadpoles tend to swim in green-illuminated areas, a preference that likely arises out of circuit-level patterns of synaptic activity.

Abstract

The *Xenopus laevis* tadpole has long been known to exhibit phototactic preferences, i.e. preferences to swim towards or away from visual stimuli. Of particular interest is the tendency for stage 48 *Xenopus* tadpoles to prefer green light but for older tadpoles to prefer blue light. Recent work has begun to characterize the timeline of development for *Xenopus* rod and cone cells with different light specificities, relating this developmental timeline to the shift in color-guided phototactic preferences. Yet still little is known about the neural processing of color in the developing brain. The present study's first experiment extends previous behavioral data, demonstrating that stage 48 *Xenopus* tadpoles' phototactic preference for green light persists over extended testing periods and outside of forced-choice procedures. To further investigate the neural mechanisms underlying color processing in the developing brain, a second experiment used *in vivo* electrophysiology to examine neural activity in the optic tectum during the processing of color. To our knowledge, this is the first study comparing tectal activity in response to different colors of light. No differences in response to color were observed at the single-cell level, suggesting that the color of visual stimuli is likely encoded at the circuit level or higher. Finally, two distinct populations of neurons were identified based on the pattern of synaptic inputs they received during visual stimulation. Future studies will investigate the roles of these two neuronal types in the processing of visual stimuli.

Introduction

The scientific study of color discrimination dates back to at least 1883, when Veit Graber first documented the phototactic preferences of salamanders (Graber, 1883; Laurens, 1911). The use of amphibian models for understanding visual processing tasks, such as color discrimination, continues over 100 years later (e.g. Rosencrans, 2018; Yovanovich, 2017). Indeed, the study of both visual circuit formation and the development of visual-related behaviors in amphibians has given rise to principles applicable to a wide range of vertebrates (e.g. Lambert, 2008), contributing to their continued relevance as model organisms today.

Newly-developed behavioral assays provide evidence that *Xenopus laevis* tadpoles possess "true" color vision, meaning that they detect and respond to contrasts that can only be attributed to differences in color rather than brightness (Gravot, 2017). In fact, *Xenopus* tadpole color processing is remarkably similar to humans' (Knorr, 2018), making the *Xenopus* visual system a viable model for studying the mechanisms that underlie subcortical color processing in humans.

Beyond being a merely viable model, *Xenopus* is an *attractive* model to study color processing for several reasons. First, *Xenopus* vision gives rise to complex visually-guided behaviors (Dong et al., 2009). Additionally, the development of the retinotectal circuit, which gives rise to visually-guided behaviors, is highly stereotypical and well-characterized (Liu et al., 2016). Finally, it is possible to make electrophysiological recordings from the optic tectum *in vivo* while visual stimuli are presented to the retina (van Rheede et al., 2015), allowing for visually-guided behaviors to be studied at the circuit level.

Visually-guided behaviors

The color-guided behaviors of *Xenopus laevis*, the African clawed frog, mirror those of other amphibians including *Rana temporaria*, the European common frog; *Rana pipiens*, the leopard frog; and *Bufo americanus*, the American toad: in early development, these tadpoles prefer to swim towards green light, but as development progresses, they develop a phototactic preference for blue light over green (Muntz, 1963; Jaeger & Hailman, 1976). These preferences are robust, occurring regardless of animal sex, temperature, time of day, or time of year (Hailman & Jaeger, 1974). In *Xenopus laevis*, the behavioral shift to a blue preference is known to coincide with the development of blue-sensitive green cones (Parker et al., 2010). It has been

hypothesized that the early green preference of tadpoles exists to attract them towards green plants for feeding and shelter (Muntz, 1963; Jaeger & Hailman, 1976), while the later blue preference of tadpoles is less well understood. The blue preference may serve to draw tadpoles towards more brightly-illuminated areas as they move closer to the sky (Hailman & Jaeger, 1974) or into deeper waters (Parker et al., 2010).

Yet these experiments bring about several questions regarding the nature of tadpoles' color-guided behavioral preferences. Jaeger and Hailman (1976) report a green preference among stage 46-48 *Xenopus laevis* tadpoles, yet cannot rule out the possibility that this preference is based instead on illuminance; tadpoles prefer swimming towards brighter stimuli and this preference overrode any color preferences observed. Thus, it has not been ascertained that *Xenopus laevis* tadpoles truly swim towards green light.

Additionally, experimental approaches have employed a "forced-choice" mechanism whereby the animal is forced to make a choice by moving towards one stimulus or another. Animals were forced to make a choice by physical touch if they did not spontaneously choose one stimulus over the other and only those trials in which the animal passed a certain criterion line were scored (Muntz, 1963; Hailman & Jaeger, 1974; Jaeger & Hailman, 1973, 1976). In all cases, a trial was considered complete once the animal passed the criterion line. These experiments do not adequately test for visually-guided preferences. First, it is possible that the animal would generally prefer to remain stationary and has no strong phototactic preference either way. It is worth noting that Boycott et al. (1964) report that forcing *Rana temporaria* to make a decision altered phototactic preferences, though Chapman (1966) found no such effect with *Rana catesbiana*. Second, it is possible that animals' *initial* color-guided preference may differ from their *long-term* color-guided preference. For example, perhaps an initial green preference would not persist over time.

Finally, a subsequent experiment by Jaeger and Hailman (1976) involved measuring the density of *Bufo americanus* tadpoles at different locations in a pond, a naturalistic environment for a tadpole, and measuring the spectral qualities of the locations from which tadpoles were gathered. In this experiment, it was found that *Bufo americanus* tadpoles congregated in green-illuminated regions of the pond, suggesting that *Bufo americanus* tadpoles prefer green-illuminated regions over extended periods of time. However, interpretation of this result is confounded by the presence of green plants that serve as food for the tadpoles in the green-

illuminated regions of the pond. It is possible that feeding motivated the observed behavior rather than innate color preferences.

To address these issues, we developed a new behavioral test to measure tadpole color preference beyond initial phototaxis which does not involve forced-choice elements. Using this procedure, we characterized the color preferences of *Xenopus laevis* tadpoles over time.

Neural processing of color

A question of basic scientific interest is how different colors are processed in the *Xenopus* tadpole's brain. An understanding of the mechanisms involved in color processing may reveal the computations that give rise to color-guided behaviors. To date, no studies have directly addressed how color is processed in the tadpole brain. But given the similarity between *Xenopus* tadpoles' color processing and humans' subcortical color processing (Knorr, 2018), any insights into this process may yield knowledge of principles that underlie visual processing in mammalian midbrain structures, such as the superior colliculus. A midbrain structure that receives direct input from the retina, the superior colliculus orchestrates visual reflexes such as orienting or saccading towards a stimulus of interest (Schneider, 1969).

Studies of the non-human primate superior colliculus have found it sensitive to red-green contrast as well as blue-yellow contrast (White et al., 2009; Hall & Colby, 2014; Herman & Krauzlis, 2014). This information, taken together with the existence of a simple vertebrate that has similar color sensitivities to its mammalian counterparts, gives rise to interesting questions. For instance, because the optic tectum is an amphibian structure homologous to the superior colliculus in mammals, the *Xenopus* retinotectal circuit provides a unique opportunity to investigate whether color encoding and processing are evolutionarily conserved across phylogenetic classes. This knowledge would provide novel insights into the evolution of color vision.

