Research Article

Wavelength Discrimination in the Zebrafish (*Danio rerio*): Evidence for Functional Color Vision

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ABSTRACT: *The zebrafish (Danio rerio) is a popular vertebrate model in several fields of research, especially visual neuroscience, where it has been used for anatomical, physiological, genetic, developmental, and behavioral research. Anatomical and physiological studies have shown the zebrafish has the necessary mechanisms for color vision, but it is not known whether zebrafish can use color vision to regulate behavior. Recently, studies have shown that zebrafish can learn an instrumental discrimination task. The study reported here used instrumental discrimination learning procedures with wavelength as the discriminanda. The results indicate that the zebrafish does, indeed, have functional color vision. The methods used here could be further developed to investigate the functionality of UV visual processing in zebrafish, color perception thresholds, and similar phenomena.*

The zebrafish, because of numerous advantageous characteristics, has come to be used extensively as the vertebrate model of choice in many areas of research. Its advantageous characteristics include transparent chorions, which allow for unobtrusive observation of the developing embryo, the capacity to maintain a large subject pool due to prolific breeding and rapid development, and general hardiness, making the zebrafish an economical, easy-to-maintain subject. The zebrafish is an ideal vertebrate model for visual neuroscience because it has a retinal anatomy and physiology similar to that of other vertebrates so that the results of research on zebrafish can be generalized to other vertebrates, including humans.

Some of the most useful data from zebrafish have been obtained when anatomical, physiological, or genetic procedures were combined with behavioral methods. For example, Taylor, Hurley, Van Epps, and Brockerhoff (2004) used behavioral genetic screens to show that a deficit in pyruvate dehydrogenase (PHD, a normally lethal condition due to abnormal mitochondrial metabolism), could be countered by adding ketogenic substrates to the housing water, a result with implications for the treatment of PHD and other congenital diseases that affect early embryonic development in humans. Darland and Dowling (2001) combined behavioral techniques with genetic mutations to identify zebrafish with decreased sensitivity to cocaine. They suggested that such studies could potentially identify specific genes associated with addiction. Muto et al. (2005) combined genetic mutations with psychophysical measurements to show the effectiveness of using mutant zebrafish in identifying specific genes associated with visual functioning. Ren, McCarthy, Zhang, Adolph, and Li (2002) also combined genetic mutations with behavioral measures and found that retinal screening pigments help regulate behavioral responses in zebrafish. Finally, Page-McCaw et al. (2004) combined genetic and physiological data with optokinetic behavioral data to study light adaptation in zebrafish.

Recently, Bilotta, Risner, Davis, and Haggbloom (2005) suggested that more behavioral techniques need to be developed to fully realize the potential of the zebrafish as a vertebrate model for visual neuroscience. To that end, they developed procedures for investigating instrumental choice discrimination learning in zebrafish. In their task, subjects were rewarded for swimming into a chamber lit by a white-light stimulus (the positive discriminative cue, S+) and received no reward for entering a dark chamber (the negative discriminative cue, S-), a stimulus arrangement in opposition to the natural tendency of zebrafish to prefer a dark environment. They reported that the zebrafish learned this discrimination to a criterion of at least 80% correct. Colwill, Raymond, Ferreira, and Escudero (2005) also reported evidence of instrumental discrimination learning in zebrafish.

In two of the experiments reported by Colwill et al. (2005), the S+ and S- discriminanda were colored sleeves (purple vs. green or blue vs. red) fitted over the arms of a T-maze. However, there was no control for possible differences in brightness between the discriminanda. Consequently, it is possible that color differences were confounded with brightness differences. If the natural zebrafish preference for a darker environment also manifests in a preference for darker stimuli, the functional discriminanda in the Colwill et al. experiments could have been brightness rather than color. To date, there have been no other investigations of color vision-regulated discrimination learning in zebrafish.

The purpose of this experiment was to investigate the capacity of the zebrafish to learn an instrumental discrimination task with differently colored but equally luminant lights as the S+ and S- discriminanda. To equate the discriminanda on luminance, idiosyncratic isoluminant values were behaviorally determined for each fish for two monochromatic stimulus lights. Those light were then used as the S+ and S- cues in an instrumental discrimination learning task modeled after that used by Bilotta et al. (2005).

METHOD

Subjects

Eight adult (> 1 yr.) male and female zebrafish were used in this experiment. The fish were purchased from a local pet store and housed in an aquarium housing system (Aquaneering Incorporated, San Diego, CA) which maintained a water temperature of 28° to 30°C, a pH of 6.8 to 7.2, and a light cycle of 14 hours on and 10 hours off. Fish were housed individually for at least 2 weeks prior to the start of conditioning procedures in order to accustom each zebrafish, a naturally schooling fish, to being alone and to provide a means of identifying each fish. This was done because fish in the present study were trained individually rather than in groups. All fish were approximately the same size. These procedures were adapted from those used by Bilotta et al. (2005).

