

WAVELENGTH DISCRIMINATION BY THE GOLDFISH NEAR ABSOLUTE VISUAL THRESHOLD

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Abstract—Goldfish were classically conditioned to discriminate between 532 nm and 636 nm lights while light adapted, and then tested for their ability to continue the discrimination as the intensities were reduced. In all tests, the two stimuli were balanced for effectiveness at absolute threshold. We find that goldfish can discriminate between these wavelengths about 1 log unit above absolute threshold. In contrast, fish who were trained to discriminate between 532 nm and 441 nm stopped discriminating 2-3 log units above absolute threshold. There are two conclusions from these results. First, some aspect of the neural signals generated by the rod and red cone mechanisms must be different, despite electrophysiological evidence that suggests they are the same. Second, the goldfish has color vision near rod threshold.

Key Words—goldfish; rod-cone interactions; color discrimination; respiratory conditioning; scotopic vision; retina.

INTRODUCTION

A single visual receptor cannot tell the wavelength of quanta that influence it. Color vision—the ability to distinguish wavelengths independent of their intensities—requires at least two receptor mechanisms with different spectral sensitivities. In goldfish, two spectrally different receptor mechanisms—probably rods and red cones—mediate absolute threshold (Powers and Easter, 1978); therefore this animal has the potential for color vision at very low intensities.

Although color vision cannot occur unless there are two kinds of receptor, their existence does not guarantee it. Neural activity beyond the receptors must maintain the distinctions between them. Evidence suggests that the messages from the goldfish's rods and red cones share similar anatomical and physiological channels to the ganglion cells, and thus do not remain separate at higher levels of processing. Rods and red cones affect ganglion cell responses similarly (Raynauld, 1969, 1972; Adams and Afanador, 1971; Beauchamp and Daw, 1972), and they may use common bipolar cell pathways to the ganglion cells (Scholes and Morris, 1973; Scholes, 1975; Stell, 1976). Because the ganglion cell axons form the optic nerve, which is the only exit from the retina, these studies suggest that the goldfish should not have color

vision near absolute threshold, despite the presence of two receptor mechanisms.

The similarity between rod and red cone signals suggested to us that a conditioned response to rod stimulation should generalize easily when red cones are stimulated, and vice versa. Our early experiments were designed to test this hypothesis.

Our initial test for generalization between rod and red cone signals yielded encouraging results. Dark-adapted goldfish which were trained to slow their heart rate when they detected an increment of 532 nm light near absolute threshold generalized more strongly to an increment of 636 nm light than to an increment of 532 nm light during light-adapted tests (Powers and Easter, 1975). When we attempted to replicate and expand these findings, however, we met with little success. Extensive testing showed that while generalization was almost always in the predicted direction, it was not strong and only rarely attained statistical significance (Powers, 1977). These results led us to consider a complementary hypothesis: that the neural messages from rods and red cones are sufficiently different to allow the fish to discriminate between them. This paper describes a test of that hypothesis.

In the experiments reported here, light-adapted goldfish were classically conditioned to discriminate between red and green lights. Then the intensities (of both stimuli) were reduced until the fish no longer discriminated between the two wavelengths. Some of the same fish were trained to discriminate between blue and green, and tested in the same way. The results show that, close to absolute threshold, the goldfish can discriminate long from medium wavelengths but not short from medium.

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METHODS

Apparatus

The apparatus was the same as described in Powers and Easter (1978), with the following two exceptions: (1) only one channel of the optical stimulator was used; (2) stimulus and shock presentation were controlled by hand.

Stimuli

Calibration procedures were described previously (Powers and Easter, 1978). All stimuli in this study were 13' in diameter, and were presented on a dark field. During light-adapted training sessions stimulus intensity was 6-8 log units above absolute threshold (Powers and Easter, 1978), and will hereafter be referred to as "photopic." The intensities of all stimulus pairs were adjusted to produce equal visual effectiveness, according to the appropriate spectral sensitivity curve (light-adapted: Powers, 1978; dark-adapted: Powers and Easter, 1978). Interference and neutral density filters (Optics Technology) set wavelength and intensity of the stimuli. The half bandwidths of the interference filters were 25-55 nm.