Implications for artificial intelligence

Synthetic biology is an emerging field to which dozens of reviews (e.g. Drubin et al., 2007), special issues in journals (e.g. Hanson et al., 2019), and chapters in books (e.g. Luisi, 2016) have been devoted. While great endeavors in synthetic biology have been made to synthetically model intracellular processes (Benner & Sismour, 2005; Drubin et al., 2007),

attention is increasingly turning to modeling multi-cellular circuits (Regot et al., 2011; Tamsir et al., 2011; Urios et al., 2016; Toda et al., 2018). Indeed, private sector corporations may also see promise in multicellular synthetic biology, as is reflected in recent patent filings for synthetic biology technologies (e.g. Prindle et al., 2016).

The retinotectal circuit is responsible for generating visually-guided behaviors in the *Xenopus* tadpole. Astoundingly, the retinotectal circuit is able to self-assemble into a circuit capable of perceiving and responding to the external environment in a matter of only 4-5 days (Liu et al., 2016). Studying the *Xenopus* retinotectal circuit provides the opportunity to learn more not only about the assembly of circuits, but also the interfaces between perception and action, an area that has long been (Horn et al., 1986; Goodale, 1990) and continues to be (Haazebroek et al., 2011; Palm, 2012) of interest to robotics and artificial intelligence.

Materials and Methods

Animals

All animal husbandry and experimental procedures were carried out in compliance with the University of Wyoming Animal Care and Use Policies. Tadpoles resulting from in-house breeding of adult *Xenopus laevis* frogs were reared in 10% Steinberg's solution (0.067 KCl, 0.034 $Ca(NO_3)_2 \cdot 4H_2O$, 0.083 $MgSO_4 \cdot 7H_2O$, 5.8 NaCl, 4.9 HEPES; Liu et al., 2018a). Tadpoles were housed on a 12-hour light/dark cycle in white-walled incubators at 22°C. All experiments were performed on stage 47-48 tadpoles (staging per Nieuwkoop & Faber, 1956).

Behavioral testing

All green preference tests were carried out in a 5.5"-diameter circular petri dish filled with 75 mL Steinberg's solution. The dish was placed on a computer monitor (HP ZR22W) that projected white light onto 75% of the dish floor and green light onto 25% of the dish floor. 10 wild type *Xenopus* tadpoles were then pipetted into the dish, allowed to acclimate for one minute, and then recorded for one hour using a GoPro Hero 7 Black or GoPro Hero+ LCD video camera. At the end of every minute of recording, the number of tadpoles in the green and white sections of the dish was counted (Figure 1). Tadpoles from any given clutch were not tested more than twice.

To control for schooling effects, in which the presence of one or a few tadpoles in one region of the dish attracts others to the same region, a parallel procedure was carried out under a similar protocol to that of the green preference test described above. However, in this control condition, the entire dish was illuminated with white light. An arbitrary 25% section of the dish was marked during analysis and the number of tadpoles in that section was counted over the course of an hour under the same methodological procedure.

Tadpole position was defined by the region where the tadpole's eyes were. For example, during a green preference test, if both eyes were in the white section of the dish yet the tadpole's tail was in the green section, the tadpole would still be counted as being in the white section. On the uncommon occurrence that one eye was in one section and the other eye was in another section of the dish, the tadpole was visually inspected and a determination was made as to which section a majority of the tadpole's head resided in.

In vivo whole cell electrophysiology

Xenopus tadpoles were prepared for *in vivo* electrophysiological recordings as described by Liu et al. (2018a). First, tadpoles were anesthetized with 0.01% tricaine methane sulphonate (MS-222). Tadpoles were then transferred to a recording dish containing a bath of HEPES-buffered extracellular saline with the acetylcholine receptor blocker tubocurarine (comprised of, in mM: 115 NaCl, 2 KCl, 3 $CaCl_2$, 3 $MgCl_2$, 5 HEPES, 1 glucose, and 0.1 tubocurarine; pH 7.25; osmolarity: 255 mOsm). The tadpole was secured with insect pins before the skin overlying the brain was removed and the brain was filleted along the midline. Finally, a portion of the ventricular membrane covering the optic tectum was removed with a broken glass micropipette to provide access to tectal somas. It is worth noting that in the classic *in vivo* preparation, only tectal neurons from the superficial layers of the optic tectum are accessible for recording.

Once tadpoles were prepared for recording, whole cell patch clamp electrophysiology was conducted (Figure 2A-B) as described by Liu et al. (2018b). Incoming spontaneous synaptic activity (Figure 3) was recorded while the cell was held in voltage-clamp at -60 mV for a period of 370-400 seconds. Recordings were made while the tadpole received visual stimulation, described below. Tectal neurons were identified using the 60X water-immersion objective on a Zeiss Axio Examiner A1 upright microscope connected to a Hamamatsu CCD camera. Only neurons from the middle third of the tectum were selected for recording, to control for potential

variability along the rostral-caudal axis of the tectum. Consistent with selection techniques reported by Ciarleglio et al. (2015), unhealthy neurons, identified by their particularly granulated and dark coloration, were not recorded. Additionally, mesencephalic trigeminal neurons, identified by their large somas, light coloration, and visible nuclei (Pratt & Aizenman, 2009), were not selected for recording.

Whole cell recordings were recorded using glass micropipettes (7-12 MΩ resistance) containing potassium gluconate internal saline (comprised of, in mM: 100 K-gluconate, 8 KCl, 5 NaCl, 1.5 MgCl2, 20 HEPES, 10 EGTA, 2 ATP, and 0.3 GTP, pH 7.2; osmolarity: 255 mOsm; Liu et al., 2018b). While recording, an Axon Instruments MultiClamp 700B microelectrode amplifier (Molecular Devices, San Jose, CA) was used to measure signals, which were then digitized using a Digidata 1322A digitizer (Molecular Devices, San Jose, CA). Recordings were captured using pCLAMP 10.3 software. For inclusion in the dataset, cells needed to have <50 MΩ access resistance during recording and needed to receive spontaneous synaptic input during the presentation of visual stimulation.

Visual stimulation for whole cell electrophysiology

Tadpoles were presented with different colors of static, equiluminant visual stimuli while whole cell recordings were made (Figure 2C). During each recording, the tadpole was presented with four colors, red, grey, blue, and green, projected from a Samsung SP-P310ME projector. The first color presented was selected randomly, to control for possible duration-related effects of visual stimulation. Each color was presented for a total of 60 seconds while colors were interleaved by 30-second periods of projecting a black screen. It should be noted that the black screen projection was not truly black (i.e. the absence of light), and thus some light was still being projected onto the tadpole's eye between colored stimuli.

Data analysis

Electrophysiological data was analyzed using AxoGraph 1.7.2. Figure 4 was produced in ImageJ using the method described in Appendix A. All statistical analysis was performed in Microsoft Excel 2016 and R 3.5.3.

Results

Tadpoles prefer green over extended time frames

If *Xenopus* tadpoles showed no preference between white and green, we would expect an average of 2.50 out of 10 tadpoles to be in the green section over the course of an hour, as the green section comprised 25% of the dish. However, across one-hour trials (N = 12), we observed significantly more tadpoles in the green section on average (median = 3.425; Wilcoxon signed rank test: V = 71, p = 0.009277). Indeed, this is illustrated qualitatively as well: Figure 4 shows a heat map of tadpole positions over time, illustrating where tadpoles were located over the course of a one-hour trial. Additionally, plotting a histogram of trial results reveals that results were clustered around 4.00, which is significantly higher than the 2.50 we would expect if tadpoles had no preference for or against green (Figure 5).