Behavioral Apparatus

The behavioral apparatus, shown in Figure 1A, was the same modified 19 L fish aquarium used by Bilotta et al. (2005). The apparatus was divided into three areas: a reservoir area, a home area, and a chamber area. The reservoir area was divided from the home area by a removable divider, which restricted an individual subject's movement to the home area and chamber area. A removable heater was placed in the reservoir area to help maintain a water temperature of 25° to 29°C during all conditioning procedures. The subjects remained in the home area between trials. A gate stabilizer divided the home and chamber areas and held an adjustable gate (see Figure 1B) which could be raised and lowered to permit or prevent a fish from accessing the chamber or home areas. The gate had three "portholes" through which the fish could view the visual stimuli presented in the chamber area while still being confined to the home area. Although the chamber area was divided into three separate units, the middle chamber was always blocked and only the two side units were used in this experiment. A liquid light-guide holder was placed outside the chamber area of the apparatus (Figure 1A).

Prior to the start of a session of data collection, the apparatus was filled with 4 L of conditioned water taken from the fish-housing system.

Optical System

Monochromatic visual stimuli were produced by two light sources. A 500nm stimulus was always produced by a 150-W xenon arc lamp (Model LH 150, Spectral Energy, Westwood, NJ). The light was collimated, passed through a water bath, and focused by a lens onto a shutter (Model LS62M2, Uniblitz, Rochester, NY) that was controlled by a shutter driver (Model D122, Uniblitz, Rochester, NY). An interference filter (half bandwidth of 10 nm, Oriel, Stratford, CT) was used to filter the white light of the arc lamp to produce a 500 nm stimulus wavelength. Stimulus luminance was controlled by neutral density filters (Model 398, Reynard, San Clemente, CA). The 500 nm stimulus

was then focused onto a liquid light guide (Model 77556, Oriel), which was directed into the selected chamber.

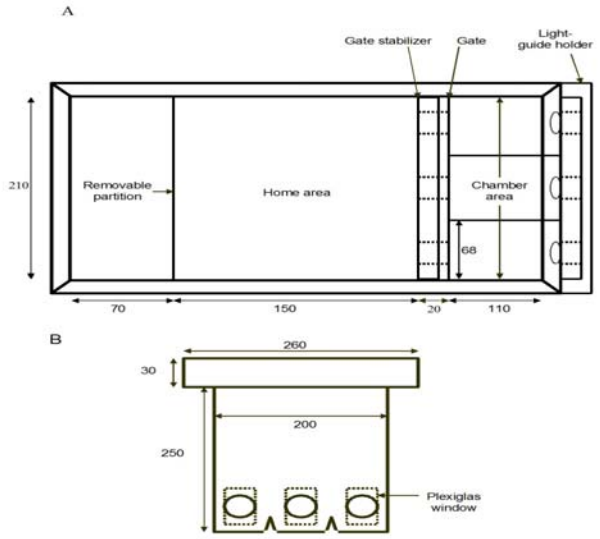

Fig 1. Schematic of the behavioral apparatus. Details can be found in Bilotta et al. (2005). **(A)** Top view. **(B)** Side view of the removable gate.

The second light stimulus was produced by a halogen light (World Precision Instruments, Sarasota, FL) passed through a liquid light guide (World Precision Instruments, Model SI-72-8, Sarasota, FL). The light was passed through interference filters (half bandwidth of 10 nm, Oriel, Stratford, CT) that produced either a 460 or 540 nm monochromatic stimulus. This light was then aimed at another liquid light guide (World Precision Instruments, Model SI-72-8, Sarasota, FL) that was directed into the second chamber. Stimulus luminance from this light source was adjusted via a rotary dimmer attached to the light source. A 50-W tungsten lamp (Model 1575, Underwriters Laboratories, Northbrook, IL) was placed above the behavioral apparatus in order to produce a 2 lux background illuminance.

Procedures

There were five distinct training phases in this experiment. These were: habituation, chamber-entry training, stimulus-association training, isoluminance training, and wavelength-discrimination training.

During training, the subjects' diets were restricted to a small amount of flake food daily. The training procedures were adapted from those used by Bilotta et al. (2005).

Habituation

After subjects' diets were restricted to a small amount of flake food each day for two days, apparatus-habituation training commenced. Habituation training, consisting of one session per day over two consecutive days, was used to familiarize the subjects with the behavioral apparatus. During each session, the room lights were turned off, and a background light of 2 lux was present. Each fish was individually placed into the home area of the behavioral apparatus, and the gate was raised to allow the subject access to the chamber areas. The fish was allowed to swim freely in the apparatus for 20 min. After this time, the session was terminated, the gate was lowered to restrict the subject's movement to the home chamber, the room lights were turned on, and the subject was removed from the behavioral apparatus and placed back into its individual container in the housing system.