Subjects

Twenty-one goldfish (*Carassius auratus*, Ozark Fisheries, Stoutland, MO) 13-15 cm tip-to-tip, were subjects in this study. They were dark adapted in their home aquaria for at least 40 min before sessions at low intensities, and were put into the test box 10-15 min before training or testing began.

Training procedure

Although the experiments reported here used a discrimination paradigm, many of the training procedures were similar to those used in the detection paradigm described earlier (Powers and Easter, 1978); the reader is referred to that paper for details omitted here.

All fish were given preliminary habituation sessions during which no stimulus was presented. Then they were exposed for at least one session (20 trials) to the stimulus that was not to be paired with shock—the nondiscriminative stimulus (NDS). The stimulus that was paired with shock—the discriminative stimulus (DS)—was introduced in the next session. This session was considered to be the first training session.

There were 5 DS trials and 15 or 16 NDS trials in a discrimination training session. The order of presentation was random except that DS trials were never consecutive and there were never more than 4 consecutive NDS trials.

The response criterion for single trials was the same as described in Powers and Easter (1978): respiration rate had to decrease to half its usual rate. The percent of trials that evoked a criterion response was calculated for the DS and for the NDS for each fish following each session. The quantity (percent response to DS minus percent response to NDS) will be called ΔR , and is an indicator of the discriminability of the two lights. An animal was considered trained when $\Delta R \geq 60$ for two consecutive sessions.

Testing procedures are described in the Results section.

RESULTS

Photopic discrimination training

Three wavelength pairs were used: 441 nm/532 nm, 472 nm/532 nm and 636 nm/532 nm. The first discrimination attempted was 472 nm/532 nm. Four fish were subjects; for two the DS was 532 nm and for the other two it was 472 nm. Despite lengthy training,

none of the fish performed at our criterion level: their final ΔR 's were -7, 3, 12 and 33.

A group of ten fish, also counterbalanced for wavelength of DS, was then trained to discriminate between 636 nm and 532 nm. We will refer to this as the red/green discrimination. Seven of them learned in an average of about six sessions. This success rate (70%) is comparable to that for a detection task (Powers and Easter, 1978). Using yet another group of subjects, we tried to condition the same red/green discrimination when the intensity of each wavelength was randomly varied ± 0.5 log units around photopic equivalence; varying the intensity was an attempt to increase the probability that the fish were discriminating on the basis of wavelength alone. This task was apparently more difficult for the fish, because only two out of the seven subjects learned it in an average of ten sessions.

After the nine fish who learned the red/green discrimination had been tested (see below), eight of them³ were trained to discriminate between 441 nm

