Weak stimulus generalization using sinusoidal gratings: A cautionary note in animal psychophysics

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Psychophysical techniques involving operant and classical conditioning are used commonly to determine the visual thresholds of nonverbal subjects. Typically, subjects are conditioned to respond to a particular stimulus, and once trained sufficiently, the subject's sensitivity to this and similar stimuli can be determined by decreasing the stimulus intensity until the animal no longer responds. However, this assumes that the animal's tendency to respond correlates with its sensory abilities. Stimulus generalization to sinusoidal gratings of different spatial frequencies was examined in goldfish. Subjects were classically conditioned to suppress respiration upon presentation of a sinusoidal grating. Animals' learning curves to each spatial frequency grating were compared in order to determine whether the animals generalized across stimuli. Results indicate that fish show weak stimulus generalization across spatial frequencies. Also, the shape of the contrast sensitivity function, a common measure of the animal's visual capabilities, is distorted by insufficient training. We conclude that when the goal is to obtain accurate data on visual capabilities, either subjects should be trained to each stimulus to be tested or their generalization gradients should be measured.

Behavioral conditioning techniques are valuable in visual psychophysics because they provide a means by which nonverbal subjects can communicate with experimenters. In most experimental paradigms, a subject first learns to respond in some manner when a particular stimulus is presented. After the response probability reaches some reliable level, stimuli that differ from the training stimulus along a dimension of interest are presented, and the probability of response is assessed for each stimulus. In the spatial domain, test stimuli may vary in spatial frequency, contrast, or drift rate.

Conditioning techniques have been used to examine the limits of spatial vision in several species, including primates (De Valois, Morgan, & Snodderly, 1974), cat (Pasternak & Merigan, 1981), and goldfish (Bilotta & Powers, 1991; Northmore & Dvorak, 1979). Typically, animals are conditioned initially to respond to a high-contrast, sinusoidal grating of a particular spatial frequency well above the resolving capabilities of the animal's visual system. After training is complete, and the animal responds at a relatively high rate to the training stimulus, sensitivity is determined by manipulating stimulus contrast until the animal no longer responds to the

stimulus. This procedure is repeated with gratings of different spatial frequencies, and the resultant thresholds are used to estimate the animal's contrast sensitivity function (CSF).

Typically, psychophysical studies rely, to some degree, on the phenomenon of stimulus generalization: An animal that has learned to respond to one stimulus will respond (within the limits of its visual system) when similar stimuli are presented. A potential danger with these procedures is to assume that what constitute "similar" stimuli to the human experimenter also are "similar" to the nonhuman subject. If this assumption is incorrect, then measures of the animal's visual ability will be confounded with the animal's ability to generalize across that particular stimulus dimension. This paper examines the implicit assumption that training on one stimulus pattern renders a subject immediately aware that the same response should be given to other, similar stimuli. We demonstrate that this assumption is unwarranted for goldfish trained in a classical conditioning paradigm. The results suggest that if the generalization gradient for the particular stimulus dimension of interest is not known, then subjects should be trained at each stimulus to be tested.

METHOD

Subjects and Apparatus

Four common goldfish (*Carassius auratus*; Ozark Fisheries, Stoutland, MO), measuring 8–10 cm in length, were maintained on a 12-h light:dark cycle throughout the experiments. A complete description of the apparatus appears elsewhere (Bilotta & Powers, 1991). Briefly, the fish were restrained in a Plexiglas box suspended from a glass aquarium. Respiration was monitored with a thermis-

This work was supported by NIH Eye Institute Grants F32-EY06088 (J.B.) and K04-EY00246 (M.K.P.). We thank R. Blake, A. B. Bonds, R. Fox, J. H. Kaas, and J. S. Lappin for generous loans of equipment. We also thank Francesca Lynd for her help in some of the data collection and Elizabeth A. Lemerise, Paul J. DeMarco, and Francesca Lynd for their comments on this manuscript. Correspondence should be addressed to J. Bilotta, Department of Psychology, Western Kentucky University, Bowling Green, KY 42101.

tor placed in front of the fish's mouth; the voltage change across the thermistor was recorded on an oscilloscope (Tektronix 5113) and observed by an experimenter. Spatially modulated sinusoidal gratings were produced by an image generator (Innisfree, Picasso) and were displayed on a high-resolution oscilloscope (Tektronix 608; P31 phosphor) at a viewing distance of 18 cm and a mean luminance of 10 cd/m². Stimulus contrast was defined as (maximum luminance - minimum luminance)/(maximum luminance + minimum luminance).

