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Assessing Wavelength Discrimination Abilities in the Zebrafish (Danio rerio) Using Appetitive Choice Discrimination Learning

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A Thesis Submitted to the Western Kentucky University Honors Program

By Tim Thornberry, Jr.

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Acknowledgements

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Abstract

In the past few decades, the zebrafish has become a popular vertebrate model in various fields of research, especially visual neuroscience, where the versatile zebrafish model has been used for anatomical, physiological, genetic, developmental, and behavioral research. Anatomical and physiological studies have shown that the zebrafish has the necessary mechanisms required for color vision. However, to date, there is no evidence that zebrafish behavior is regulated by color vision. This project used an appetitive choice discrimination paradigm to assess the ability of the zebrafish to modify its behavior based exclusively on color cues. Subjects were conditioned to associate a food reward with a particular colored stimulus and were then required to discriminate between the visual stimulus associated with food and another, equiluminant visual stimulus of a different wavelength. Results showed that the zebrafish can modify its behavior on the basis of stimulus wavelength. The methods used here could be further developed to determine color perception thresholds and examine behavioral modification based on UV visual processing in zebrafish. Also, this and other research involving the zebrafish can be used as a model system to investigate disorders associated with human visual processing.

Introduction

The zebrafish has been used extensively as a vertebrate model in various research disciplines. It has been the choice vertebrate model for many fields due to numerous advantageous characteristics, such as: transparent chorions, which allow for unobtrusive observation of the developing embryo; prolific breeding and rapid development, which allow researchers to maintain a large subject pool; and general hardiness, which makes the zebrafish an economical, easy-to-maintain subject for many areas of research. The field of embryology has benefited from use of the zebrafish model, as is evident from Taylor, Hurley, Van Epps, and Brockerhoff's (2004) experiment. They determined through behavioral genetic screens that a deficit in pyruvate dehydrogenase (PHD), a normally lethal condition, could be countered by adding ketogenic substrates to the housing water. Taylor et al. (2004) went on to suggest the therapeutic implications of such research in treating PHD and other congenital diseases that affect early embryonic development in humans. Additional therapeutic interventions may be developed from genetic research with zebrafish, a booming area of interest at present. Guo (2004) reviewed existing genetic research and also offered possible future directions in genetic research with zebrafish. Tropepe and Sive's (2003) review suggested that zebrafish could be used to study the genetic factors of autism.

The zebrafish is an ideal vertebrate model for visual neuroscience because it has a retinal anatomy and physiology similar to that of other vertebrates. That similarity allows the results of research on zebrafish to be generalized to other vertebrates, including humans.

The zebrafish has been a favorite vertebrate vision model in developmental anatomical and physiological studies for decades. Branchek and Bremiller (1984) tracked the anatomical development of zebrafish rod and cone photoreceptors, determining that rod and cone photoreceptors reached anatomical maturity at different times in development, the rods by 15-40 days postfertilization (dpf) and the cones by 10 dpf. Recently, Bilotta, Thornberry, Jr., and Saszik (2006) found that physiological maturity of the rod photoreceptors occurs much later in life than anatomical maturity would suggest. Russell (2003) promoted the zebrafish model for physiological research as well, specifically for studying how signaling pathways interact.

Perhaps the most impressive visual data from zebrafish are obtained when anatomical, physiological, or genetic procedures are combined with psychophysical methods. Along with Taylor et al.'s (2004) findings regarding PHD, which combined behavioral and genetic procedures, 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. And Page-McCaw et al. (2004) combined genetic and physiological data with optokinetic behavioral data to study light adaptation in zebrafish.

As Bilotta, Risner, Davis, and Haggbloom (2005) suggested, however, 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, Bilotta et al. 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 (the negative discriminative cue, S-) for entering either of two dark chambers. They reported that the zebrafish could learn this discrimination, which defies the fish's natural tendency to prefer darker environments. The purpose of this experiment was to determine, using procedures similar to those of Bilotta et al. (2005), whether or not the zebrafish can learn a relatively complex discrimination task to obtain food based entirely on stimulus color. Colwill, Raymond, Ferreira, and Escudero (2005) reported a series of three experiments that investigated visual discrimination learning in the zebrafish using a T-maze and a series of sleeves that were fitted over the two arms of the maze. However, during the first two experiments, which analyzed color discrimination abilities between green versus purple and red versus blue sleeves, no attempt was made to control for difference in stimulus brightness between the stimuli. Thus, the apparent findings by Colwill et al. that the zebrafish has an innate preference for purple over green and a preference for blue over red may be explained by the zebrafish's innate preference for darker environments instead. Colwill et al.'s data also show a great deal of variability that was most likely

caused by variability between subjects as well as within subjects. Thus, to date, there are no demonstrations that investigate color vision-regulated behavior in zebrafish that also control for stimuli brightness and individual differences. The current project controls for these possible confounds by examining individual learning curves and determining idiosyncratic isoluminant points.

In the present study, eight adult zebrafish were trained on an instrumental discrimination learning task with wavelength as the discriminative stimulus. Prior to discrimination training, isoluminance training was used to determine at what point two monochromatic stimuli were perceived as equally bright by each subject. The subject was then tested to see if it could discriminate between the two stimuli by learning to choose the correct stimulus 80% of the time in two consecutive sessions of ten trials.

Materials and Methods

The procedures used in this experiment were modeled after those used by Bilotta et al. (2005).

Subjects

Eight adult (> 1 yr.) male and female zebrafish were used in this study. Fish were purchased from a local pet store and housed in an aquarium housing system (Aquaneering Incorporated, San Diego, CA). The system 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. Subjects 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 subject. All subjects were approximately the same size. No information regarding age or sex was recorded.

Behavioral Apparatus

The behavioral apparatus used was the same modified 19 L fish aquarium used by Bilotta et al. (2005, Figure 1A). 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 the 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 (Figure 1B) which could be raised and lowered to permit/prevent the fish's access to 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, allowing fish access only to two chambers whenever the gate was lifted. A liquid light-guide holder was placed outside the chamber area of the apparatus (see Bilotta et al., 2005).

Before conditioning began, the behavioral apparatus was filled with 4 L of conditioned water taken from the fish-housing system.

Optical System

Monochromatic visual stimuli were produced by one of two light sources. The 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 led into the selected chamber via the liquid light-guide holder.

The second light source was produced by a halogen light (World Precision Instruments, Sarasota, FL). A liquid light guide (World Precision Instruments, Model SI-

72-8, Sarasota, FL) attached to this light source was held in place by clamps. The other end of the liquid light guide led to 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), held by another clamp. The other end of this liquid light guide led to the selected chamber via the liquid light-guide holder. 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 training phases that included habituation, food-delivery training, stimulus-association training, isoluminance training, and wavelength-discrimination training. Prior to the start of training, subjects were housed individually to encourage individual behavior and to provide a means of identification. During training, the subjects' diets were restricted to a small amount of flake food daily to encourage the association of a food reward with the visual stimulus.