Chamber-Entry Training

Immediately following habituation training, each fish received one 20-trial session of chamber-entry training daily for three consecutive days. At the beginning of each chamber-entry training session, the subject was re-habituated to the apparatus for 5 min. Following habituation, and while the fish was in the home area, the gate was lowered. After 10 s, the gate was raised, allowing the subject to swim into one of the two chambers. If the subject swam into one of the chambers, the gate was lowered to restrict the subject to the chamber it chose. One of the three monochromatic stimuli (460, 500, or 540 nm) was then presented in conjunction with a food reward of 5-10 live brine shrimp administered with a glass eye dropper. The fish was given 30 s to consume the brine shrimp. The visual stimulus was then terminated, the gate was raised, and the fish was allowed to swim back into the home area. The gate was then lowered, marking the end of the trial. After a 10-s intertrial interval (ITI), a new trial began. In the event that a subject did not swim into one of the two chambers after 90 s, the gate was lowered and the trial was terminated. At the end of the session, the subject was returned to the housing system. Fish that did not enter one of the two chambers on all 20 trials in the last training session were replaced.

Stimulus-Association Training

After chamber-entry training concluded, subjects began stimulus-association training. Again, subjects were habituated to the apparatus for 5 min, and then confined to the home area. The monochromatic stimulus later to be used as S+ was then presented in one of the two chamber areas for 10 s (this was the 460nm light for two fish, the 500nm light for four fish, and the 540nm light for the remaining two fish). The gate was then raised, and the subject was allowed to swim into either the illuminated or the dark chamber. If the subject swam into the illuminated S+ chamber area, this was scored as a correct response. The gate was then lowered, restricting the subject's movement to that chamber, the subject was reinforced with a live, brine shrimp food reward, and it was allowed 30 s to consume the food. Afterwards, the visual stimulus was terminated, the gate was raised, and the fish was allowed back into the home area ending the trial. If the subject swam into the dark chamber area, the gate was lowered, the visual stimulus was terminated, and the subject was confined to the dark chamber area for 30 s without food reinforcement. The gate was then raised and the subject was allowed back into the home area ending the trial. If the subject failed to choose either of the two chambers after 90 s, the visual stimulus was terminated, the gate was lowered, and the subject remained in the home area until a new trial began. Each stimulus-association training session consisted of 20 trials separated by a 10-s ITI. A quasi-random process was used to designate a chamber as S+, and each chamber was designated S+ for 10 of the 20 trials to prevent development of a chamber preference. At the end of the 20 trials, the subject was removed from the apparatus and returned to the housing system. Each fish was trained to a criterion of 80% correct responses per session for two consecutive sessions.

Isoluminance Training

The purpose of this experiment was to determine whether zebrafish could learn an instrumental discrimination with different wavelengths of light as the discriminative cues. Isoluminance training was used to determine luminance values at which the S+ stimulus, associated with a food reward in the previous training phase, and a second monochromatic stimulus that would serve as the S- cue during discrimination training, were perceived as equally bright. By determining these isoluminant values, we eliminated any potential confound between color and brightness. By identifying idiosyncratic isoluminant values, as opposed to a single isoluminant point for each pair of wavelengths, we also controlled for the possibility that the perception of brightness could differ among subjects.

The methodology used for isoluminance training was essentially the same as that used for stimulus-association training. However, in these sessions, the previously dark chamber now contained the monochromatic stimulus to be used as S- during discrimination learning. Table 1 shows the stimulus combinations used as S+ and S- for each fish.

After 5 min of habituation, the subject was confined to the home area by lowering the gate. The S+ and S- stimuli were then presented simultaneously. After 10 sec, the gate was raised and the subject was allowed to swim into either the S+ or S- chamber. In the event the subject entered the S+ chamber, the gate was lowered, the S- cue was terminated, and the subject was rewarded with 5-10 live brine shrimp. After 30 s of feeding, the gate was raised, the subject was allowed back into the home area, and the gate was lowered. If the subject entered the S- chamber, both stimuli were terminated and the fish was confined to the S- chamber for 30 s without food reinforcement. The gate was then raised, allowing the subject to return to the home area. If the fish did not enter a chamber after 90 s of swimming in the home area, the trial was terminated by turning the stimuli off, lowering the gate, and confining the fish in the home area until the next trial. All trials were separated by a 10-s ITI.

The isoluminance point for each pair of stimuli for each fish was determined by varying the illuminance of the 500 nm stimulus between trials in steps of 0.3 log units of attenuation. Six different illuminance values were tested per session.