Subsequent analysis showed that the number of tadpoles in the green section did not differ significantly over the course of the hour, both qualitatively (Figure 6) and quantitatively (one-way ANOVA: $F(59,660) = 0.80$, $p = 0.86$).

All-white dish test rules out schooling effects

The all-white control test resulted in an average of 2.05 tadpoles in the arbitrary 25% section of the dish over the course of an hour. This is similar to the value of 2.50 that would be expected with a random distribution of tadpoles throughout the dish, implying that schooling effects cannot account for the persistent number of tadpoles in the green. If schooling were occurring, we would expect more or fewer tadpoles to be present in the arbitrary region of the dish, as tadpoles would be drawn towards other tadpoles, either in that section of the dish or another section.

No differences in synaptic transmission during presentation of different colors

The presentation of different colors was not associated with any differences in the following measures: frequency of synaptic events, amplitude of synaptic events, charge (pA*s) of synaptic events during the transition from black to the color, or charge (pA*s) of synaptic events during the transition from the color to black (N = 7 cells; one-way MANOVA: $F(3,54) = 0.56$, $p = 0.91$; Wilk's $\Lambda = 0.615$).

Two cell types identified based on pattern of synaptic input

Analysis of electrophysiological recordings suggests the presence of at least two types of tectal neurons based on the pattern of synaptic input they receive. Some cells seemed to receive strong activity during the transition between two stimuli of highly contrasting luminance ("Contrast Detectors;" Figure 7A), and no patterned activity during the presentation of static stimuli, while other cells seemed to receive patterned activity during the presentation of static stimuli ("Oscillators;" Figure 7B). The patterned activity was qualitatively deemed to resemble oscillatory activity, hence the name of these cells.

Discussion

Further characterization of Xenopus laevis visually-guided behaviors

Previous studies have examined the initial phototactic choice of tadpoles in forced-choice procedures. Our work builds on these findings to further characterize color-based behavioral preferences in *Xenopus laevis*. We found that, over the course of hour-long trials, *Xenopus* tadpoles preferred to swim in green-illuminated areas at a rate higher than chance, even outside of a forced-choice procedure. This finding provides a more complete picture of visually-guided behaviors in the *Xenopus* tadpole and extends our understanding of the innate color preferences of organisms.

Additionally, it is an open question whether the green preference exhibited by *Xenopus* tadpoles is necessarily a color-based response, or if it is a brightness-based response (Jaeger & Hailman, 1976). However, the methodology employed here uses a green light that is less bright than the surrounding white light. Although our methodology does not address initial phototaxis, we found that *over time*, tadpoles prefer green despite it being the dimmer of the two stimuli, in contrast to previous studies of *Xenopus* phototaxis in which brightness preference overshadowed any color preference.

Future research will characterize the nuances of long-term visually-guided behavior. For example, the long-term attractiveness or aversiveness of colors besides green is as yet unexamined. Additionally, further controls must be run. An alternative explanation of our finding is that *Xenopus* tadpoles simply prefer to swim in the dimmer region of a dish over the course of an hour. Thus, further experiments will test tadpole preference in a dish in which 75% is illuminated black and 25% green. The sample size of all-white dish controls will also be increased, to verify the veracity of our initial finding regarding schooling. Finally, as we only

tested groups of 10 tadpoles, the preference for green could arise from a slight preference among all tadpoles or from the averaging of opposing preferences among tadpoles. To further investigate our observations, future tests will examine individual tadpole behavior, either by testing solitary tadpoles in the testing apparatus or by tracking the position of individual tadpoles from recordings involving 10 tadpoles.

One remaining question is what gives rise to the increased time spent in the green section of the dish within an individual *Xenopus* tadpole. One explanation could be that tadpoles simply cross into the green section more frequently than chance. Another explanation may be that tadpoles choose to swim only in the green section of the dish, while maintaining the same rate of swimming. A third possibility is that tadpoles reduce their rate of swimming while in the green section of the dish. These explanations are certainly not mutually exclusive, but in any case, it is currently unknown which explanation or combination of explanations gives rise to the observation of more time spent in the green. Follow-up inquiry will aim to quantify tadpole swimming speed to begin to address this question.

Neuronal encoding of colored stimuli

Our electrophysiology experiments failed to find evidence of color-based processing at the single-cell level in the optic tectum of the *Xenopus* tadpole. One of the following two explanations may account for this result, though this certainly is not an exhaustive list of possible explanations: *(1)* Color of visual stimuli is not encoded for at the single-cell level. Perhaps features such as color are encoded for via circuit dynamics. If this is the case, we believe oscillator cells are a more likely candidate for involvement in circuit processes that encode for color, as it is hard to imagine how color might be encoded for by a contrast detector cell that only receives robust input during changes in luminance contrast. *(2)* The superficial tectal neurons we recorded from do not encode for color at all. Consistent with this, it has been found in non-human primates that neurons in the superficial superior colliculus do not show much color-related activity (White et al., 2009). If the *Xenopus* optic tectum is homologous to the mammalian superior colliculus in organization, color processing would be expected take place in the intermediate layers of the optic tectum.

Both explanations may be addressed by Ca^{2+} imaging. If circuit dynamics encode for color, different patterns of circuit activity might be identified during the presentation of different colors by two-photon Ca^{2+} imaging. Indeed, this technique has previously been used to identify synchronous oscillatory activity in the *Xenopus* optic tectum (Imaizumi et al., 2013). Yet if intermediate, but not superficial tectal neurons encode for color information, then we would predict to identify robust activity in intermediate, but not superficial tectal neurons during a transition between equiluminant stimuli. Thus, future research on the neural processing of color in the *Xenopus* tadpole will aim to characterize the neural processing of color by two-photon Ca^{2+} imaging.

If Ca^{2+} imaging finds evidence that intermediate tectal neurons encode for color information, we will utilize our modified tadpole brain preparation (Hamodi & Pratt, 2015) to further investigate the properties of these neurons. The classic *in vivo* preparation only allows access to neurons in the most superficial layer, but this modified preparation would allow us to record color-evoked responses of neurons from intermediate layers of the tectum.

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References

Benner, S. A., & Sismour, A. M. (2005). Synthetic biology. *Nature Reviews Genetics*, *6*(7), 533.

Boycott, B. B., Mrosovsky, N., & Muntz, W. R. A. (1964). Black and white preferences in the frog, Rana temporaria, and other Anura. *Journal of Experimental Biology*, *41*(4), 865-877.

Chapman, R. M. (1966). Light wavelength and energy preferences of the bullfrog: Evidence for color vision. *Journal of comparative and physiological psychology, 61*(3), 429.

Ciarleglio, C. M., Khakhalin, A. S., Wang, A. F., Constantino, A. C., Yip, S. P., & Aizenman, C. D. (2015). Multivariate analysis of electrophysiological diversity of Xenopus visual neurons during development and plasticity. *eLife, 4*.

Dong, W., Lee, R. H., Xu, H., Yang, S., Pratt, K. G., Cao, V., ... & Aizenman, C. D. (2009). Visual avoidance in Xenopus tadpoles is correlated with the maturation of visual responses in the optic tectum. *Journal of neurophysiology, 101*(2), 803-815.