Habituation

After two days of food restriction, apparatus-habituation training commenced. Habituation training consisted of one session per day over two consecutive days. Habituation sessions were 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 subject 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

subject 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.

Food-Delivery Training

Immediately following habituation training, each subject received one daily session of food-delivery training for three consecutive days. The purposes of this training were to reinforce the subject's behavior of swimming into one of the two chamber areas and to counter the zebrafish's innate preference for darker environments by associating the monochromatic stimulus with a food reward. At the beginning of each food-delivery training session, the subject was re-habituated to the apparatus for 5 min. Following habituation, the gate was lowered, restricting the subject's movement to the home area. After 10 sec, the gate was raised, allowing the subject to swim into one of the two chamber areas. If the subject swam into one of the chambers, the gate was lowered, restricting the subject's movement to the chamber area 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 sec to consume the brine shrimp. The visual stimulus was then terminated by removing the liquid light guide from the liquid light-guide holder (for 460 or 540 nm stimuli), or the shutter was closed (for the 500 nm stimulus). 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-sec intertrial interval (ITI), a new trial began. In the event that a subject did not swim into one of the two chambers after 90 sec, the gate was

lowered, the trial was terminated, and it was recorded as a no-response trial. After 20 trials, the session was ended and the subject was returned to the housing system. The subject had to undergo food-delivery training for at least three days and successfully swim into one of the two chambers for all 20 trials in the last training session before moving on to stimulus-association training.

Stimulus-Association Training

After food-delivery training concluded, the subject began stimulus-association training. After being habituated for 5 min, the subject was confined to the home area. The monochromatic stimulus was then presented in one of the two chamber areas designated as the positive (S+) stimulus for 10 sec. 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 sec to consume the food. Afterwards, the visual stimulus was terminated, the gate was raised, and the fish was allowed back into the home area. This concluded the stimulus-association trial. If the subject swam into the dark chamber area, this was scored as an incorrect response. In the event of an incorrect response, the gate was lowered, the visual stimulus was terminated, and the subject was confined to the dark chamber area for 30 sec without food reinforcement. The gate was then raised and the subject was allowed back into the home area, signaling the end of the trial. If the subject failed to choose either of the two chambers after 90 sec, the trial was scored as having a no-response result. 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 sec 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 subject had to meet or exceed a criterion of 80% correct responses per session for two consecutive sessions in stimulus-association training in order to proceed to isoluminance training.

Isoluminance Training

The purpose of this experiment was to determine whether zebrafish could learn an instrumental discrimination with different wavelengths as the discriminative cues. The purpose of isoluminance training was to determine at which luminance the S+ stimulus associated with a food reward and a second monochromatic stimulus were perceived as equally bright. By determining these isoluminant values for each subject, this study was able to control for the potential confound that a subject would learn to discriminate the two monochromatic stimuli using brightness cues. To ensure that the subject used only color cues when differentiating between the two visual stimuli, isoluminance values were determined for each subject for the two given wavelengths. Idiosyncratic isoluminant points were determined as opposed to a single isoluminant point for each pair of wavelengths for all subjects because the perception of brightness may differ among subjects. An isoluminant point was defined as the illuminance value at which a subject's performance was at or below chance.

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 a monochromatic stimulus (460, 500, or 540 nm) and that was designated S- (because responses to this stimulus were not rewarded); for subjects Z4 and Z8, the 500 nm stimulus was designated as the S+ and the 460 nm stimulus was designated S-; for subjects Z3 and Z9, the S+ was 500 nm and the S- was 540 nm; for Z30 and Z28, the S+ was 460 nm and the S- was 500 nm; and for subjects Z25 and Z33, the S+ was 540 nm and the S- was 500 nm. The illuminance of the 500 nm stimulus varied between trials in steps of 0.3 log units of attenuation. Six different illuminance values were tested per session. After 5 min of habituation, the subject was confined to the home area by lowering the gate. Stimuli were presented simultaneously; the monochromatic stimulus associated with the food reward was designated the S+ again and the new monochromatic stimulus was designated S-. After 10 sec, the gate was raised and the subject was allowed to swim either into the S+ or S- chamber. In the event of the subject's swimming into the S+ chamber, the response was scored as a correct response. The gate was lowered, the S- was terminated, and the subject was rewarded with 5-10 live brine shrimp. After 30 sec of feeding, the gate was raised, the subject was allowed back into the home area, the gate was lowered, and a new trial began after a 10 sec ITI.

If the subject responded by swimming into the S- chamber, it was scored as an incorrect response. Visual stimuli were terminated, and the fish was confined to the S- chamber for 30 sec without food reinforcement. The gate was then raised, allowing the subject to return to the home area. After a 10-sec ITI, the next trial was administered. In the event of a no-response trial, which consisted of 90 sec of swimming in the home area without swimming into either the S+ or S- chamber, the trial was terminated. The stimuli were then terminated, the gate was lowered, and the subject remained in the home area

until the next trial. 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.

Wavelength-Discrimination Training

After isoluminance training determined the subject's isoluminant point for the two given monochromatic stimuli, the subject began the final phase of training: wavelength-discrimination training. During these sessions, the illuminance of the 500 nm stimulus was fixed at the isoluminant value determined during isoluminance training. The training methodology was essentially the same as that used for isoluminance training. The S+ and S- designations were the same as those used in isoluminance training. The subject began 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 trial resulted in a correct response by the subject's swimming into 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. After 30 sec, the gate was raised, and the subject was allowed to reenter the home area. The gate was lowered, and an ITI of 10 sec passed before a new trial began. If the trial resulted in an incorrect response, meaning the subject swam into the S- chamber, the gate was closed, the stimuli were terminated, and the subject remained in the dark chamber for 30 sec without food reinforcement. After this time passed, the gate was raised, the subject was allowed back into the home area, and the gate was lowered, ending the trial. Again, there was an ITI of 10 sec. If the subject refused to swim into either chamber after 90 sec, stimuli were

terminated, the gate was lowered, and the trial was scored as a no-response result. Wavelength-discrimination training consisted of two consecutive, 10-trial sessions per day. Training ended when the subject achieved an 80% correct-response criterion for both of the consecutive, 10-trial sessions.

Results

Stimulus-Association Training

Figure 2 shows the mean learning curve for all subjects that were conditioned to their respective monochromatic stimulus during stimulus-association training. The X-axis represents training sessions required to reach criterion, and the Y-axis represents the percent-correct response of the subjects. Each filled circle represents the mean percent correct responses for all subjects which had not yet reached criterion. Error bars represent ± 1 standard deviation. Variability was relatively high until the 7^{th} training session. After the 7^{th} training session, there was no variability because only one subject (Z9) had not reached criterion at this time. The dashed line represents the 80% correct-response criterion necessary for the subject to continue to isoluminance training. The average percent-correct response at the onset of training was 42.5%. On average, it took subjects 6.75 sessions to reach criterion. Not including data obtained from fish Z9, which took many more sessions to reach criterion than all other subjects, it took an average of 5.71 sessions to reach criterion. All subjects reached criterion performance by 14 sessions.

