

*AUDITORY FREQUENCY GENERALIZATION
IN THE GOLDFISH
(CARASSIUS AURATUS)*¹

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Auditory frequency generalization in the goldfish was studied at five points within the best hearing range through the use of classical respiratory conditioning. Each experimental group received single-stimulus conditioning sessions at one of five stimulus frequencies (100, 200, 400, 800, and 1600 Hz), and were subsequently tested for generalization at eight neighboring frequencies. All stimuli were presented 30 db above absolute threshold. Significant generalization decrements were found for all subjects. For the subjects conditioned in the range between 100 and 800 Hz, a nearly complete failure to generalize was found at one octave above and below the training frequency. The subjects conditioned at 1600 Hz produced relatively more flat gradients between 900 and 2000 Hz. The widths of the generalization gradients, expressed in Hz, increased as a power function of frequency with a slope greater than one.

The stimulus generalization gradient has been a powerful dependent variable for the quantitative study of perceptual processes in animals, especially vision in the pigeon (Guttman, 1956; Blough, 1961; Thomas, 1969). Stimulus generalization methods have also been valuable in investigations of visual processes in the goldfish where the problems of color vision (McCleary and Bernstein, 1959; Yarczower and Bitterman, 1965) and pattern recognition (Sutherland, 1969) have been analyzed with some success. In a pilot attempt to extend the stimulus generalization paradigm to the problems of auditory perception in the goldfish (Fay, 1969*a*), it was found that single stimulus classical respiratory conditioning procedures yielded steep frequency generalization gradients around a 40-Hz training stimulus. Recent psychophysical studies of auditory frequency discrimination (Jacobs and Tavalga, 1968; Fay, 1970*a, b*) have also shown a remarkable degree of differential sensitivity across a wide frequency range which would not be predicted from what is known about the goldfish's rather crude otolithic ear (von Frisch, 1938).

These data suggest that the auditory modality is an important one for the goldfish and that the frequency dimension, in particular, can be used to control behavior quite selectively. The present experiment was designed to study the auditory frequency generalization behavior of the goldfish in greater detail. Five groups of four goldfish were classically conditioned to tones of various frequencies within their best hearing range and immediately tested for generalization at neighboring frequencies.

METHOD

Subjects

Twenty experimentally naive common goldfish (*Carassius auratus*), about 150 to 180 mm long, were maintained in four large aquaria that were continuously supplied with filtered and aerated water.

Apparatus and Conditioning Methods

The conditioning methods have been described in detail elsewhere (Fay, 1969*a, b*; Fay and MacKinnon, 1969). Briefly, complete inhibition of respiration in goldfish is an unconditioned response to electric shock. This response can be conditioned to a neutral auditory stimulus within 10 delay conditioning trials. Recording respiration rate was accomplished by means of a nylon thread connecting the lower lip of the restrained fish to a sensitive leaf-switch.

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A conditioning trial consisted of the presentation of a 6.3-sec tone and a 0.5-sec shock (2 msec square pulses repeated at 40 pulses per sec) that terminated with the tone. The degree of conditioned respiratory suppression was expressed as the number of respiratory mouth movements occurring during the 6.3-sec stimulus presentation divided by the sum of the number of mouth movements occurring 10 sec before and during the stimulus presentation. The unequal measurement intervals before and during the stimulus presentation allowed this suppression ratio to vary between 0 and about 0.4. Thus, a suppression ratio of 0 indicates that no respiratory mouth movements occurred during the stimulus (the numerator of the suppression ratio is 0 in this case). A suppression ratio of 0.39 indicates that the respiration rate during the stimulus did not differ from the rate as measured 10 sec before the stimulus onset (the suppression ratio is equal to the ratio 6.3/16.3).

Shock was delivered across the caudal portion of the fish by two wire screen electrodes. A shock intensity was chosen that elicited a 5- to 10-sec unconditioned inhibition of respiration. Due to the tendency for the unconditioned response to habituate, shock levels were adjusted as necessary throughout the training sessions.