Drubin, D. A., Way, J. C., & Silver, P. A. (2007). Designing biological systems. Genes & development, 21(3), 242-254.

Graber, V. (1883). Fundamentalversuche über die Helligkeits- und Farbenempfindlichkeit augenloser und geblendeter Thiere. *Sitzungsber. Math-Nat. Kl. kaiser. Akad. Wiss 87,* 201-236.

Gravot, C. M. (2017). *I can see it in your eyes: what the Xenopus laevis eye can teach us about motion perception.* (Doctoral dissertation). Retrieved from Ludwig-Maximilians-Universität München Elektronische Hochschulschriften.

Goodale, M. A. (Ed.). (1990). *Vision and action: The control of grasping* (Vol. 2). Intellect Books.

Haazebroek, P., Van Dantzig, S., & Hommel, B. (2011). A computational model of perception and action for cognitive robotics. *Cognitive processing*, *12*(4), 355.

Hailman, J. P., & Jaeger, R. G. (1974). Phototactic responses to spectrally dominant stimuli and use of colour vision by adult anuran amphibians: a comparative survey. *Animal Behaviour, 22,* 757-795.

Hall, N., & Colby, C. (2014). S-cone visual stimuli activate superior colliculus neurons in old world monkeys: Implications for understanding blindsight. *Journal of Cognitive Neuroscience, 26*(6), 1234-1256.

Hamodi, A. S., & Pratt, K. G. (2014). The horizontal brain slice preparation: a novel approach for visualizing and recording from all layers of the tadpole tectum. *Journal of neurophysiology, 113*(1), 400-407.

Hanson, A. D., Hibberd, J. M., Koffas, M. A. G., Kopka, J., & Wurtzel, E. T. (2019). Focus issue editorial: Synthetic biology. *Plant physiology, 179*(3), 772.

Herman, J., & Krauzlis, R. (2014). Attention to color in the primate superior colliculus. *Journal of Vision*, 14(10), 514-514.

Horn, B., Klaus, B., & Horn, P. (1986). *Robot vision.* Cambridge, MA: MIT press.

Imaizumi, K., Shih, J. Y., & Farris, H. E. (2013). Global hyper-synchronous spontaneous activity in the developing optic tectum. *Scientific reports, 3*, 1552.

Jaeger, R. G., & Hailman, J. P. (1973). Effects of intensity on the phototactic responses of adult anuran amphibians: a comparative survey. *Zeitschrift fur Tierpsychologie, 33*(3), 352.

Jaeger, R. G., & Hailman, J. P. (1976). Ontogenetic shift of spectral phototactic preferences in anuran tadpoles. *Journal of Comparative and Physiological Psychology, 90*(10), 930.

Knorr, A. G., Gravot, C. M., Gordy, C., Glasauer, S., & Straka, H. (2018). I spy with my little eye: a simple behavioral assay to test color sensitivity on digital displays. *Biology open, 7*(10), bio035725.

Lambert, F. M., Beck, J. C., Baker, R., & Straka, H. (2008). Semicircular canal size determines the developmental onset of angular vestibuloocular reflexes in larval Xenopus. *Journal of Neuroscience, 28*(32), 8086-8095.

Laurens, H. (1911). The reactions of amphibians to monochromatic lights of equal intensity. *Bull. Mus. Comp. Zoöl. 53*, 253-302.

Liu, Z., Hamodi, A. S., & Pratt, K. G. (2016). Early development and function of the Xenopus tadpole retinotectal circuit. *Current opinion in neurobiology, 41*, 17-23.

Liu, Z., Donnelly, K. B., & Pratt, K. G. (2018). Preparations and protocols for whole cell patch clamp recording of Xenopus laevis tectal neurons. *Journal of visualized experiments: JoVE*, (133).

Liu, Z., Thakar, A., Santoro, S. W., & Pratt, K. G. (2018). Presenilin regulates retinotectal synapse formation through EphB2 receptor processing. *Developmental neurobiology, 78*(12), 1171-1190.

Luisi, P. L. (2016). *The emergence of life: from chemical origins to synthetic biology.* Cambridge, UK: Cambridge University Press.

Muntz, W. R. A. (1963). The development of phototaxis in the frog (Rana temporaria). *Journal of Experimental Biology*, *40*(2), 371-379.

Nieuwkoop, P. D. & Faber, J. (1956). *Normal table of Xenopus laevis (Daudin).* Amsterdam, NL: North-Holland Publishing Company.

Palm, G. (2012). The visual input to the cortex. In *Neural assemblies: An alternative approach to artificial intelligence* (Vol. 7; pp. 87-91). Heidelberg, DE: Springer Science & Business Media.

Parker, R. O., Mccarragher, B., Crouch, R., & Darden, A. G. (2010). Photoreceptor development in premetamorphic and metamorphic Xenopus laevis. *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology*, *293*(3), 383-387.

Pratt, K. G., & Aizenman, C. D. (2009). Multisensory integration in mesencephalic trigeminal neurons in Xenopus tadpoles. *Journal of Neurophysiology*, *102*(1), 399-412.

Prindle, A., Samayoa, P., & Hasty, J. (2016). *U.S. Patent No. 9,500,636.* Washington, DC: U.S. Patent and Trademark Office.

Regot, S., Macia, J., Conde, N., Furukawa, K., Kjellén, J., Peeters, T., ... & Solé, R. (2011). Distributed biological computation with multicellular engineered networks. *Nature, 469*(7329), 207.

Rosencrans, R. F., Leslie, C. E., Perkins, K. A., Walkowski, W., Gordon, W. C., Richards-Zawacki, C. L., ... & Farris, H. E. (2018). Quantifying the relationship between optical anatomy and retinal physiological sensitivity: A comparative approach. *Journal of Comparative Neurology, 526*(18), 3045-3057.

Schneider, G. E. (1969). Two visual systems. *Science, 163,* 895-902.

Tamsir, A., Tabor, J. J., & Voigt, C. A. (2011). Robust multicellular computing using genetically encoded NOR gates and chemical 'wires'. *Nature, 469*(7329), 212.

Toda, S., Blauch, L. R., Tang, S. K., Morsut, L., & Lim, W. A. (2018). Programming self-organizing multicellular structures with synthetic cell-cell signaling. *Science, 361*(6398), 156-162.

Urrios, A., Macia, J., Manzoni, R., Conde, N., Bonforti, A., de Nadal, E., ... & Solé, R. (2016). A synthetic multicellular memory device. *ACS synthetic biology, 5*(8), 862-873.

van Rheede, J. J., Richards, B. A., & Akerman, C. J. (2015). Sensory-evoked spiking behavior emerges via an experience-dependent plasticity mechanism. *Neuron, 87*(5), 1050-1062.

White, B. J., Boehnke, S. E., Marino, R. A., Itti, L., & Munoz, D. P. (2009). Color-related signals in the primate superior colliculus. *Journal of Neuroscience, 29*(39), 12159-12166.

Yovanovich, C. A., Koskela, S. M., Nevala, N., Kondrashev, S. L., Kelber, A., & Donner, K. (2017). The dual rod system of amphibians supports colour discrimination at the absolute visual threshold. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*(1717).