The variability among fish in rate of learning can be seen in Figure 3, which presents individual learning curves for the stimulus-association training. Again, the X-axis represents training sessions, and the Y-axis represents percent-correct responses.

Each subject's individual learning curve is uniquely represented by a symbol (filled/unfilled circles, triangles, squares, etc.) and solid lines. The dashed line represents the 80% learning criterion required to complete stimulus-association training. All subjects reached the learning criterion; however, the number of sessions to do so varied from 4 (Z4) to 14 (Z9). All subjects initially performed below chance and improved performance until reaching criterion.

Isoluminance Training

Figures 4-11 display the results of isoluminance training for each subject. In all figures, the X-axis is log-stimulus attenuation and the Y-axis is percent-correct response. Filled circles and lines represent mean percent-correct response values for each irradiance. Error bars represent ± 1 standard error of the mean. The dashed line represents chance performance. The isoluminant point was defined as the attenuation at which the average percent-correct response fell closest to chance levels (50%). The arrow indicates which attenuation was defined as the isoluminant point for that particular fish and that particular discrimination task.

As can be seen, isoluminant values varied between subjects even when performing the same discrimination task. For example, as shown in Figure 4, subject Z4 performed, on average, below chance (47.86%) when -1.5 log units of attenuation were applied to the 500 nm S+ stimulus. As shown in Figure 5, subject Z8's isoluminant point was defined at -0.6 log units of attenuation when performing the same discrimination task as subject Z4. When -0.6 log units of attenuation were applied to the S+, Z8's average correct performance fell closest to chance (57.5%). Figure 6 shows that subject Z3's isoluminant point when the 500 nm S+ was paired with a 540 nm S- occurred when -1.5

log units of attenuation were applied to the S+. At this attenuation, Z3's successful performance of the discrimination task fell to 52%, just above chance expectations. Figure 7 shows subject Z9's isoluminance training results at the same discrimination task as Z3. This time, when the S+ (500 nm) was attenuated by -1.2 log units and paired with the S- (540 nm), the subject's discrimination success fell to 60%. Thus, -1.2 was identified as Z9's isoluminant point. In Figure 8, subject Z30 performed closest to chance (64%) when the 500 nm (now S-) stimulus was attenuated with -0.6 log units of attenuation and paired with the 460 nm S+. When performing the same discrimination task, subject Z28 performed closest to chance (54%) when -0.9 log units of attenuation were applied to the 500 nm S-, as can be seen in Figure 9. In Figure 10, subject Z25 performed at chance expectations (50%) when the 460 nm S+ was paired with a 500 nm S- stimulus that was attenuated by -0.3 log units. Finally, as shown in Figure 11, when performing the same discrimination task, subject Z33 performed closest to chance when the S- was combined with -0.6 log units of attenuation. This pattern of results illustrates the importance of using idiosyncratic isoluminance values for wavelength-discrimination training.

Wavelength-Discrimination Training

Figure 12 shows the results of the wavelength-discrimination training that took place after isoluminance training. Here, the X-axis represents training session and the Y-axis represents percent correct response. The various shapes (filled/unfilled circles, squares, triangles, etc) and solid lines represent the individual discrimination-learning curves for each subject. The dashed line represents the criterion of 80% correct, and the dotted line represents chance. As can be seen, all subjects reached criterion, although

after different amounts of training. All subjects fell to chance performance at some point in training except subjects Z8, Z25, and Z3, who never fell below criterion. Subjects took an average of 6.88 sessions to attain criterion, and all subjects reached criterion by 16 sessions.

Discussion

Stimulus-Association Training

The present study supports the findings of Bilotta et al. (2005) and Colwill et al. (2005), 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, individual learning fish did vary in number of sessions required to reach the learning criterion. Thus, zebrafish, like other organisms, display individual differences in learning rate.

Isoluminance Training

One possible confound of the Colwill et al. (2005) study that was addressed in this work was that the subject would use visual cues other than stimulus wavelength to correctly identify the stimulus paired with food reward. In other words, in Colwill et al. (2005), it is possible that the subject used both stimulus color and brightness to determine the location of the food reward. In order to ensure that only color cues would be available to the subjects to discriminate stimuli, an isoluminant point was determined for each subject for the given discrimination task. The isoluminant point was defined as the attenuation at which the subject performed correctly, on average, closest to chance when discriminating between two monochromatic stimuli. At this point, it was assumed that the

subject could no longer use brightness cues to differentiate between visual stimuli. While it is impossible to know if the stimuli were actually perceived by the subject to be equally bright, the isoluminance training ensured the stimuli were functionally equivalent. Furthermore, the use of all three monochromatic stimuli as both S+ and S- (across different fish) countered any innate tendency to approach a certain color and countered any possible brightness preference that might remain after an isoluminant point was determined. Individual isoluminant values were obtained for all subjects, and it was found that isoluminant values varied not only between discrimination tasks but also between subjects performing the same discrimination task. This finding suggests that visual perception abilities may vary between individual zebrafish, and individual differences should be taken into account when one is using psychophysical paradigms to measure visual abilities.

Wavelength-Discrimination Training

The purpose of this experiment was to determine if the zebrafish is capable of changing its behavior to obtain food based entirely on color cues. In the present study, all subjects reached the learning criterion when discriminating equiluminant stimuli of varying wavelengths. This is interpreted to mean that all subjects were able to discriminate two visual stimuli based entirely on color cues. As was seen in stimulus-association training, the number of required learning sessions varied among individuals, emphasizing the importance of taking individual differences into account when using behavioral paradigms to measure visual performance. These data confirm anatomical and physiological data that suggest zebrafish have color vision capabilities. This is the first and only behavioral study to demonstrate that zebrafish have functional color vision, i.e.,

fish use color cues to alter their behavior in order to obtain a food reward.

Implications for Future Research

Future studies of zebrafish vision and visual perception can be performed using the procedure used here. Such research could determine whether wavelength discrimination is possible at different wavelengths other than those used in the present study. 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 may lead to the development of new models for vertebrate visual deficits such as color blindness and night blindness.