The sound stimuli were generated by a Philco 301-A wave analyzer, electronically switched (rise and fall times were 0.25 sec), attenuated, amplified, and transduced by a 10 in. (25 cm) acoustic suspension loudspeaker. The test tank was a glass cylinder, 9 in. (23 cm) high and 9 in. (23 cm) in diameter, with a lucite bottom to which the fish restrainer and the shock electrodes could be attached. The loudspeaker was mounted facing inside the top of a plywood box, 12 in. (30.5 cm) on a side. A hole, 9.5 in. (23.5 cm) in diameter, was cut in the bottom of the box through which the top of the cylindrical test tank was inserted. The test tank was filled to a height of 7 in. (18 cm) so that the water surface was flush with the inside bottom surface of the box. This method for producing underwater sound presumably minimizes the nearfield effect normally produced by underwater sound projectors (van Bergeijk, 1967). The test tank and box were supported on steel rails inside a sound-attenuated, anechoic chamber.

For sound pressure calibration, the output

of an Atlantic LC-10 calibrated hydrophone was preamplified by 20 db and measured by the wave analyzer. Several measurements were made at each stimulus frequency with the hydrophone in various orientations within the fish restrainer. On the basis of audiograms determined previously for four goldfish in the same apparatus (Fay, 1969b), intensity levels were determined so that each stimulus would be presented 30 db above threshold. On the basis of inter-subject threshold variability, the error in determining equal sensation levels was probably no greater than ± 5 db.

Initial Conditioning Procedure

The 20 subjects were divided randomly into five groups of four fish so that each group was trained at only one of five frequencies (100, 200, 400, 800, and 1600 Hz). Before training, there were several trials in which the unconditioned stimulus was presented alone in order to determine optimal shock levels. Training then began immediately. Each fish received conditioning trials until five successive conditioned responses occurred (suppression ratio less than 0.2). All subjects reached this criterion within 25 trials. In addition, each subject received 40 more conditioning trials. The intertrial interval varied randomly from 1 to 3 min and averaged 2 min.

Generalization Testing Procedure

Immediately following the 40 additional conditioning trials, the generalization tests were run. For each experimental group, eight test frequencies were chosen such that four

Table 1
Generalization Test Stimuli for Each Group

<i>Group Training Frequency</i>	<i>Test Frequencies (Hz)</i>								
Pilot (40 Hz)	20	25	30	35	40	45	50	60	70
1 (100 Hz)	40	50	70	85	100	125	150	175	200
2 (200 Hz)	100	125	150	175	200	250	300	350	400
3 (400 Hz)	200	250	300	350	400	500	600	700	800
4 (800 Hz)	400	500	600	700	800	900	1100	1250	1600
5 (1600 Hz)	800	900	1100	1250	1600	1800	2000		

were above and four were below the training frequency (Table 1). The extreme test frequencies were located at one octave above and below the training frequency, and all stimuli were spaced at approximately equal logarithmic intervals. For the group trained at 1600 Hz, only two test tones were presented above the training frequency because stimuli of sufficient intensity could not be generated above 2000 Hz. The order of presentation of the eight test tones for each group was randomized,

and this random series was presented four times to each subject without shock. In order to minimize suppression decrements due to extinction, every fourth trial involved reinforcement at the original training frequency. The intertrial interval was reduced to 1 min for the generalization tests in order to reduce the total duration of the test session. The initial learning, overtraining, and generalization testing were carried out in one session lasting about 2.5 hr.