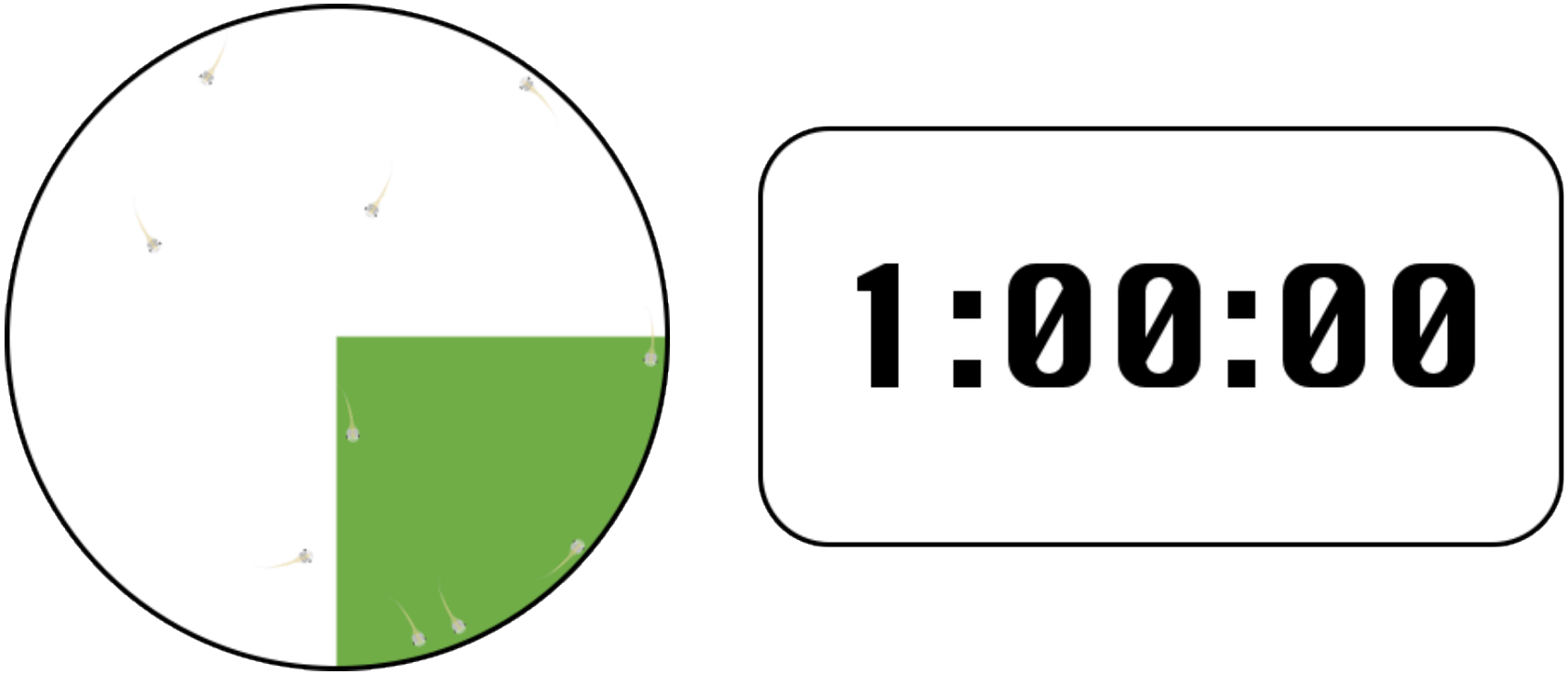

Figure 1. Schematic of the green preference behavioral testing procedure. *The position of 10 tadpoles was recorded for one hour to determine color preference over time. The number of tadpoles in the green and white sections of the dish was counted once per minute for one hour, resulting in 60 equally spaced time points counted per test. In this example frame, five tadpoles would be counted as in the green section and five in the white section.*

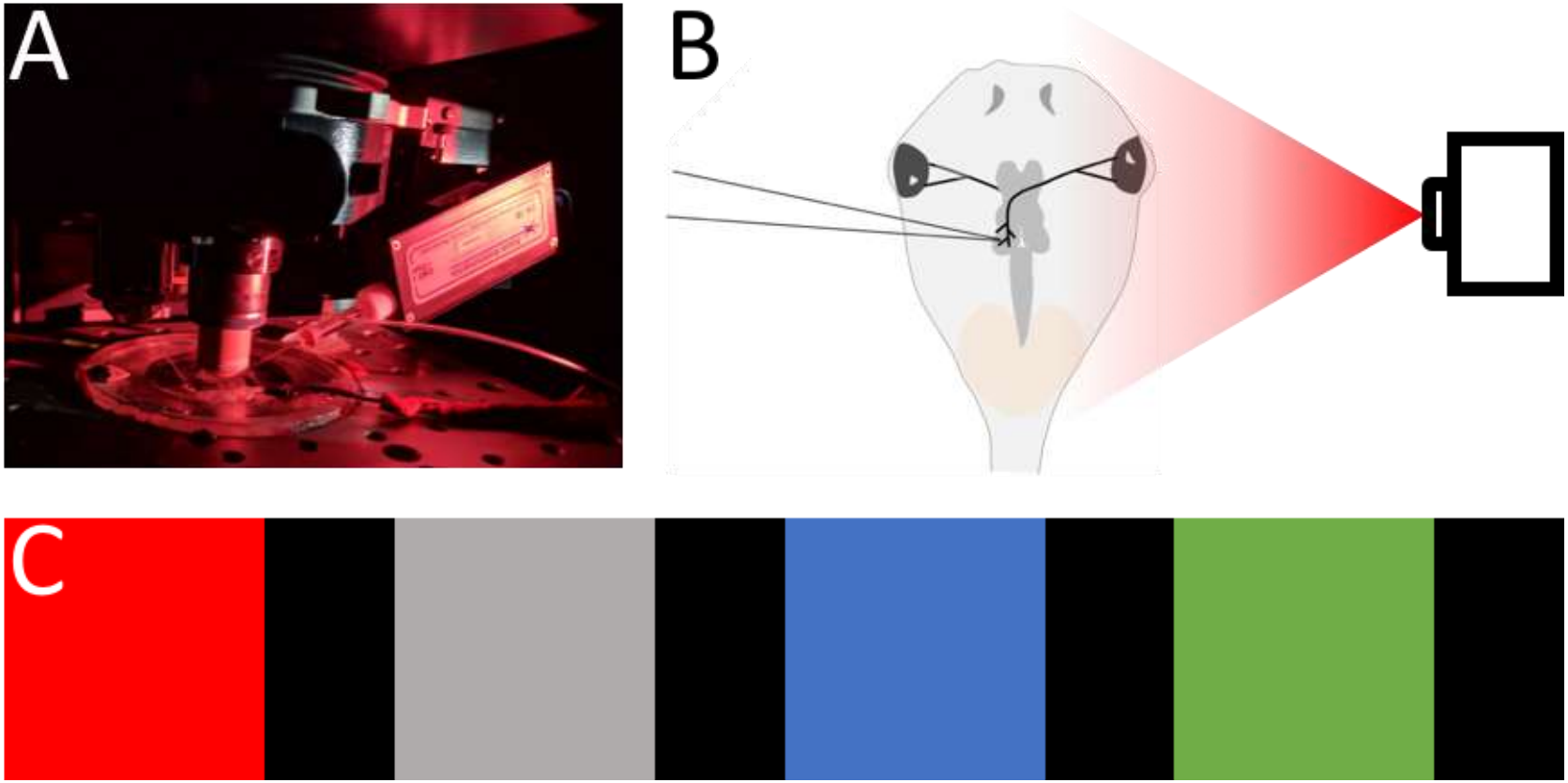

Figure 2. Electrophysiology Procedure and Visual Stimuli. ***(A)*** *Photograph of the electrophysiology recording rig. A 60X water immersion objective was used to visualize tectal neurons while a glass micropipette was used to record from cells.* ***(B)*** *Visual stimuli were presented to the tadpole's eye, filling the entire visual field, while patch clamp recordings were made from neurons in the contralateral optic tectum.* ***(C)*** *Sample order of visual stimulus presentation. Visual stimuli consisted of four colors projected onto the tadpole's eye, interleaved with black between each color. Each color was projected for 60 seconds while inter-color black periods had a duration of 30 seconds.*