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Figures

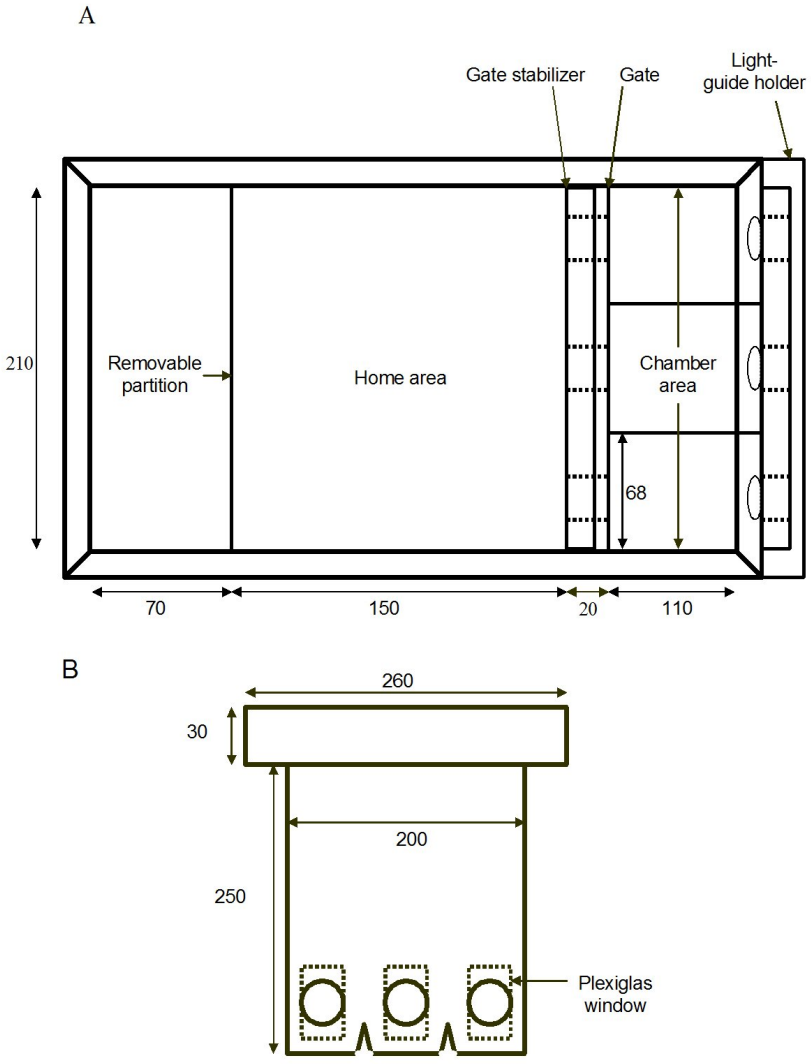

Figure 1. Schematic of the behavioral apparatus. Details can be found in Bilotta et al. (2005). **(A)** Top view. **(B)** Side view of the removable gate.

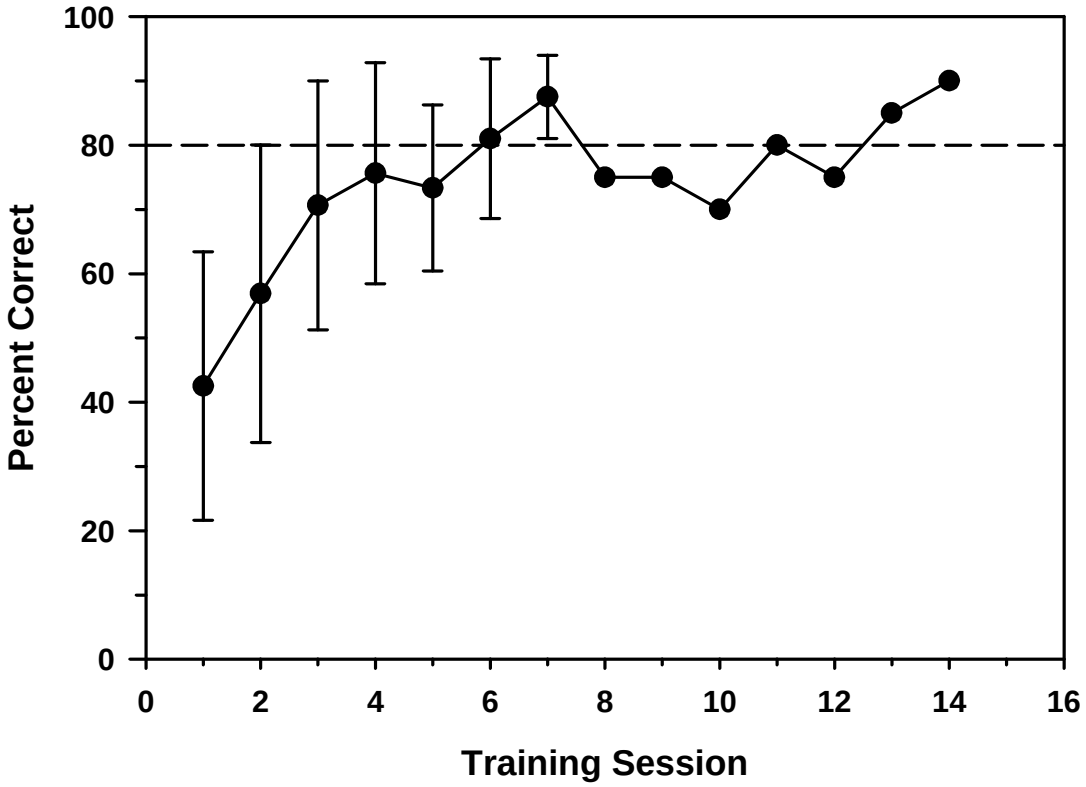

Figure 2. The mean learning curve for stimulus-association training.

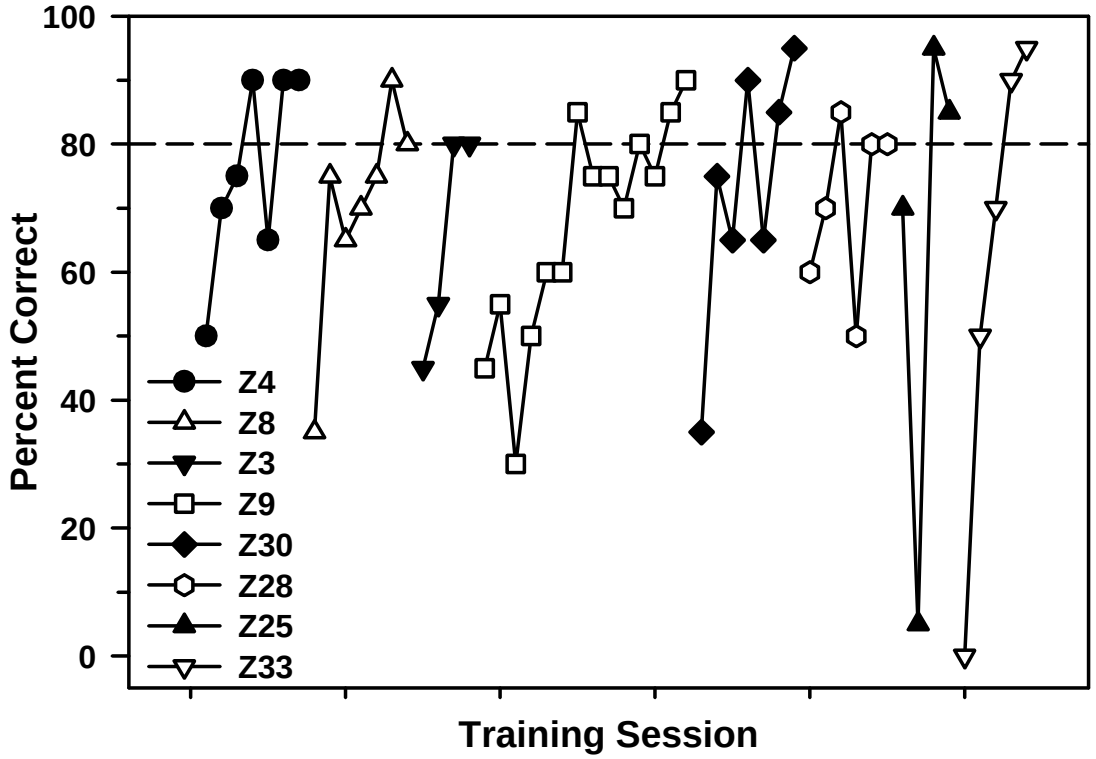

Figure 3. Individual learning curves for stimulus-association training.