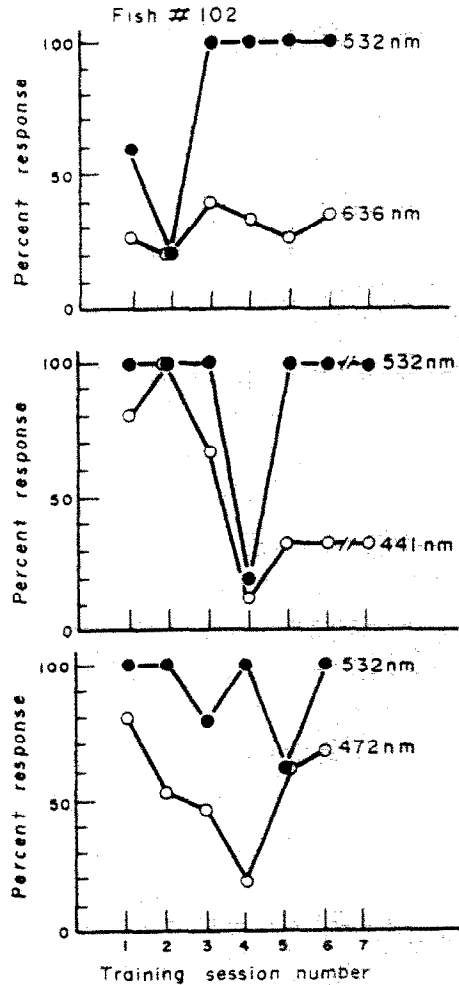


Fig. 1. Discrimination learning curves for one light-adapted fish (No. 102). Ordinate: percent of trials during which response criterion was met. Filled circles: stimulus associated with shock (DS). Open circles: stimulus not associated with shock (NDS). 532 nm was always DS for this fish. There were 7 days between sessions 6 and 7 for 532 nm/441 nm training; session 7 was 1 day after the last 532 nm/472 nm session.

³ One had since died.

Table 1. Photopic wavelength discrimination

	Between 532 nm and:			636 nm variable intensity
	441 nm	472 nm	636 nm	
Percent success ^a	63	0	70	29
Mean sessions to criterion (learners)	4.0	—	6.4	10
Mean sessions (nonlearners)	5.7	8.6	6.7	8.4
Total <i>N</i>	8 ^b	5 ^c	10	7

^a Percent of subjects who performed at criterion levels.

^b All eight had previously learned to discriminate 636 nm from 532 nm.

^c One fish had previously learned to discriminate both 636 nm and 441 nm from 532 nm.

and 532 nm. We will refer to this as the blue/green discrimination. For each fish, the response to green during blue/green retraining was the same as it had been during red/green training. That is, if green was DS for a given fish in the red/green discrimination, it remained DS in the blue/green discrimination. Five of the eight fish learned the blue/green discrimination in an average of four sessions each.

Finally, one of the most reliable fish (No. 102) was again retrained, this time on the original 472 nm/532 nm discrimination. The fish did not meet criterion within six sessions, despite its extensive experience. The learning curves for No. 102 are in Fig. 1.

Table 1 summarizes the results of photopic discrimination.

Discrimination testing

Subjects who had learned red/green or blue/green discriminations while light adapted were tested for

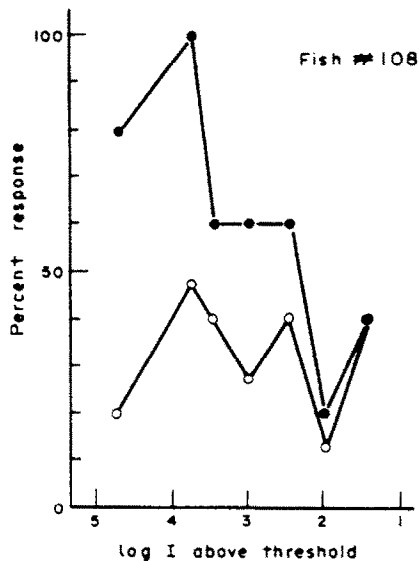


Fig. 2. Breakdown of a discrimination at low intensities in one subject (No. 108). Ordinate: Same as Fig. 1. Abscissa: log intensity of the test stimuli, relative to absolute threshold. Filled circles: DS (532 nm). Open circles: NDS (636 nm). Stimuli were balanced for equal dark-adapted effectiveness. Each pair of points at a given intensity is from one session on one day.

their ability to discriminate between the same wavelengths at progressively lower intensities. The test was a modified staircase psychophysical procedure. Each day an animal was tested for its response to the conditioned wavelength pair, but at an intensity 0.5 or 1.0 log units less than on the previous day. The procedure for any given session was the same as the light-adapted training these fish had already received: there were 20–21 trials/day, and reinforcement followed all DS presentations. ΔR was calculated for each session and any positive value during testing was taken to imply discrimination, however weak. When ΔR reached zero, testing was terminated.

Figure 2 shows the performance of one subject during such a series of daily tests. As the intensity decreased, ΔR gradually diminished, but it stayed positive until between 1 and 2 log units above absolute threshold (Powers and Easter, 1978). Note that when ΔR reached zero, the fish still responded to individual stimuli. This argues against the possibility that the basic light-shock association was extinguished at the end of testing.