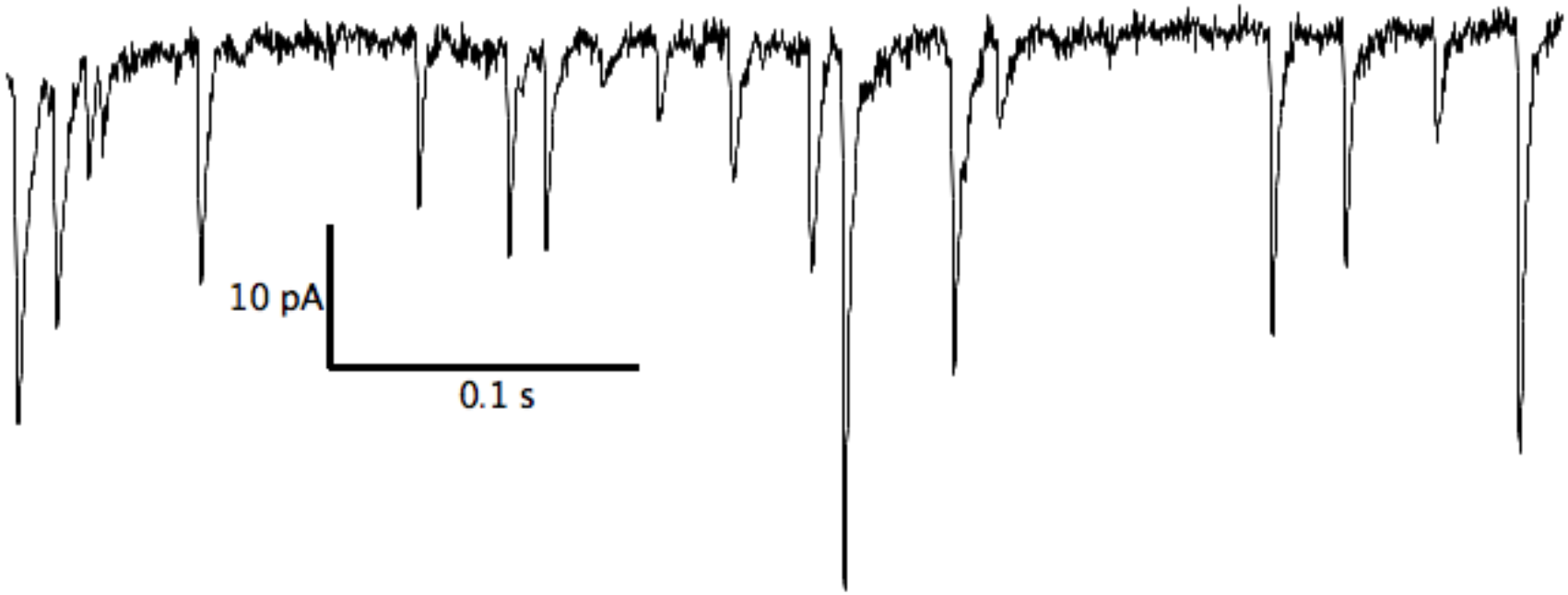

Figure 3. Sample trace of spontaneous synaptic activity. *Spontaneous synaptic events were recorded in patch clamp. Each synaptic event captured is a synaptic transmission received by the neuron we are recording from.*

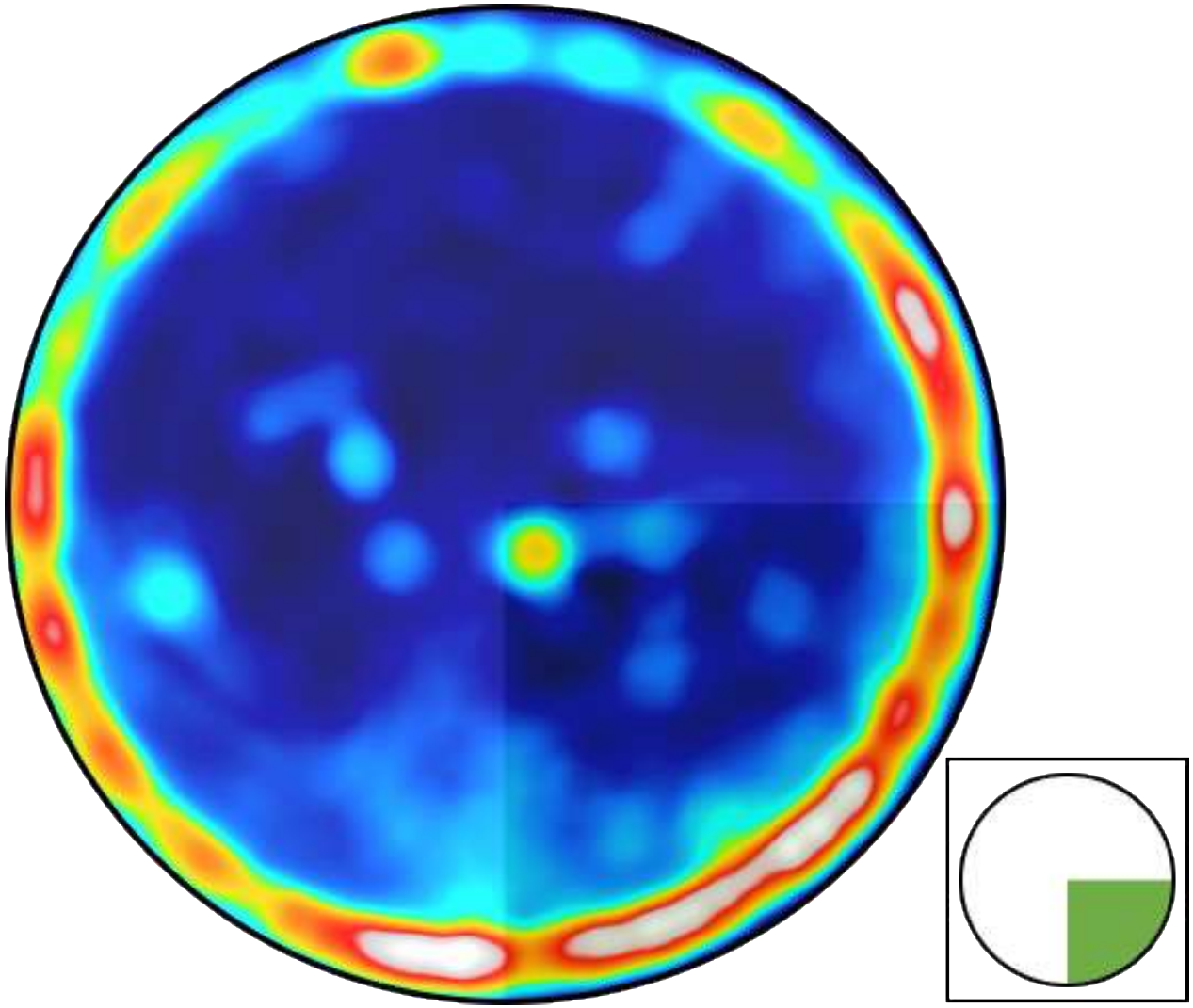

Figure 4. Heat Map Illustrating Tadpole Positions Over Time. *Inset: diagram of the testing dish. The bottom-right corner is the green sector of the dish. Tadpoles preferred to swim along the edge of the dish overall. Most time was spent on the edge of the dish in the green section (bottom-right 25%), with another area of moderate tadpole presence at the edge of the green section near the center of the dish. From least to most tadpole presence during the hour: dark blue, cyan, green, yellow, orange, red, white.*