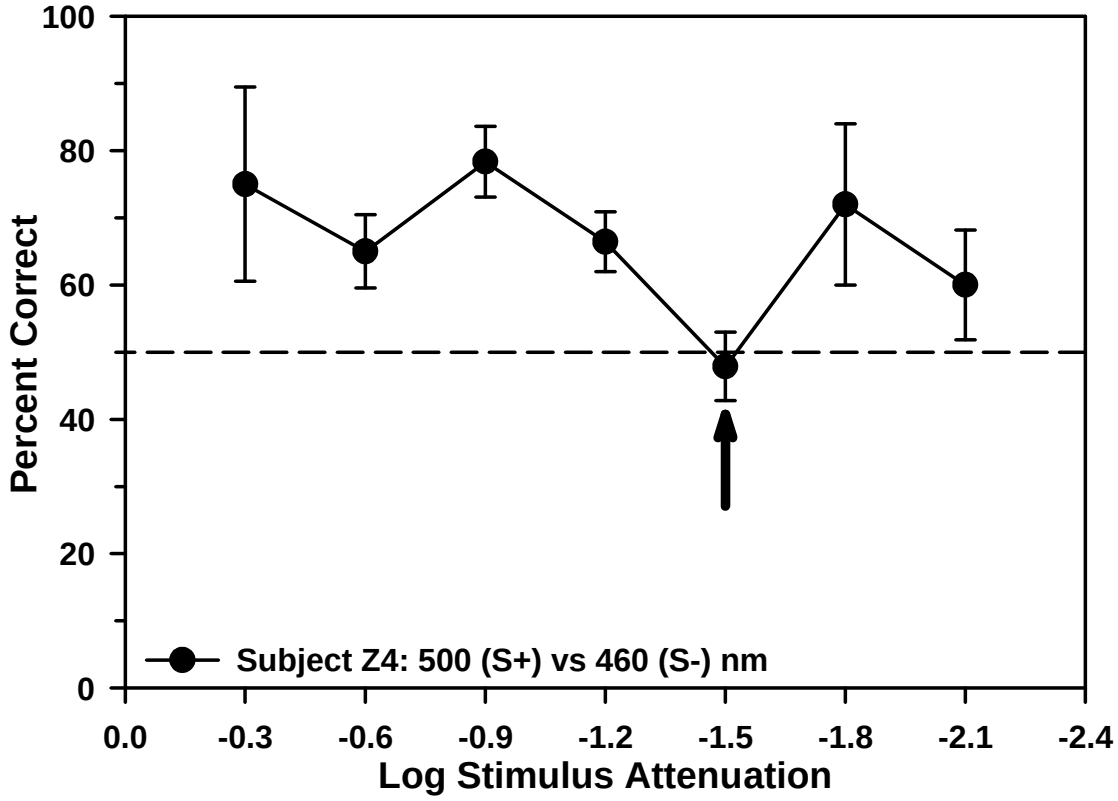

Figure 4. Isoluminance training results and isoluminant point determined for subject Z4, which was instrumentally trained to swim towards a 500 nm (S+) stimulus during stimulus-association training.

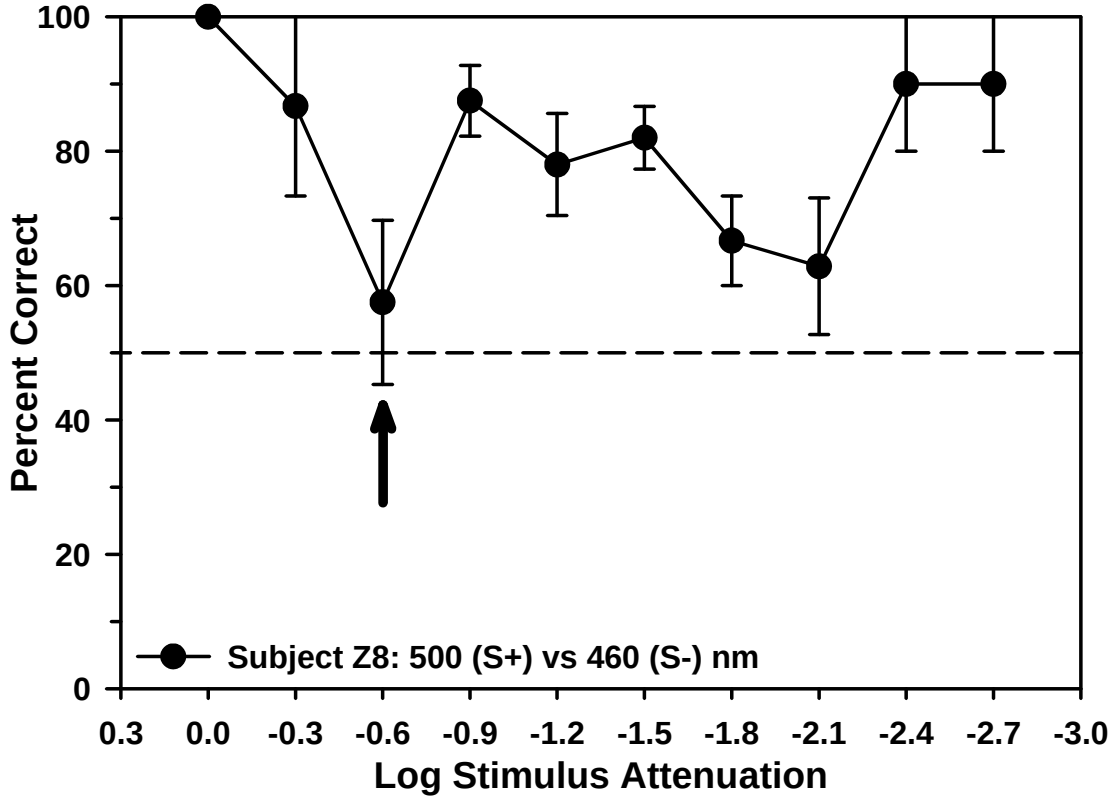

Figure 5. Isoluminant training results for subject Z8 who was conditioned to swim towards a 500 nm (S+) stimulus for a food reward as opposed to a 460 nm (S-) monochromatic stimulus.