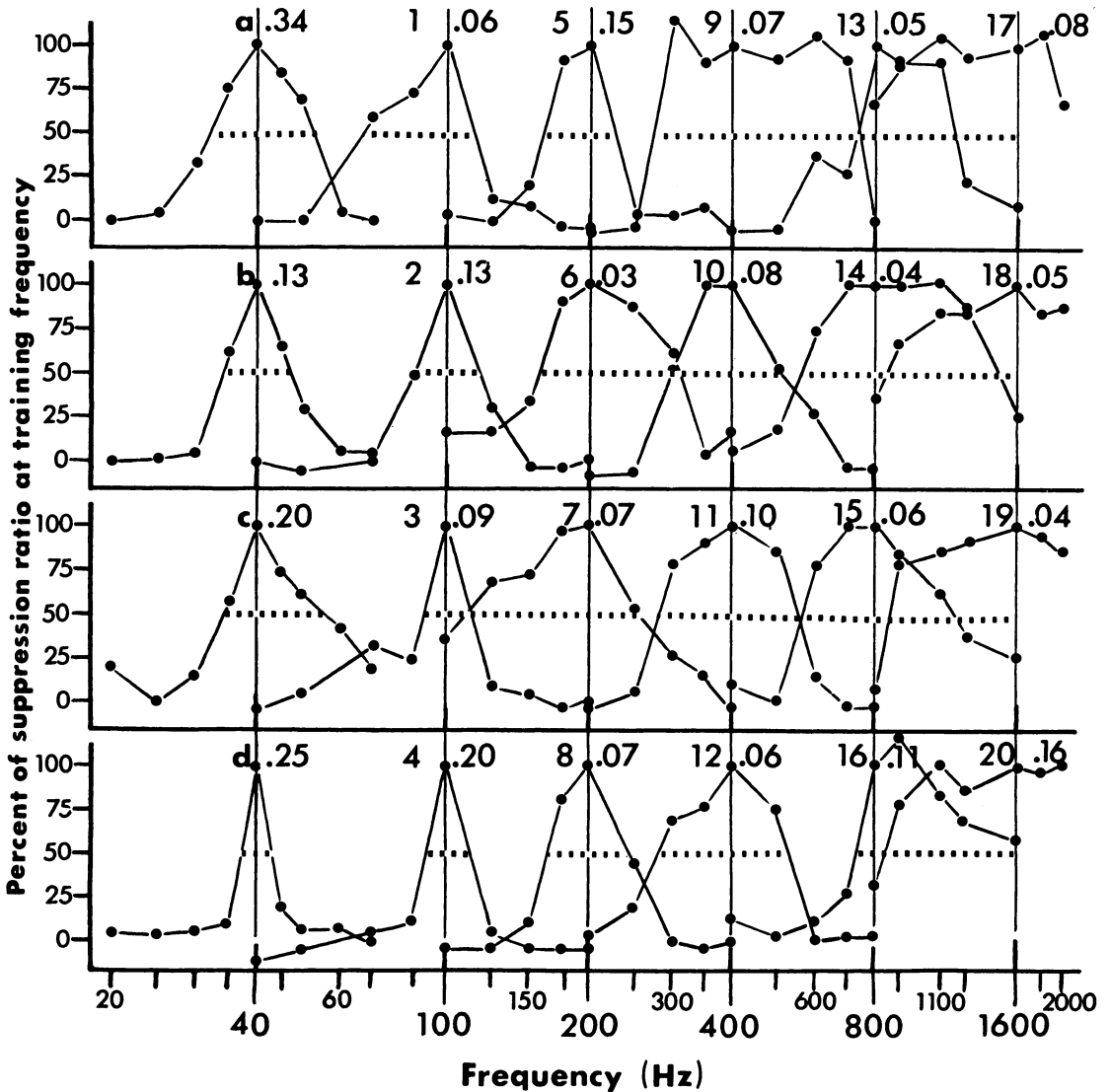


Fig. 1. Individual relative generalization gradients. The numbers to the left at the top of each gradient identify the subjects. The numbers to the right indicate each subject's mean suppression ratio at the training frequency, defined as 100% response strength. The dashed lines indicate the widths of the gradients at 50% response strength. Each point is the mean of four trials.

RESULTS

Suppression ratios were computed for each generalization test trial, including the trials that were reinforced at the training frequency. The mean suppression ratio at the training frequency and at each test frequency were computed for each subject. The test frequency means were then converted to a percentage of the training frequency mean for each subject as follows: the mean suppression ratio at the training frequency was set equal to 100%, and the expected value of the suppression ratio if no respiratory suppression occurred (about 0.39) was set equal to 0%. The mean suppression ratio at each test frequency was then subtracted from 0.39, and the remainder was divided by the range of suppression ratio values between 0.39 and the suppression ratio at the training frequency. This value was then multiplied by 100.

These individual relative generalization gradients are plotted on a logarithmic scale for frequency in Fig. 1. The group mean generalization gradients are plotted on a logarithmic scale for frequency in Fig. 2. Included in these figures are the results for another group of four subjects trained at 40 Hz and tested similarly in a previous pilot study (Fay, 1969a).

For the subjects trained at 40, 100, 200, and 400 Hz, the relative generalization drops to near 0% within one octave above and below the training frequency. The gradients produced by the subjects trained at 1600 Hz are generally less steep with real decrements oc-

curing only at 800 Hz. Although the group mean gradients are generally asymmetrical with steeper slopes toward the lower frequencies, there is some individual variability in this respect. Subject 9, for example, generalized considerably within the 300 to 700 Hz range, but produced rather steep gradients below 300 Hz.

The gradients for subjects C, 3, 5, 6, 8, 15, and 16 suggest some octave generalization but this tendency is not at all robust. Subjects 9, 14, 16, 17, and 20 responded more strongly to one or more of the test tones than to the training tone. There is no systematic tendency for subjects within groups to respond similarly in this respect, however.