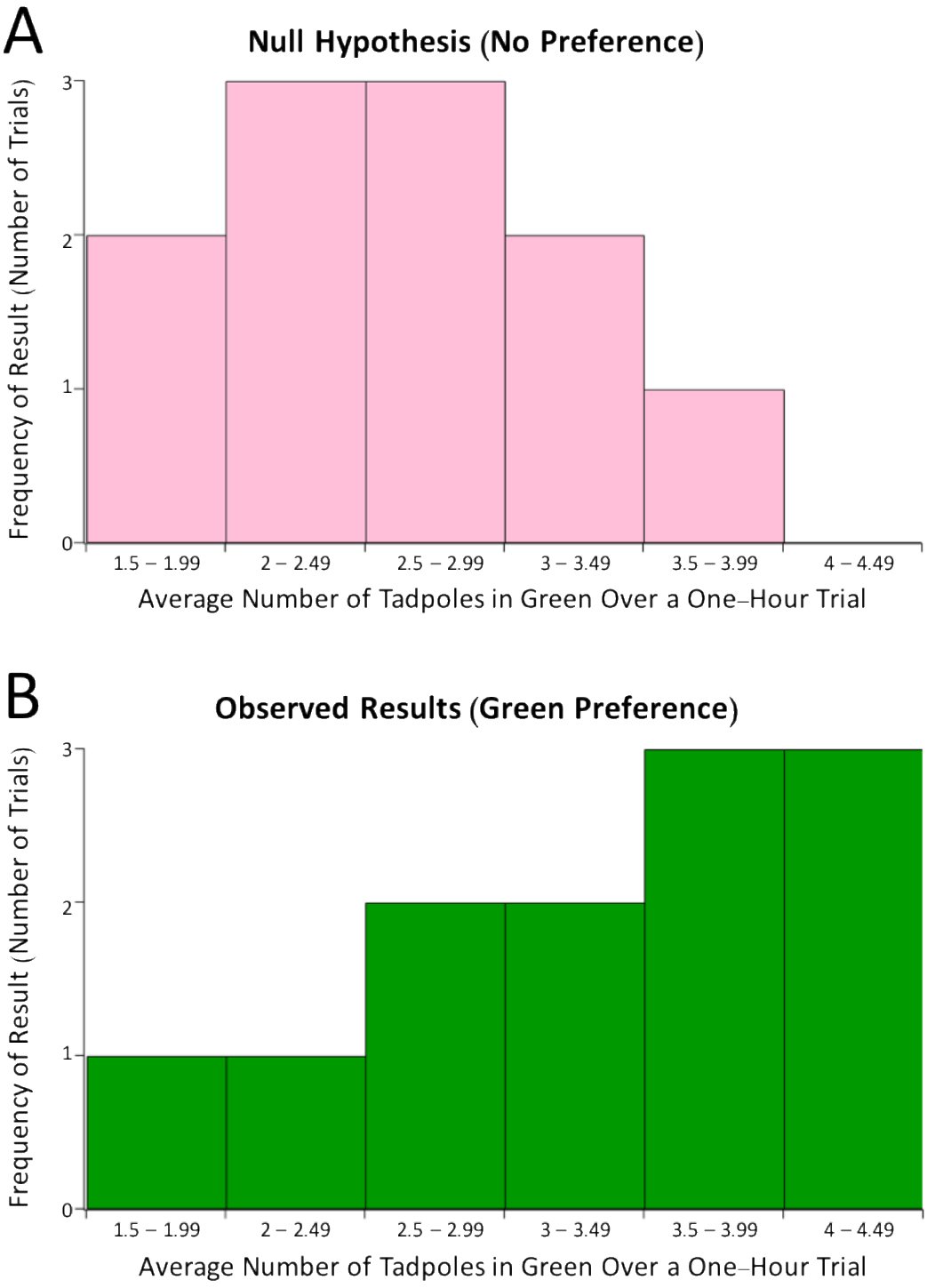

Figure 5. Histograms showing theoretical and observed result distributions for green preference tests. (A) *Theoretical results predicted by the null hypothesis, that tadpoles have no preference for or against the color green. Results are clustered around 2.50.* ***(B)*** *Observed results from 12 behavioral trials. Results are clustered around 4.00.*