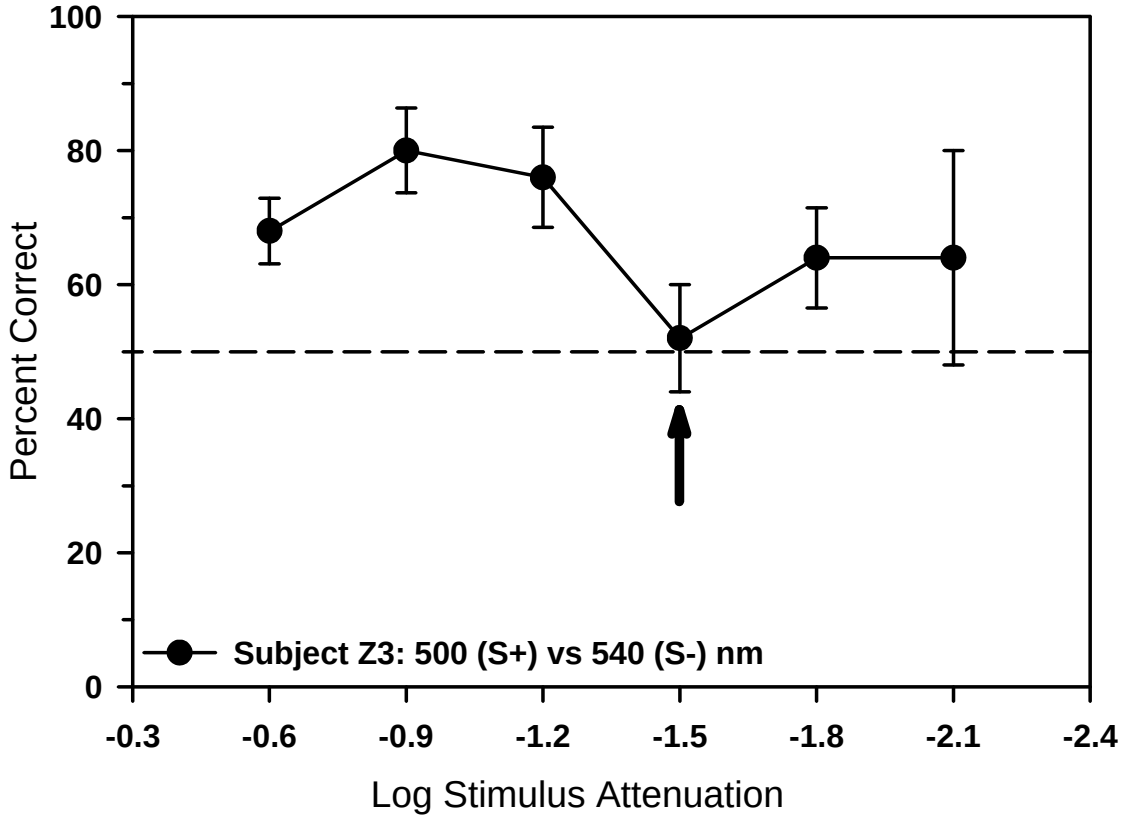

Figure 6. Subject Z3's results of isoluminance training, which determined an isoluminant point at -1.5 log units of attenuation when the S+ (500 nm) was paired with a 540 nm S-.

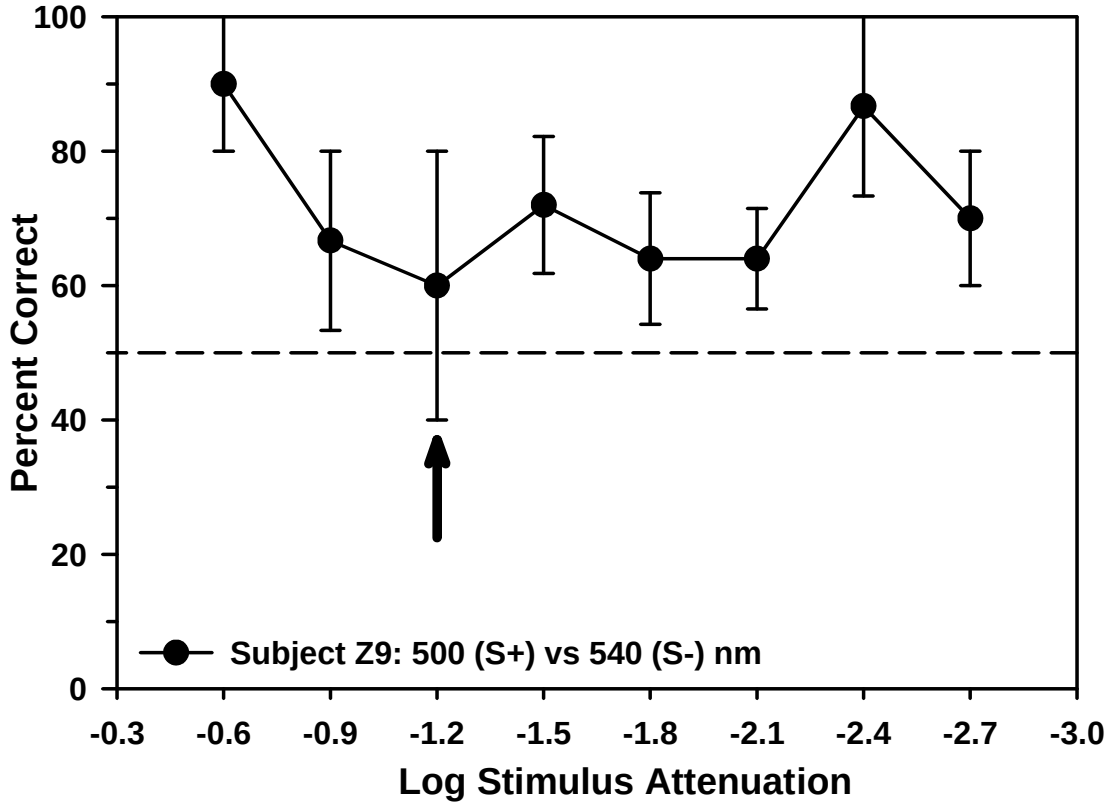

Figure 7. The results of isoluminance training for subject Z9, which was conditioned to associate a 500 nm stimulus with a food reward during stimulus-association training.