Procedures

The animals were first placed into the test aquarium for two to four habituation sessions (20 min), during which the right eye was exposed to the display oscilloscope with no grating present (*uniform field*). Following habituation, the fish were classically conditioned to suppress respiration when the uniform field changed to a vertical 0.10 cycles/degree (cpd) sinusoidal grating of 50% contrast drifting in a posterior-to-anterior direction at 1 Hz (the conditioned stimulus, or CS). Grating duration was 5 sec; 50 msec after the uniform field reappeared, the fish received a mild tailshock (the unconditioned stimulus, or UCS; 50 msec, 5–10 V ac). Both the stimulus generator and the shock were controlled by a computer (Data Translation 11/23). The interval between grating presentations was about 1 min. Baseline respiration measurements were obtained between stimulus presentations by monitoring fish respiration for 5 sec while the uniform field was present.

Training. Daily training sessions with the 0.10-cpd grating consisted of 20 trials each. A *response* was defined as a reduction in the number of breaths during the stimulus interval to one half (or less) the number of breaths during the previous baseline interval. The animal was considered *trained* at a particular spatial frequency when at least 80% of the trials per session reached the response criterion for two consecutive sessions (see Powers & Easter, 1978).

Generalization. Once the animal was trained to the 0.10-cpd stimulus, sinusoidal gratings of different spatial frequencies were presented, always at 50% contrast and drifting at 1 Hz. For these sessions, percent response was computed for blocks of 10 trials in two sessions per day. Sessions were continued until the fish responded on 8 out of 10 trials for two consecutive sessions. The order of presentation of the various spatial frequencies to trained animals was: 0.40, 0.80, 0.20, and 0.05 cpd. It should be mentioned that the *generalization* procedures used in this study differ from typical generalization methods (i.e., the UCS typically is not presented during generalization trials); the procedures described here were intended to examine generalization as well as to train animals to the new stimuli.

Contrast sensitivity functions. For 1 subject, CSFs were derived by means of an observer-based two-alternative forced-choice procedure (see Bilotta & Powers, 1991). In this procedure, a human observer monitored the respiration patterns of the fish during two 5-sec intervals. During one interval, the sinusoidal grating was presented; during the other interval, the uniform field was displayed. The observer decided which interval contained the stimulus grating solely on the basis of the fish's pattern of respiration. The computer determined which temporal interval would contain the stimulus grating and provided feedback to the observer following his/her decision. As in the training and generalization procedures, the UCS was delivered following every CS (Bilotta & Powers, 1991; Powers & Easter, 1978). A 20-sec interstimulus interval was used to ensure that the respiration rate returned to normal prior to the second interval in the event that the first interval contained the stimulus and, thus, the UCS. The unconditioned response to the UCS was not displayed to the observer. During CSF measurements, contrast varied in a staircase procedure (Bilotta & Powers, 1991) starting at 50%; contrast steps were 0.3 log units. The order of spatial frequencies for measuring the CSFs was 0.10, 0.40, 1.60, 0.80, 0.20, and 0.05 cpd. Contrast thresholds (75% correct) and standard devi-

ations were derived for each spatial frequency stimulus using the method of Dixon and Mood (1948; see Bassi & Powers, 1986).

RESULTS

Goldfish Do Not Generalize Across Spatial Frequencies

Figure 1 shows the learning curves of 2 fish (B10 and B12) at 5 spatial frequencies. Following training to the 0.10-cpd stimulus (open circles), the animals did not generalize to the 0.40-cpd stimulus (triangles). In fact, the percent response for the first session with the new spatial frequency was similar to the initial sessions with the 0.10-cpd stimulus.