Functions like the one in Fig. 2 were obtained for five other animals trained to discriminate between red and green, and for four of the same fish trained to discriminate between blue and green. ΔR was calculated as described earlier at each intensity tested. The values of ΔR for all individual subjects are shown in Fig. 3. For clarity, the mean ΔR of all subjects tested at a given intensity with a given wavelength pair is plotted separately in Fig. 4. The results show that the ability to discriminate between blue and green is lost before the ability to discriminate between red and green. This was true of all individual subjects. Furthermore, the ranges of individual values of $\Delta R = 0$ did not overlap between the blue/green and red/green discriminations. ΔR was zero at 0.80 (± 0.45 S.D.) log units above absolute threshold for

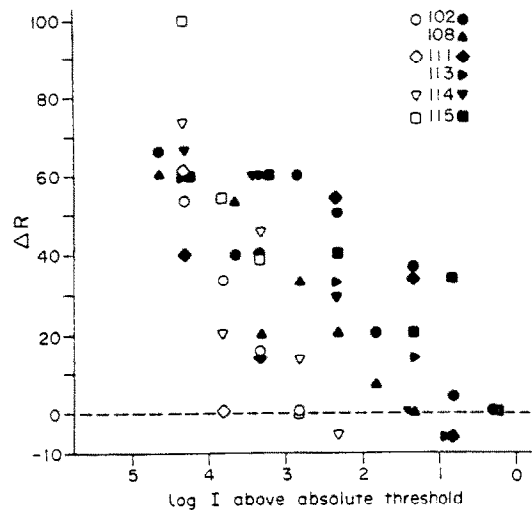


Fig. 3. Wavelength discrimination at low intensities. Ordinate: difference in percent response (percent response to DS minus percent response to NDS). Abscissa: intensity of the stimuli, relative to absolute threshold, for the balanced pair. Open symbols: blue/green discrimination. Closed symbols: red/green discrimination. *N* = 6 for red/green and 4 for blue/green. Red: 636 nm; green: 532 nm; blue: 441 nm. Multiple points at a single location have been plotted with slight horizontal displacement.

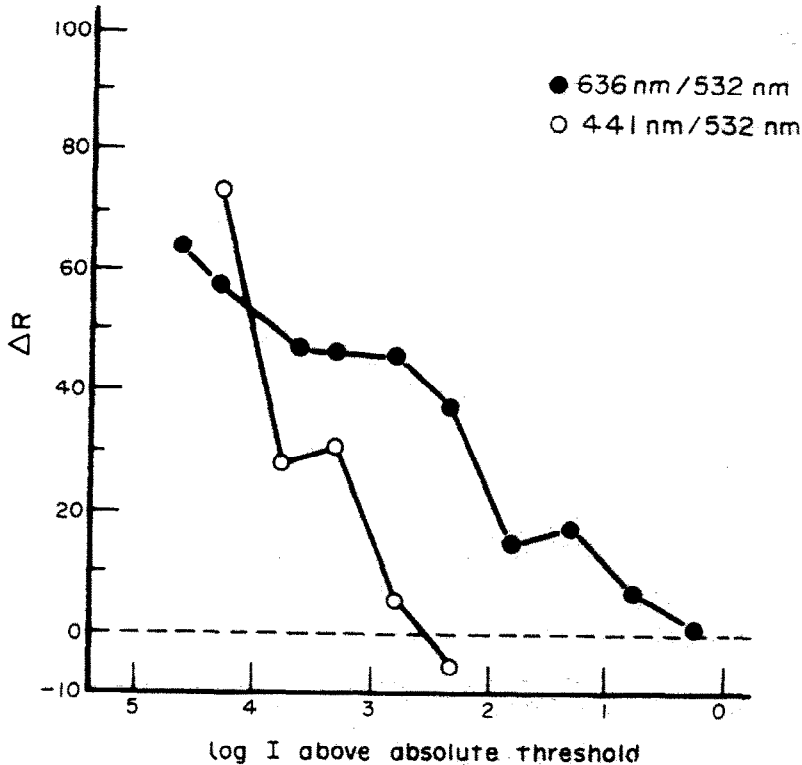


Fig. 4. Mean values for discrimination of wavelength at low intensities. Axes and data same as Fig. 3. Filled symbols: means for the red/green discrimination; open symbols: means for the blue/green discrimination.

the red/green discrimination and 2.98 (± 0.63 S.D.) log units above absolute threshold for the blue/green discrimination.