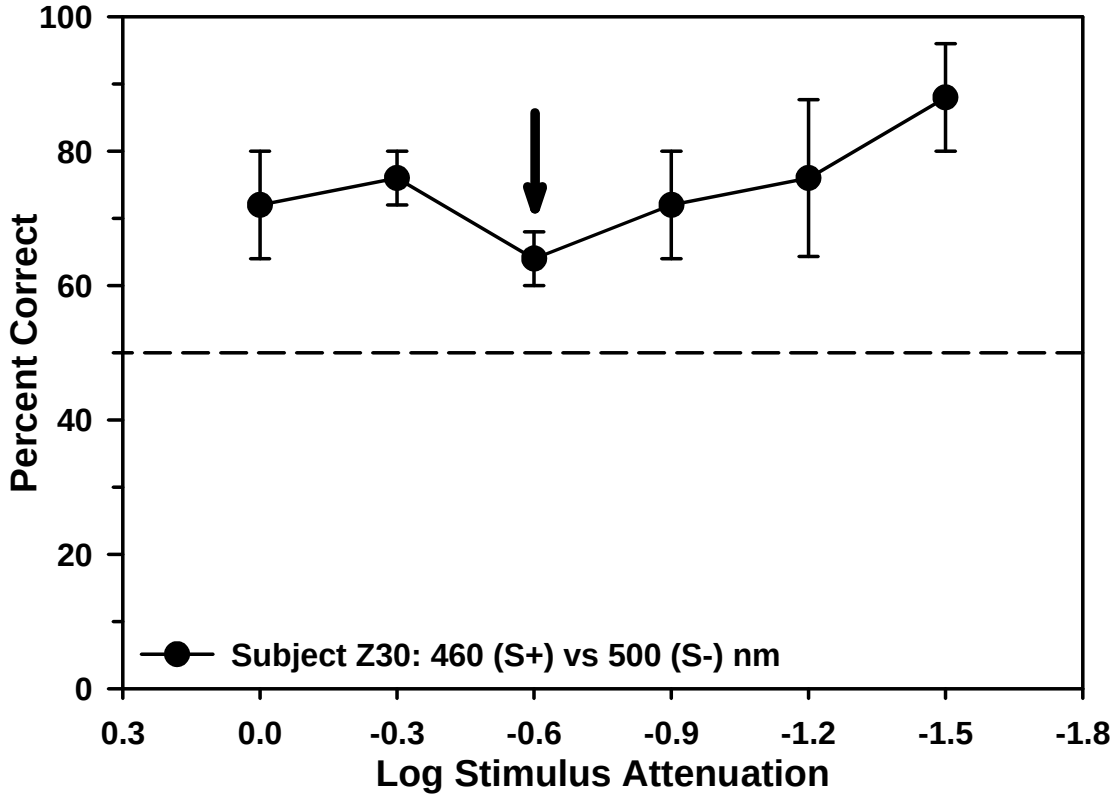

Figure 8. Results of subject Z30's isoluminance training. -0.6 log units of attenuation most impeded the subject's performance of swimming to the S+ (460 nm) when it was paired with the S- (500nm).

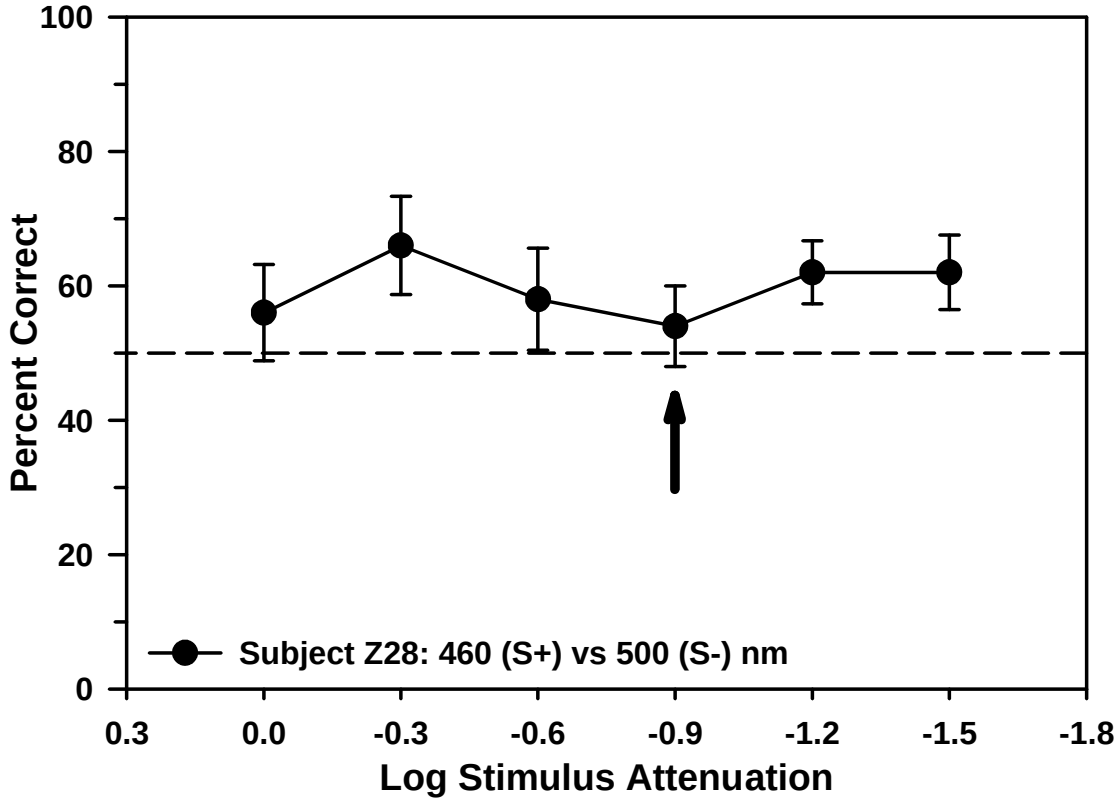

Figure 9. Isoluminance training results for Z28, which was instrumentally trained during stimulus-association training to associate a 460 nm (S+) monochromatic stimulus with a food reward.