Since the generalization tests were not carried out in complete extinction, it is possible that the generalization gradients may have become more pronounced during the course of testing. In order to examine this possibility, mean absolute generalization gradients are plotted for the first and second halves of the generalization tests in Fig. 3. The solid gradients pass through the means for the first half of the test (the first and second presentations of each test tone), and the dashed gradients pass through the means for the second half of the test (the third and fourth presentations of each test tone). There is a slight but consistent tendency for the gradients for the second half of the test to lie below the gradients for the first half. Since the overall shapes of the gradients do not change significantly, however, the differences are probably caused by a

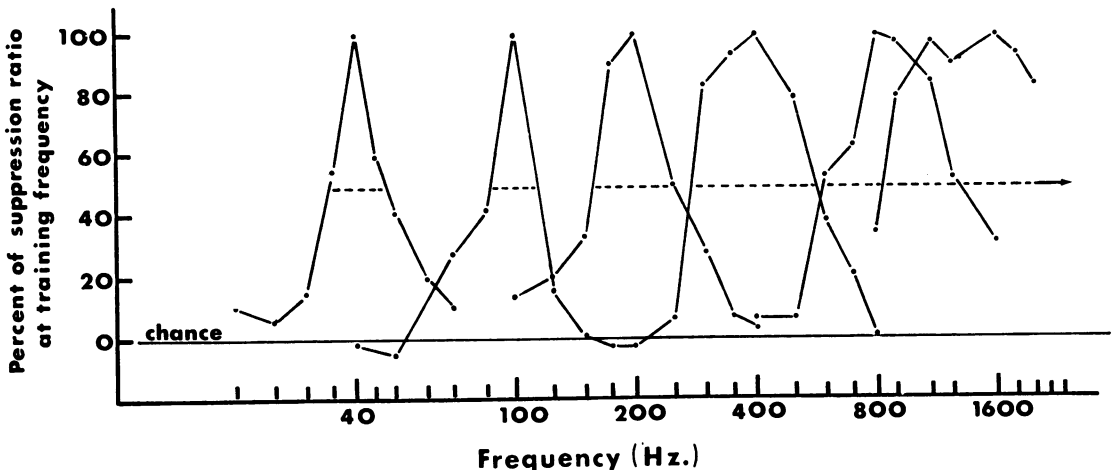


Fig. 2. Mean relative generalization gradients for each group. The dashed line indicate the widths of the gradients at 50% response strength.

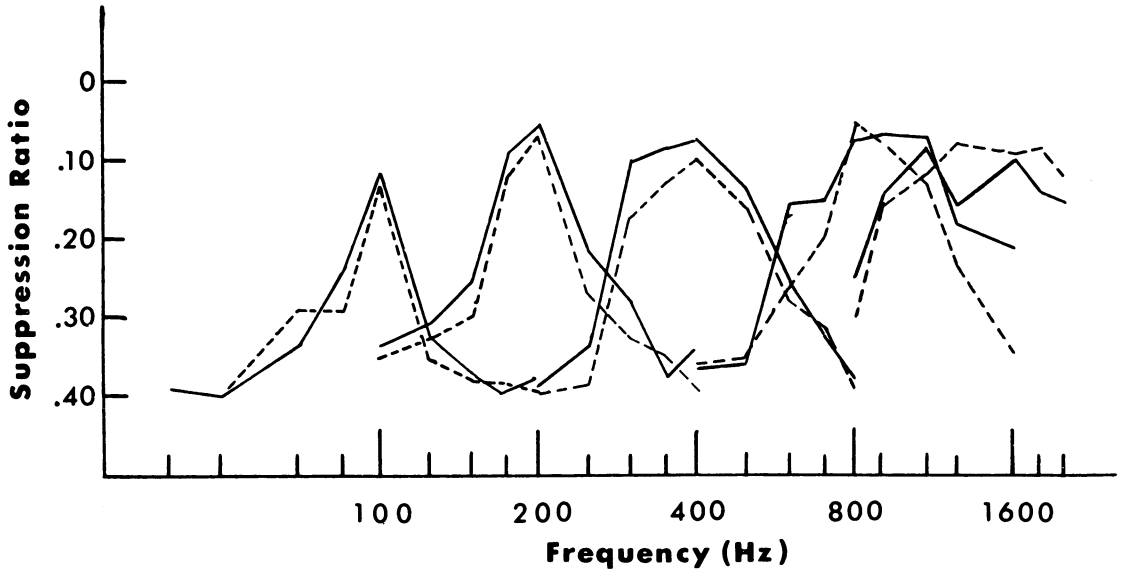


Fig. 3. Group mean absolute generalization gradients for the first (solid lines) and second (dashed lines) halves of the generalization test.

general decline in responsiveness rather than by the differential extinction of responses to the test tones.