Each isoluminance training session included 30 trials. Both the S+ chamber and the illuminance of the 500nm stimulus varied in a quasi-random fashion, with each chamber designated as S+ for 15 of the 30 trials. Each of the 6 illuminance values for the 500 nm stimulus was presented 5 times per session. Isoluminance training continued until an isoluminant point was determined, defined as the attenuation at which the average percent-correct response fell closest to chance levels (50%).

Wavelength-Discrimination Training

After isoluminance training determined the subject's isoluminant point for the two monochromatic stimuli, the subject began wavelength discriminatio training. During these sessions, the illuminance of the 500 nm stimulus was fixed at the isoluminant value determined

during isoluminance training, otherwise the training methodology was essentially the same as that used for isoluminance training, and the stimuli designated as S+ and S- were the same as in isoluminance training.

A trial began with the subject in the home area. The gate was then raised and the subject was allowed to swim into one of the two chamber areas. If the fish entered the S+ chamber, the gate was lowered, the S- was terminated, and the subject was rewarded with a food reward of 5-10 live brine shrimp. If the fish chose the S- chamber, the gate was closed, the stimuli were terminated, and the subject remained in the dark chamber for 30 s without food reinforcement. After 30 s confinement to either the S+ or S- chamber, the gate was raised and the subject was allowed to reenter the home area. The gate was then lowered, and an ITI of 10 s passed before a new trial began. If the subject refused to swim into either chamber within 90 s of trial initiation, the stimuli were terminated, the gate was lowered, and a new trial began after a 10-s ITI. Subjects received two consecutive 10-trial sessions per day until they reached a criterion of 80% correct on two consecutive sessions.

RESULTS

Stimulus-Association Training

All eight fish learned to enter the chamber illuminated by the S+ stimulus. Figure 2 shows the mean percent correct for all fish across 14 training sessions. Because training was terminated for each fish after the criterion was reached, the graph reflects an assigned score of 80% correct for that fish for the remaining sessions. Error bars represent ± 1 standard deviation. Variability was relatively high until the 7th training session, after which there was very little variability because only one subject (Z9) had not yet reached the 80% correct criterion (dashed line). On average, it took subjects 6.75 sessions to reach the learning criterion. If the data for fish Z9 are excluded, the learning criterion was reached in an average of 5.71 sessions. All subjects satisfied the learning criterion within 14 sessions. Figure 3 shows individual learning curves for each fish.

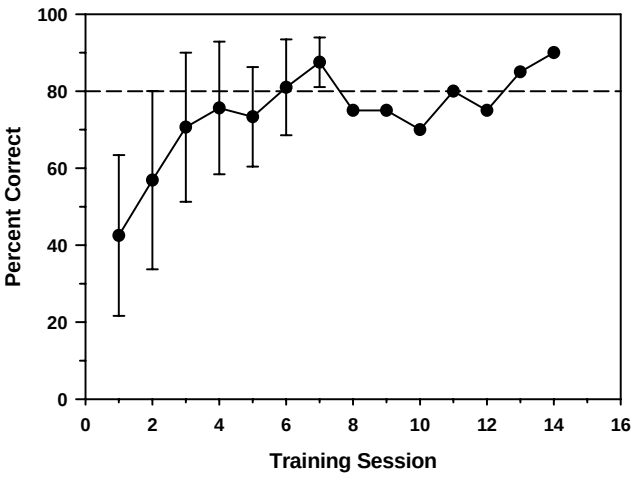

Fig 2. Mean percent correct for all eight fish over 14 sessions of stimulus-association training.

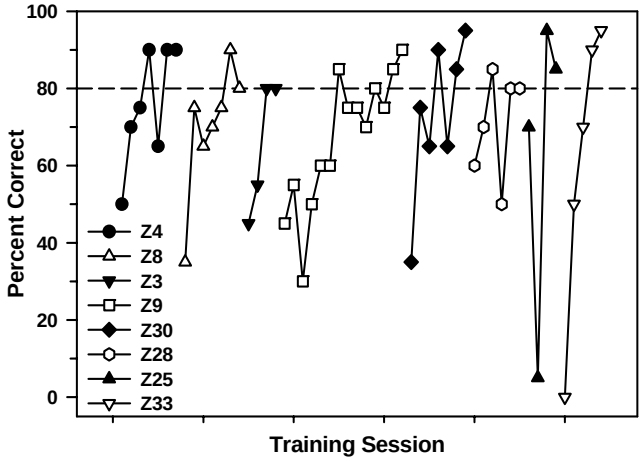

Fig 3. Individual learning curves for each fish during stimulus-association training

Isoluminance Training

Figures 4-11 show the results of isoluminance training separately for each subject. In all figures, the X-axis is log-stimulus attenuation and the Y-axis is percent-correct response for each irradiance value with the dashed line representing chance performance. Error bars represent ± 1 standard error of the mean. The isoluminant point was defined as the attenuation of the 500nm stimulus at which the average percent-correct fell closest to chance (arrow).