In order to ascertain whether the discrimination was based on wavelength or intensity, five red/green discrimination subjects were retested at 1 log unit above the intensity where ΔR was zero for each of them. The intensities of the red and green lights were varied ± 0.5 log units randomly from trial to trial. The data from Training Session No. 1 in Fig. 5 show the results of this test. The animals could discriminate between the two lights even when intensity was varied; a comparison of mean ΔR from this session with mean ΔR for the same subjects when intensity was constant shows a difference of less than 5%. These results indicate that the discrimination was not made on the basis of intensity.

Finally, we tried to improve performance at 1 log unit above the intensity where red/green discrimination ceased for each fish by repeating the procedure just described for four more sessions. As Fig. 5 shows, the training did not help.

DISCUSSION

Light-adapted wavelength discrimination

The results of the photopic discrimination training support earlier reports (McCleary and Bernstein, 1959; Muntz and Cronly-Dillon, 1966; Yager, 1967; Shefner and Levine, 1976) that the goldfish has photopic color vision, and that blue/green discrimination is more difficult than red/green (Muntz and Cronly-Dillon, 1966). The fish in our study which learned the 441 nm/532 nm discrimination were all highly ex-

perienced, having previously been trained on the red/green task. When naive, our fish did not learn the 472 nm/532 nm discrimination at all. Even the most reliable experienced fish (No. 102; see Fig. 1) did not learn the task. The electrophysiology of retinal ganglion cells suggests a basis for the difficulty: blue and green receptors have similar effects on ganglion cells

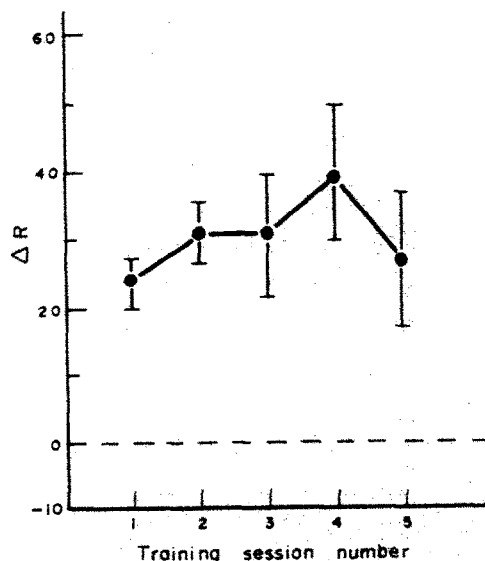


Fig. 5. Repetitive testing near the intensity where the red/green discrimination is no longer possible. The intensity of the test light was randomly varied within a ± 0.5 log unit range of equal dark-adapted effectiveness. Mean scores for five fish; bars ± 1 S.E.M.

(Beauchamp and Lovasik, 1973), while red and green receptors have opposite effects (Wagner, MacNichol and Wolbarsht, 1963; Daw, 1968).

Dark-adapted wavelength discrimination

The goldfish can no longer discriminate between medium and short wavelengths when the intensity of the light is about 3 log units above absolute threshold. Several lines of evidence suggests that this value is approximately what would be expected if rods alone were responsible for catching quanta at low intensities in this region of the spectrum. First, Yager (1968) found 3 log units difference in sensitivity between the peaks of the behavioral dark- and light-adapted action spectra he measured in goldfish. This suggests that mid-spectral lights exceeding absolute threshold by about 3 log units will stimulate cones. Second, Raynauld (1969) reported seeing cone-dominated activity in goldfish retinal ganglion cells 2.5 log units above absolute threshold when he stimulated with green light. Third, we have shown (Powers and Easter, 1978) that photomechanical movement of the cones toward their light-adapted positions occurs at about 3 log units above absolute threshold; this finding is consistent with the more quantitative analysis made by Easter and Macy (1978) in another teleost. And fourth, the rod/cone break for mid-spectral wavelengths in peripheral retina is at a comparable location in human subjects (Stiles, 1939). The inability to discriminate between short and medium wavelength lights equated for dark-adapted effectiveness means the rods are probably the only photoreceptors functioning at these wavelengths.