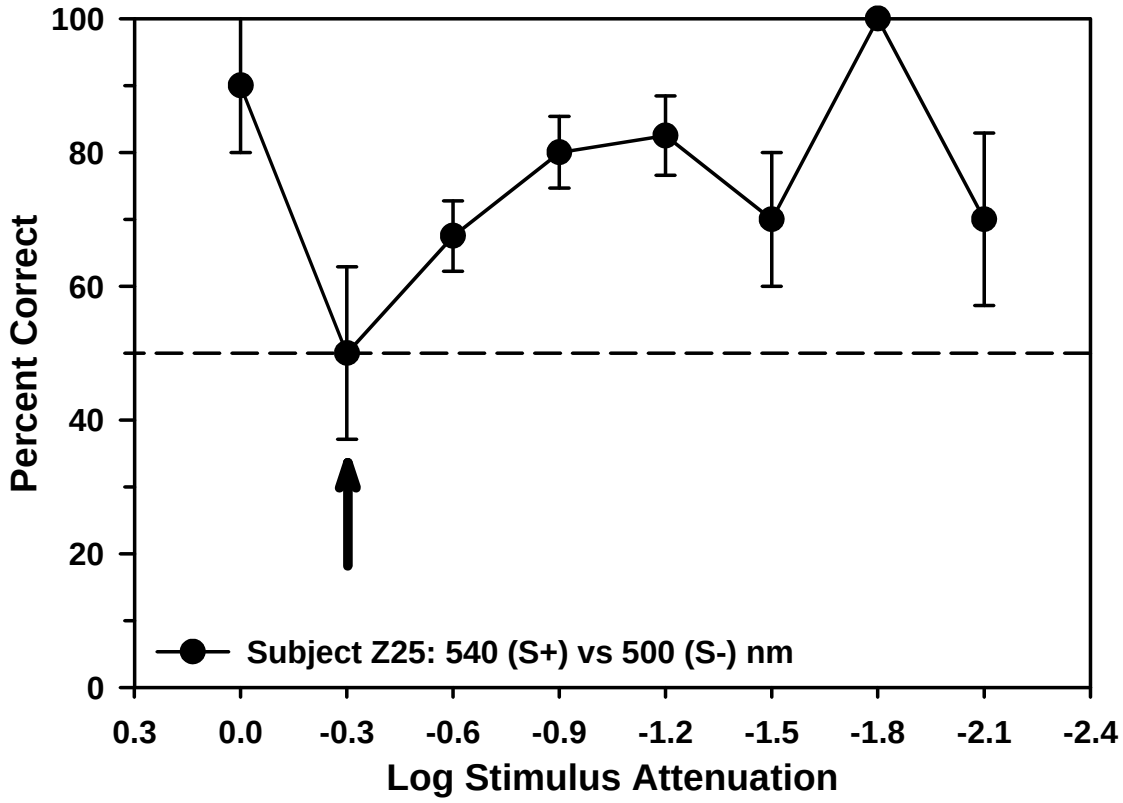

Figure 10. Results of subject Z25's isoluminance training, which identified Z25's isoluminant point at -0.3 log units of attenuation applied to the 500 nm (S-) stimulus.

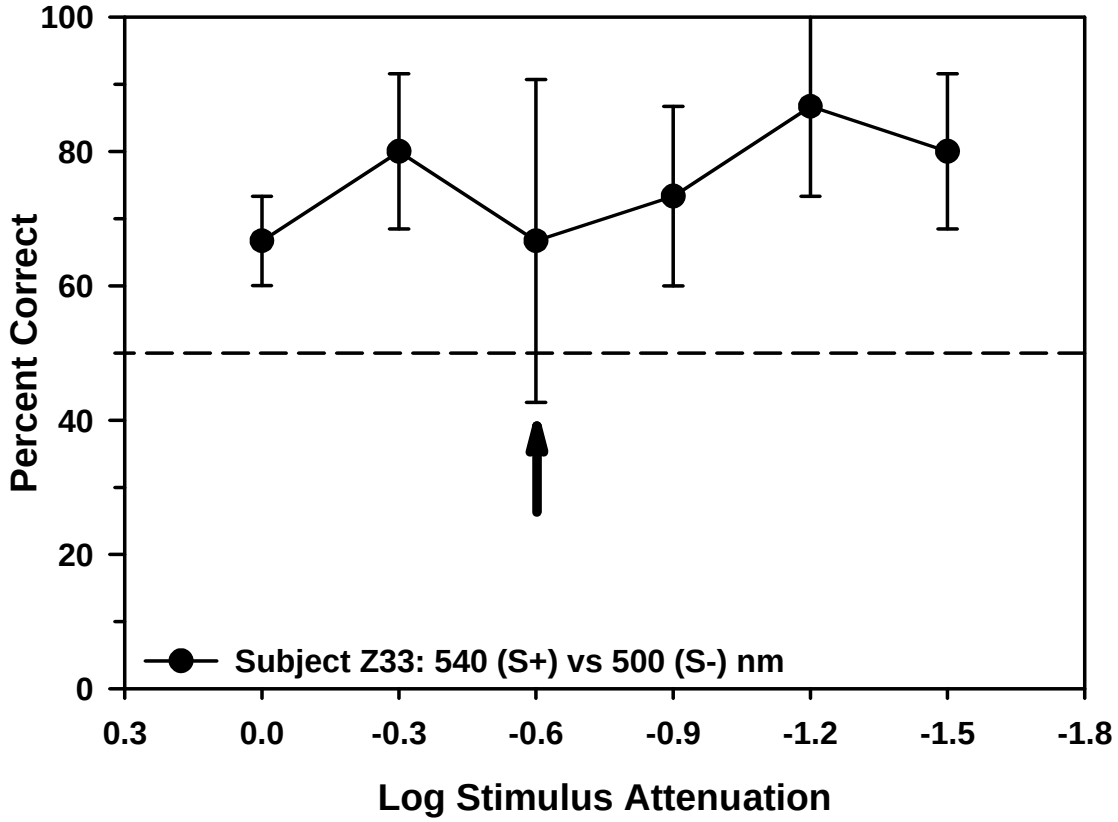

Figure 11. Isoluminance training results for Z33. The subject's isoluminant point was at -0.6 log units of attenuation.

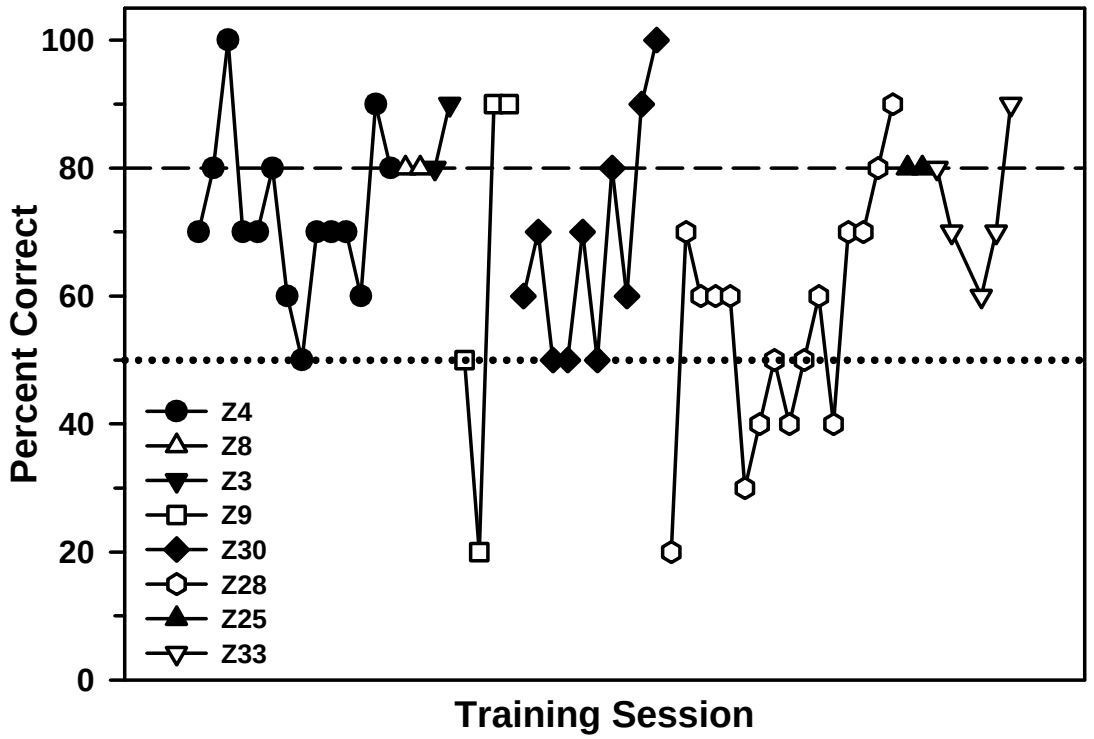

Figure 12. Individual learning curves for wavelength-discrimination training.