Comparing across figures, it can be seen that isoluminant values varied among subjects given the

same wavelength stimuli as discriminanda. For example, subjects Z4 and Z8 experienced the 500 nm stimulus as S+ and the 460nm stimulus as S-. The performance of subject Z4 was nearest chance (47.86%, Figure 4) when -1.5 log units of attenuation were applied to the 500 nm S+ stimulus, whereas, for subject Z8 the isoluminant point occurred at -0.6 log units of attenuation (57.5%, Figure 5). Subjects Z3 and Z9 experienced the 500 nm stimulus as S+ and the 540 nm stimulus as S-. As can be seen in Figures 6 and 7, the isoluminant point for subject Z3 occurred at -1.5 log units of attenuation while the isoluminant point for subject Z9 occurred at -1.2 log units of attenuation. Subjects Z30 and Z28 experienced the 460 nm stimulus as S+ and the 500 nm stimulus as S-. As can be seen in Figures 8 and 9, the isoluminant point for subject Z30 occurred at -0.6 log units of attenuation while the isoluminant point for subject Z28 occurred at an attenuation of -0.9 log units. Finally, subjects Z25 and Z33 experienced the 540 nm stimulus as S+ and the 500 nm stimulus as S-. Figures 10 and 11 show that the isoluminant point for subject Z25 occurred at -0.3 log units of attenuation while the isoluminant point for subject Z33 occurred at -0.6 log units of attenuation. This pattern of results confirms the potential importance of using idiosyncratic isoluminance values for wavelength-discrimination training.

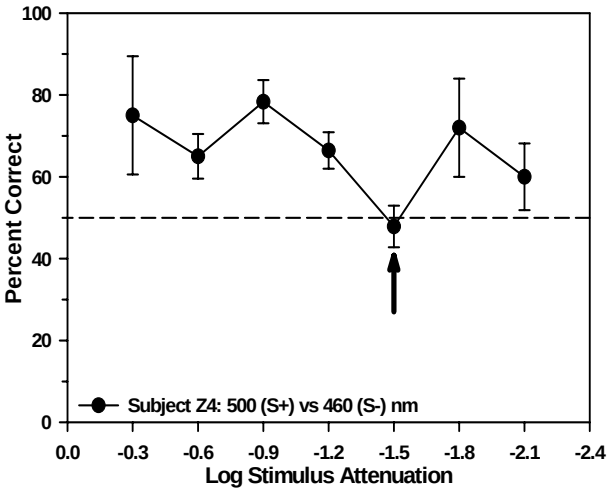

Fig 4. Isoluminance training results and isoluminant point for subject Z4 trained to approach a 500 nm (S+) stimulus during stimulus-association training.

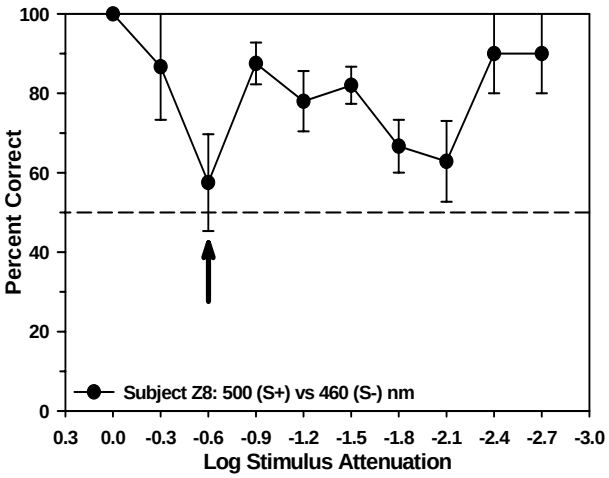

Fig 5. Isoluminance training results and isoluminant point for subject Z8 trained to approach a 500 nm (S+) stimulus during stimulus-association training

Wavelength-Discrimination Training

Figure 12 shows the wavelength-discrimination learning acquisition curves for each fish. The X-axis represents training session and the Y-axis represents percent correct responses. The dashed line represents the learning criterion of 80% correct, and the dotted line represents chance. As can be seen, all subjects reached criterion, although after different amounts of training. Subjects took an average of 6.88 sessions to reach the learning criterion, and all subjects reached criterion within 16 sessions.