There are two important points regarding this figure. First, the data point represented by the filled circle was obtained with a 0.10-cpd stimulus on the same day, just prior to the first session for the 0.40-cpd stimulus. Therefore, the difference in percent response between the final

Figure 1. The open circles show learning curves during training for 2 fish (B10 and B12) to a 0.10-cpd stimulus (see text for details). Subsequent presentation of other spatial frequency gratings (triangles = 0.40 cpd; squares = 0.80 cpd) revealed poor generalization of responding. However, once the animals became conditioned at 0.10, 0.40, and 0.80 cpd, they did generalize to gratings of 0.20 cpd (inverted triangles) and 0.05 cpd (diamonds). Open symbols show data from 20-trial sessions, and closed symbols represent 10-trial sessions. The filled circle represents a 10-trial refresher session to the 0.10-cpd stimulus just prior to generalization testing with the 0.40-cpd stimulus.

session with the 0.10-cpd stimulus and the first session with the 0.40-cpd stimulus is unlikely to be due to "forgetting" between sessions. Also, the difference is unlikely to be due to a "fatigue" factor because the total number of trials of the two sessions (10 trials with the 0.10-cpd stimulus and 10 trials with the 0.40-cpd stimulus) was the same as in the original 20-trial training sessions.

The second point is that the 0.10- and 0.40-cpd stimuli should have been equally visible to the animals because the contrast sensitivity of well-trained goldfish to these two spatial frequencies is similar at the drift rate used here (Bilotta & Powers, 1991). Thus, there was no apparent difference in sensory input between these two stimuli—the animals just did not generalize across them.

A similar phenomenon occurred when stimulus spatial frequency was changed from 0.40 cpd to 0.80 cpd. The percent response was at least as low as for the 0.10-cpd or 0.40-cpd stimuli when they were first presented. Contrast sensitivity of highly trained goldfish to 0.80-cpd stimuli is lower than to either 0.10- or 0.40-cpd stimuli (Bilotta & Powers, 1991), but the steepness of the learning curve and the lack of generalization did not seem to be affected by this difference. Subsequent presentation of 0.20- and 0.05-cpd stimuli did produce generalization; that is, the

animals responded at criterion without any additional training to these spatial frequencies. The 3rd fish gave similar results.

Weak Generalization Distorts the CSF

The effects of this failure to generalize on contrast sensitivity measurements are shown in Figure 2A. The inset in Figure 2 shows the training sessions for Subject B08. This fish was trained in the same way as the previous animals (Figure 1). However, after it reached response criterion to the 0.10-cpd stimulus (i.e., following Session 3), contrast sensitivity was measured (Figure 2A). The resulting CSF was atypical for goldfish (Bilotta & Powers, 1991; Northmore & Dvorak, 1979). The function had a peak contrast sensitivity at a spatial frequency of 0.05 cpd instead of 0.20 cpd. It is interesting to note that the variability in sensitivity (as shown by the error bars) for the first three spatial frequencies tested (0.10, 0.40, and 1.60 cpd) was much larger than that for the last three spatial frequencies (0.80, 0.20, and 0.05 cpd).

After obtaining the CSF shown in Figure 2A, training was resumed for Subject B08 starting with a 10-trial refresher to the 0.10-cpd stimulus (Figure 2 inset, Session 4). With one exception, the fish responded during

Figure 2. Inset: Training (open circles) and generalization (closed symbols) sessions of another subject (B08). Conventions are the same as in Figure 1, with the exception that following Training Session 3, contrast thresholds for six spatial frequencies were obtained (see Panel B of this figure). Generalization sessions then began at Session 4. Panel A: Contrast sensitivity function of Subject B08 following training to a 0.10-cpd stimulus. Log contrast sensitivity is defined as: $\log(1/\text{threshold contrast})$. Error bars are ± 1 SEM. Panel B: Contrast sensitivity of Subject B08, as measured in three separate sessions following the complete generalization sequence (following Session 13). The CSFs are similar to one another and comparable to those reported for overtrained fish by Bilotta and Powers (1991).

all successive sessions at response criterion without requiring retraining. That is, following the determination of the CSF, in which the subject was exposed to CS-UCS pairings at every spatial frequency, it responded at criterion across all frequencies.