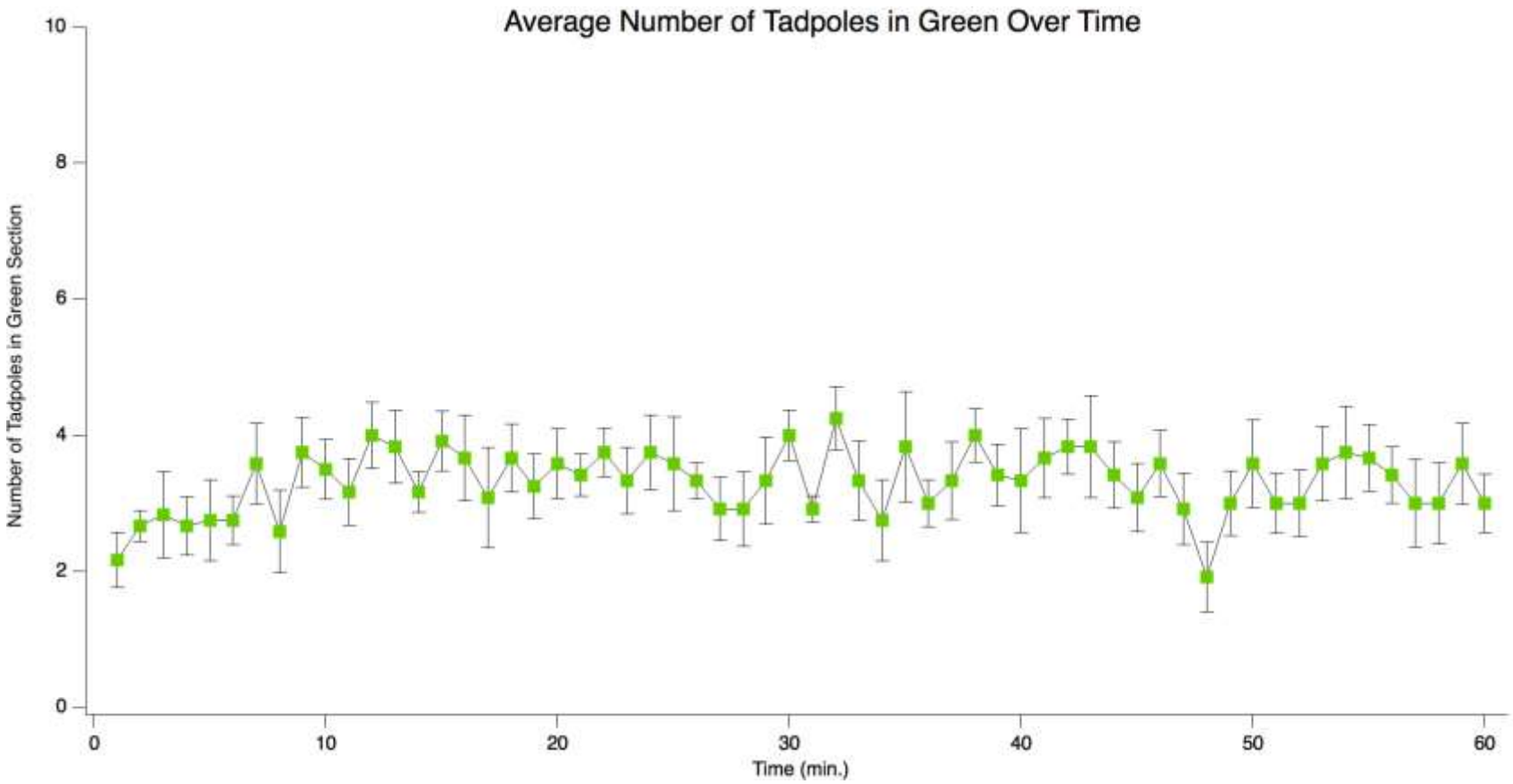

Figure 6. Average number of tadpoles in the green section over time. *The number of tadpoles in the green section of the dish did not vary over time. Green squares represent the mean number of tadpoles in the green section at each minute, averaged across trials. Error bars indicate the Standard Error of the Mean (SEM).*

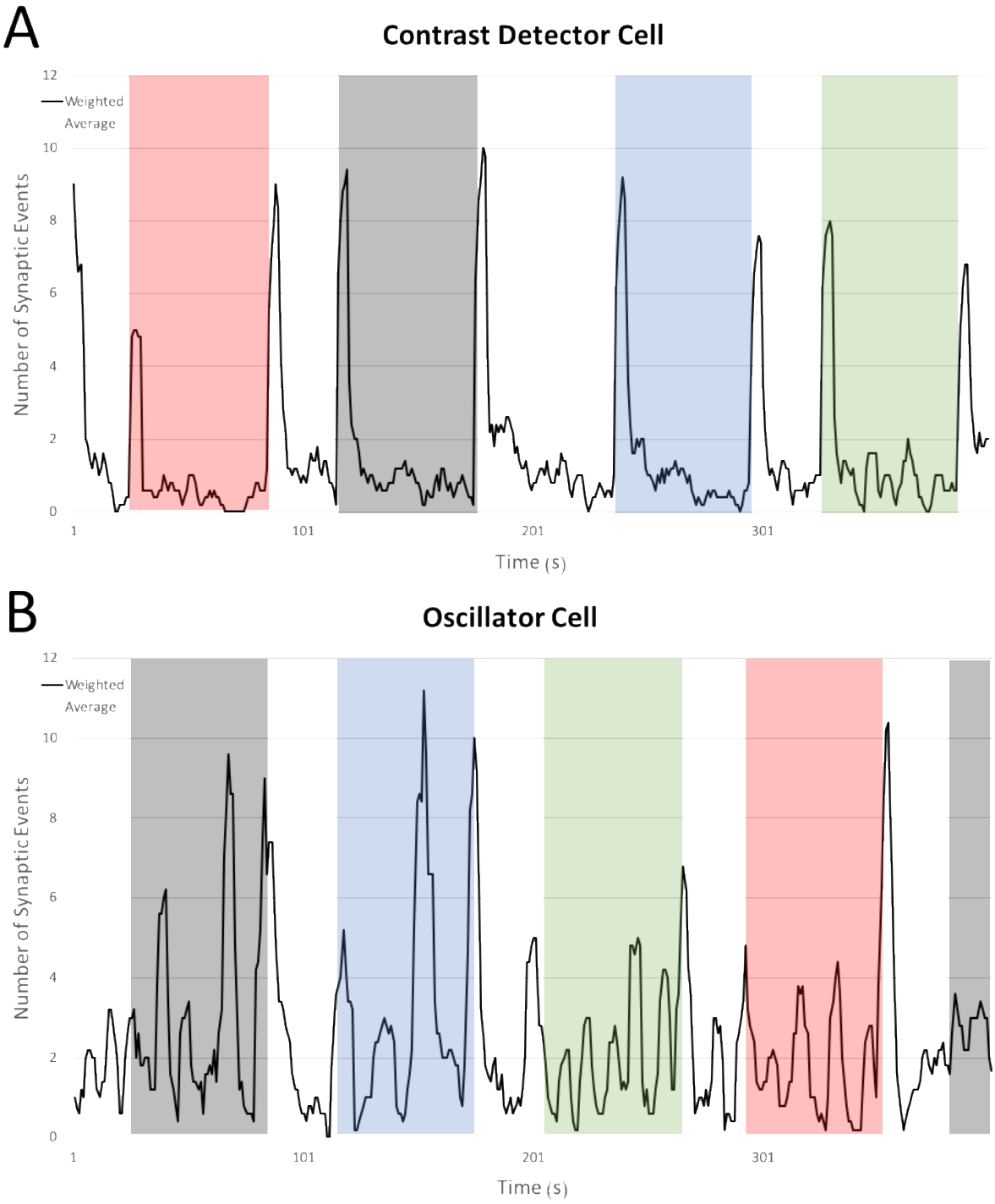

Figure 7. Representative synaptic frequency graphs for the two cell types identified. ***(A)*** *Contrast detector cells receive synaptic input primarily in response to changes in contrast, such as a color turning on or off.* ***(B)*** *Oscillator cells receive oscillatory input during the presentation of static visual stimuli, such as a static color. Oscillator cells may also, as in the representative example, receive input when changes in contrast occur.*

Appendix A – Heat Map Procedure

1. 30 FPS videos were captured with a GoPro Hero 7 Black or GoPro Hero+ LCD
2. Videos were concatenated in iMovie and exported as a .mp4 file
3. Video was converted to .avi file format (NV12 codec) in FFMPEG
4. The following process was completed in ImageJ:
    a. Video was converted to a 3 FPS .tif stack
    b. Image > Stacks > Z-project > Max Intensity [applied to all slices]
    c. Process > Image Calculator
        i. Image1: [File resulting from step 4a]
        ii. Operation: Difference
        iii. Image2: [File resulting from step 4b]
    d. [Applied to file resulting from step 4c] Image > Stacks > Z-project > Average Intensity [applied to all slices]
    e. Process > Image Calculator
        i. Image1: [File resulting from step 4c]
        ii. Operation: Subtract
        iii. Image2: [File resulting from step 4d]
    f. [Applied to file resulting from step 4e] Image > Type > 8-bit
    g. [Applied to file resulting from step 4f] Image > Adjust > Threshold > Adjust as necessary [I used MaxEntropy] > Apply
    h. [Applied to file resulting from step 4g] Process > Filters > Mean > 5.0
    i. [Applied to file resulting from step 4h] Image > Stacks > Z-project > Sum Slices
    j. [Applied to file resulting from step 4i] Process > Filters > Mean > 10.0
    k. File > Import > LUTs [I used Royal.lut]