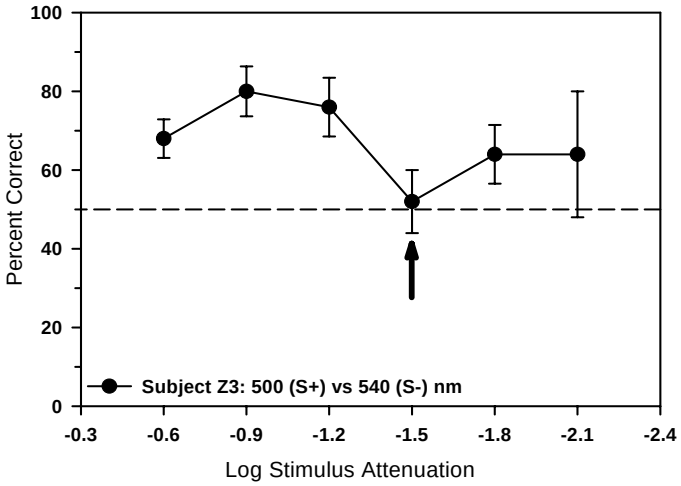

Fig 6. Isoluminance training results and isoluminant point for subject Z3 trained to approach a 500 nm (S+) stimulus during stimulus-association training.

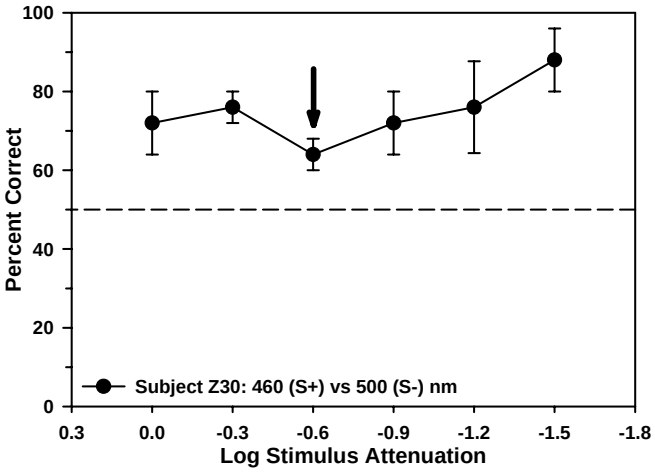

Fig 8. Isoluminance training results and isoluminant point for subject Z30 trained to approach a 460 nm (S+) stimulus during stimulus-association training.

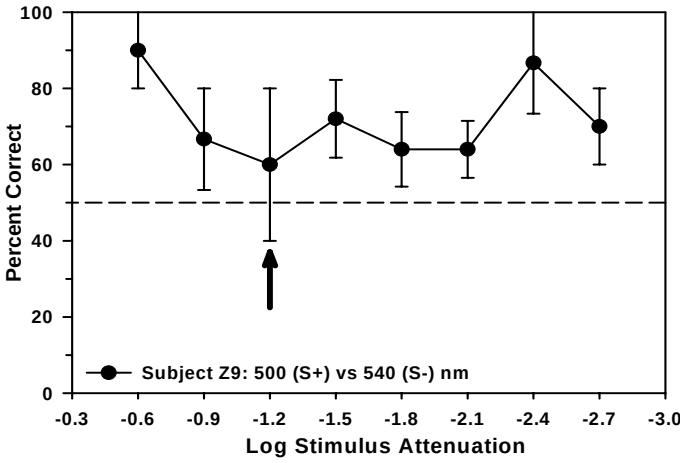

Fig 7. Isoluminance training results and isoluminant point for subject Z9 trained to approach a 500 nm (S+) stimulus during stimulus-association training.

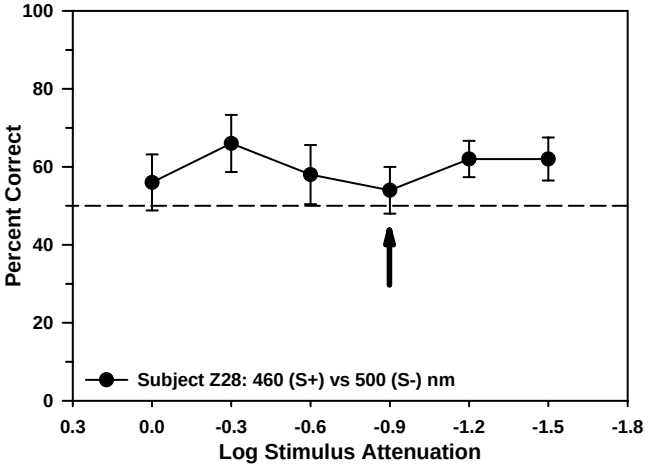

Fig 9. Isoluminance training results and isoluminant point for subject Z28 trained to approach a 460 nm (S+) stimulus during stimulus-association training.