Following the complete training sessions for Subject B08 (inset Figure 2, Session 13), CSFs were remeasured on 3 separate days (Figure 2B). The CSFs following the complete training sessions were quite similar across the three sets of measurements and are similar to typical CSFs of highly trained animals to drifting gratings of 1 Hz at a mean luminance of 10 cd/m² (Bilotta & Powers, 1991). Also note that contrast sensitivity to the 0.10- and 0.40-cpd stimuli was similar (see above). However, the three functions were quite different from the contrast sensitivity values of the *same* fish prior to training at all spatial frequencies, with the exception of contrast sensitivity to 0.10 cpd—the original training stimulus.

DISCUSSION

These results suggest that generalization does not occur readily across spatial frequencies in goldfish. Careful investigations in other domains have shown that generalization declines as the test stimulus moves away from the training stimulus along some dimension (see Mackintosh, 1974). In the context of the present study, we would speculate that the goldfish would in fact generalize more readily to stimuli that are similar to the initial training stimulus spatial frequency of 0.10 cpd (such as the 0.05- and 0.20-cpd stimuli) than to stimuli farther away from the training stimulus on the spatial frequency axis. Our results suggest that the 0.40- and 0.80-cpd stimuli may fall outside the generalization gradient for a training stimulus of 0.10 cpd, since prior training to the 0.10-cpd stimulus did not expedite learning for these stimuli. This did not appear to be the case for the 0.05- and 0.20-cpd stimuli since prior training did influence learning; perhaps these stimuli fall within the generalization gradient of the 0.10-cpd stimulus. (We note, however, that order effects also may account for the generalization to the lower spatial frequency stimuli because they were always presented last.)

Most importantly for visual studies, we have shown that weak stimulus generalization in the spatial frequency domain can dramatically alter the shape of CSFs and contribute to the variability of sensitivity estimates. High intersession variability in goldfish contrast sensitivity measurements has been reported when animals were trained at only one spatial frequency (Northmore & Dvorak, 1979).

Although one may suppose that weak generalization is found only in lower vertebrates, it is not. Studies of contrast sensitivity with other species have reported this phenomenon. For example, with sinusoidal gratings as stimuli, large training effects have been shown in monkeys (De Valois et al., 1974).

The present study has shown that weak stimulus generalization can affect contrast sensitivity measurements; however, we have demonstrated that these effects can be

eliminated by additional training. Training should continue with high-contrast gratings until a high response criterion is attained at most (preferably all) spatial frequencies to be tested. This strategy yields consistent contrast sensitivity values that are comparable to values reported in other studies (e.g., Bilotta & Powers, 1991). An additional precaution would be to train animals at all spatial frequencies at a number of stimulus contrasts (e.g., De Valois et al., 1974). However, with this method, you must already have some idea of the CSF to prevent consistent training at stimuli that are below threshold. Also, for the respiration suppression technique, it would be difficult to decide on a response criterion (such as the one-half suppression rate, see above) since respiration rate is affected only by high-contrast stimuli (Bilotta & Powers, 1991; Northmore & Dvorak, 1979). Our study shows that training with high-contrast stimuli avoids these problems and produces reliable contrast sensitivity measurements.

Weak generalization across test stimuli may not be as serious a problem with other types of visual stimuli, such as spots of light on a dark background. The animal may generalize across wavelengths of light and discriminate them from a no-light interval. (However, there is evidence to suggest that weak stimulus generalization may occur across this stimulus domain as well; see Bilotta, Kesavan, & Powers, 1990.) For grating stimuli, the animal must generalize across "stripe" sizes and discriminate them from a uniform field of the same mean luminance. This may represent a more unusual learning task. It would be interesting to examine generalization in other stimulus domains to investigate whether or not similar results would be found and to determine the generalization gradients for various stimulus dimensions. Until experiments of this nature are performed, the best strategy is to train your subjects well.

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(Manuscript received September 4, 1990;
revision accepted for publication July 10, 1992.)