The ability to discriminate between medium and long wavelengths, on the other hand, does not break down until their intensities are very close to absolute threshold. Therefore, the goldfish has color vision in the longer-wavelength portion of the spectrum at intensities near the limit of seeing. This conclusion is strengthened by our earlier demonstrations (Powers and Easter, 1978) that (1) more than one visual pigment absorption spectrum is needed to match the spectral sensitivity at absolute threshold, and (2) the long wavelength portion of the threshold spectral sensitivity curve can be adapted without reducing the peak sensitivity.

If the two mechanisms are rods and red cones, as seems likely, then there is no obvious reason for the inability to continue discriminating at absolute threshold. A clue may come from human psychophysics, where a small interval between absolute threshold and threshold for color exists even for very long wavelengths (Bouman and Walraven, 1957; Graham and Hsia, 1969). Although the achromatic interval may not occur under all experimental circumstances (see discussion in Marriott, 1976), we have observed it, using ourselves as subjects, under conditions similar to those of our goldfish: long, large flashes of 703 nm in the peripheral retina (see Powers, 1977). If flashes at threshold (or slightly above) that affect red cones do not always appear colored, and flashes that affect rods also are not colored, then the goldfish's inability to tell them apart at threshold is understandable.

Finally, we turn to an attempt to reconcile our finding that the goldfish can discriminate wavelengths

using its rods and red cones with the fact that the messages these two receptors send to ganglion cells are similar (Raynauld, 1969; 1972; Adams and Afanador, 1972; Beauchamp and Daw, 1972). We treat this problem in two ways. First, we argue that it is advantageous to this animal to have rods and red cones affect the ganglion cells similarly; and second, we suggest that the signals might differ in a subtle way as yet unobserved by electrophysiologists.

Current theories of underwater visibility (Lythgoe, 1968, 1975; Easter, 1975) contend that contrast is highest at those wavelengths where the underwater space light is least (i.e. in the long wavelengths). If only the rods were active at low levels of illumination, the band of detectable wavelengths would be narrow, excluding much of the longer wavelength, higher contrast light. We have shown, however, that the goldfish is more sensitive to long wavelength lights while dark adapted than would be possible if only its rods were active (Powers and Easter, 1978). We would argue that this extra sensitivity to long wavelength light serves to increase the width of the spectral window in a manner that favors contrast detection. If contrast is an important feature in the fish's environment, then having the ganglion cells respond in a similar way regardless of which receptor is stimulated would be advantageous; the fish could gain information from a wide spectral range without unnecessary concern about wavelength.

Despite the apparent ecological advantages of and the actual electrophysiological evidence for similarity between rod and red cone initiated signals in ganglion cells, we have shown that they cannot be identical because goldfish can discriminate between them. Raynauld's (1969, 1972) original finding, upon which our early generalization experiments were based (Powers and Easter, 1975), was that the *quality* of input from rods and red cones to ganglion cells was the same: if the cell was excited by rod input, it was also excited by red cone input. We suggest that the discrimination reported here results from *quantitative* differences in the signals from rods and red cones, perhaps in the time-course of a ganglion cell's discharge produced by each (cf. Gouras and Link, 1966, in monkey retina). Such differences are almost certainly small (because ΔR was small, and could not be improved, close to threshold), and thus could easily have been overlooked in earlier electrophysiological studies.

To summarize, we believe that the behavior and electrophysiology are not inconsistent with one another. Rods and red cones affect the ganglion cells similarly, but probably not identically, and we suggest that the small differences can be used by the animal to discriminate wavelengths. Although we have suggested that the time course of the ganglion cell discharge could be the difference in question, this remains to be demonstrated.

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