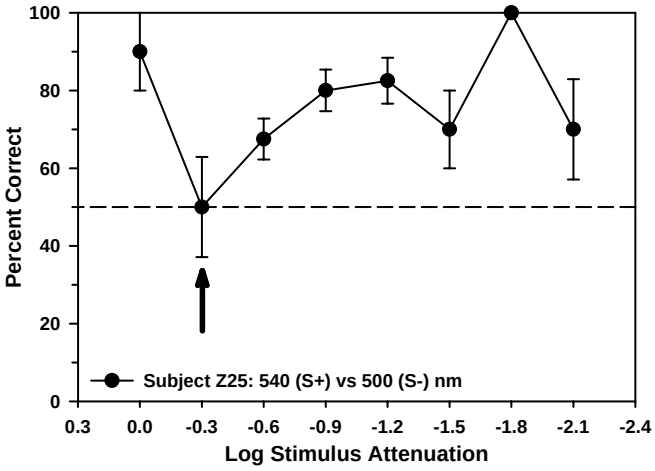

Fig 10. Isoluminance training results and isoluminant point for subject Z25 trained to approach a 540 nm (S+) stimulus during stimulus-association training.

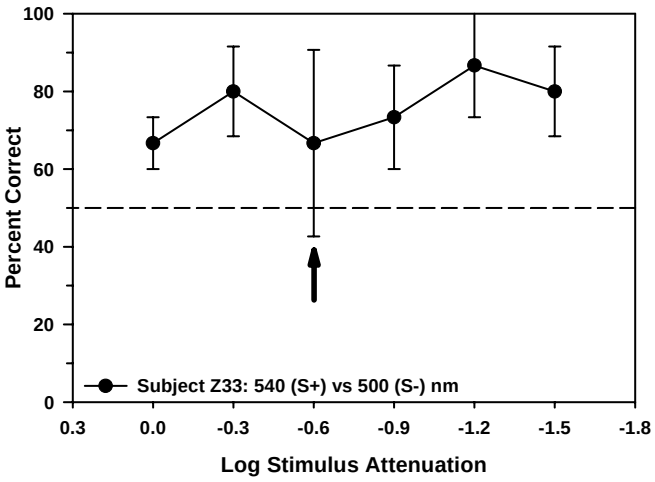

Fig 11. Isoluminance training results and isoluminant point for subject Z33 trained to approach a 540 nm (S+) stimulus during stimulus-association training.

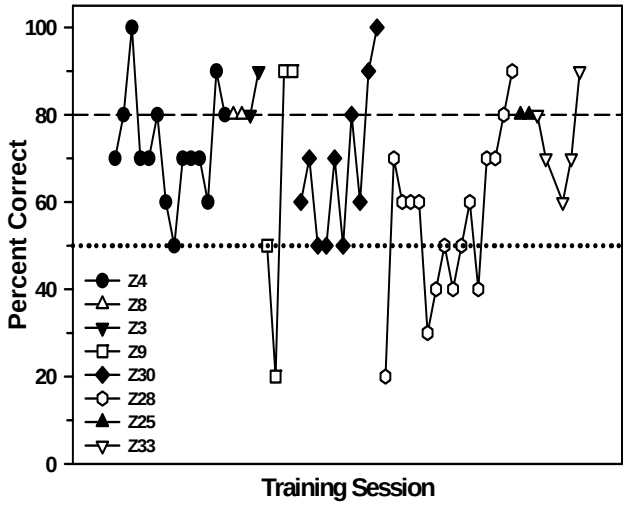

Fig 12. Individual learning curves for wavelength-discrimination training.

DISCUSSION

Stimulus-Association Training

The present study supports the findings of Bilotta et al. (2005) and Colwill et al. (2005) in demonstrating that the zebrafish can learn a relatively difficult appetitive instrumental discrimination learning problem. All subjects in the present study were able to associate a monochromatic visual stimulus with a food reward by overcoming their inherent preference for dark environments over lit environments. As was seen in Bilotta et al.'s (2005) study, there was considerable variability across fish in the number of sessions required to reach the learning criterion.

The main purpose of this experiment was to determine if the zebrafish is capable of using color (wavelength) information to regulate behavior (i.e., to determine whether the zebrafish has functional color vision). Although Colwill et al. (2005) used color cues as the putative discriminanda, they did not control for the possibility that color differences between discriminanda were confounded with brightness differences. In order to eliminate possible brightness differences between the discriminanda and help ensure that color was the functional discriminative stimulus dimension in the present experiment, we identified idiosyncratic isoluminant values for the S+ and S- wavelength stimuli. The isoluminant point was defined as the degree of attenuation of the 500 nm stimulus that resulted in nearly chance discrimination between

it and another monochromatic stimulus. At the isoluminant point, which was determined prior to wavelength discrimination training; it was assumed that the subject could no longer use brightness cues to differentiate between the S+ and S- cues. While it is impossible to know if the isoluminant stimuli were actually perceived by a subject to be equally bright, the isoluminance training ensured the stimuli were functionally equivalent. Furthermore, the use of all three monochromatic stimuli in different combinations as both S+ and S- (across different fish) controlled for a possible innate tendency to approach a certain color, and also countered any possible brightness preference that might remain after the isoluminant points were determined.

The identification of individual isoluminant values for all subjects showed that the isoluminant values varied between subjects tested with the same pair of discriminanda. This finding confirms the potential importance of using idiosyncratic isoluminant values to control for potential brightness differences between discriminanda. The results obtained here with idiosyncratic isoluminant discriminanda of different wavelengths show that zebrafish can, indeed, learn an appetitive instrumental discrimination problem with color as the functional discriminanda. These results are consistent with a conclusion that the zebrafish has functional color vision as would be expected given its retinal anatomy.

Future studies of zebrafish vision and visual perception can be performed using the procedure used here. Such research should determine whether wavelength discrimination is possible at wavelengths other than those used here. The present study only investigated discrimination abilities at 460, 500, and 540 nm wavelengths. These wavelengths were chosen based on Risner, Bilotta, Vukmanic, and Moore's (2006) study, which determined behavioral spectral sensitivity thresholds for zebrafish. In the Risner et al. study, zebrafish were most sensitive to monochromatic stimuli of 500 nm wavelength. Also, they found that zebrafish were relatively insensitive to wavelengths of 460 and 540 nm. The present study sought to determine if wavelength discrimination was possible at all in zebrafish. Had the present study used wavelengths that were relatively the same in spectral sensitivity, it may have been more difficult to determine if color discrimination was possible in zebrafish. Further studies could also use this paradigm to determine visual stimulus-generalization thresholds in zebrafish by using wavelengths of monochromatic light that differ by less than 40 nm, the wavelength differences used in this study. The zebrafish's unique ability to see UV light could also be studied, as future studies using this paradigm could examine wavelength-discrimination abilities of zebrafish in the UV spectrum, an examination that has yet to be performed. Combining such threshold information with pharmacological and genetic techniques may help determine the effects certain drugs and mutations have on visual perceptual abilities as measured by psychophysical techniques. Such studies could lead to the development of new models for vertebrate visual deficits such as color blindness and night blindness.

Acknowledgements

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REFERENCES

Bilotta, J., Risner, M. L., Davis, E. C., & Haggbloom, S. J. (2005). Assessing appetitive choice discrimination learning in zebrafish. *Zebrafish*, *2*, 259-268.

Bilotta, J., Thornberry, Jr., T., & Saszik, S. (2008). Dark-adaptation functions of the developing zebrafish. Unpublished manuscript.

Branchek, T., & Bremiller, R. (1984). The development of photoreceptors in the zebrafish, *Brachydanio rerio*. I. Structure. *The Journal of Comparative Neurology*, *224*, 107-115.

Colwill, R. M., Raymond, M. P., Ferreira, L., & Escudero, H. (2005). Visual discrimination learning in zebrafish (*Danio rerio*). *Behavioural Processes*, *70*, 19-31.

Darland, T. & Dowling, J. E. (2001). Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 11691-11696.

Guo, S. (2004). Linking genes to brain, behavior and neurological diseases: What can we learn from zebrafish? *Genes, Brain and Behavior*, *3*, 63-74.

Muto, A., Orger, M. B., Wehman, A. M., Smear, M. C., Kay, J. N., Page-McCaw, P. S., Gahtan, E., Xiao, T., Nevin, L. M., Gosse, N. J., Staub, W., Finger-Baier, K., & Baier, H. (2005). Forward genetic analysis of visual behavior in zebrafish. *Public Library of Science Genetics*, *1*, 575-588.

Page-McCaw, P. S., Chung, S. C., Muto, A., Roeser, T., Staub, W., Finger-Baier, K. C., Korenbrot, J. I., & Baier, H. (2004). Retinal network adaptation to bright light requires tyrosinase. *Nature Neuroscience*, *7*, 1329-1336.

Ren, J. Q., McCarthy, W. R., Zhang, H., Adolph, A. R., & Li, L. (2002). Behavioral visual responses of wild-type and hypo pigmented zebrafish. *Vision Research*, *42*, 293-299.

Risner, M. L., Bilotta, J., E., Vukmanic, E. V., & Moore, A. (2006). Behavioral spectral sensitivity of the zebrafish (*Danio rerio*). *Vision Research, 46*, 2625-2635.

Russell, C. (2003). The roles of hedgehogs and fibroblast growth factors in eye development and retinal cell rescue. *Vision Research, 43*, 899-912.

Taylor, M. R., Hurley, J. B., Van Epps, H. A., & Brockerhoff, S. E. (2004). A zebrafish model for pyruvate dehydrogenase deficiency: Rescue of neurological dysfunction and embryonic lethality using a ketogenic diet. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 4584-4589.

Tropepe, V. & Sive, H. L. (2003). Can zebrafish be used as a model to study the neurodevelopment causes of autism? *Genes, Brain and Behavior*, *2*, 268-281.