The individual relative gradients (Fig. 1), as well as the group mean gradients (Fig. 2), suggest that the absolute amount of generalization is a rapidly increasing function of training frequency. That is, for a given percentage generalization decrement, the widths of the gradients, in Hz, increases systematically with the training frequency. Figure 4 shows the relationship between the training frequency and the interpolated frequency change necessary to produce a 50% response decrement. Note that on both axes, frequency is represented on a logarithmic scale. The filled circles represent the frequency change upward from the training frequency and the squares represent the change downward, for each subject. The two solid lines are linear regression lines that are derived from a logarithmic transformation of the upward and downward generalization points. Using orthogonal polynomials in tests for trend (Winer, 1962), it was found that the variation due to linear trend is 98% of the total between-group variation, and is significant beyond the 0.1% level ($F = 202; 1, 15$ df.) for the upward generalization points. For generalization downwards, the linear trend variation is 95% of the between-group variation, and is significant beyond the 0.1% level ($F = 247; 1, 18$ df.).

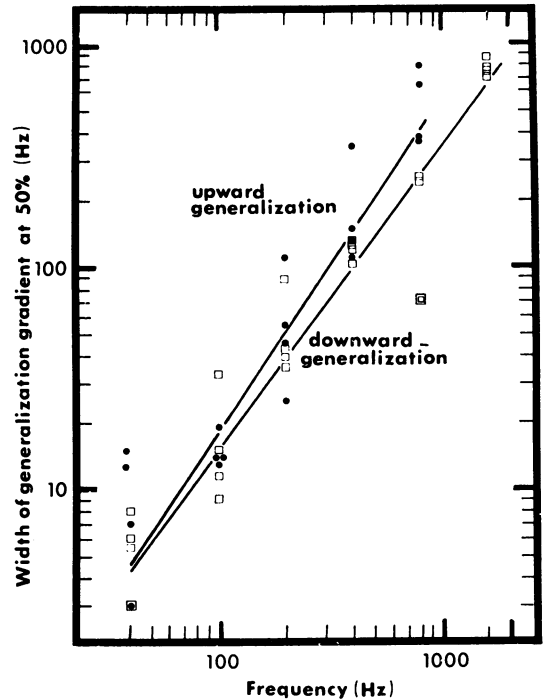


Fig. 4. The widths of individual generalization gradients at 50% response strength as a function of the training frequency. The filled circles and the upper linear regression line represent generalization upward from the training frequency. The squares and the lower regression line represent generalization downward from the training frequency.

The fact that the logarithmically transformed data are fairly well described by straight lines suggests that the relation between frequency and the amount of generalization is a power function. That is, equal frequency ratios correspond to equal ratios of generalization (in Hz). The exponents (slopes) of the upward and downward generalization lines are 1.48 and 1.35, respectively.

DISCUSSION

The well defined stimulus generalization gradients for the subjects trained at frequencies up to 800 Hz indicate the remarkable degree to which the conditioned respiratory suppression response comes under the control of sound frequency, at least for the frequencies below 1000 Hz. The conditioning and testing procedures used in this study would actually appear to reduce, rather than to increase, the probability of significant generalization decrements. Each subject had a very limited experience with pure tones, consisting entirely of the 1-hr conditioning session immediately preceding the generalization test. In addition, the generalization tests were short, each stimulus being presented only four times. In auditory stimulus generalization tests with pigeons, in which the response is conditioned suppression of pecking (Hoffman, 1969), significant generalization decrements typically appear only after several test sessions, consisting of many responses. The group data for the goldfish, on the other hand, reveal striking and consistent absolute generalization decrements after only two presentations of each test tone (Fig. 3).

Perhaps the most basic question here, then, is why generalization failed to occur to any appreciable extent. The data indicate that while the goldfish is conditioned to respond at the onset of a brief tonal stimulus, it is the frequency of the tone, not the presence or absence of an auditory stimulus, that gains primary control over the animal's response. This can be seen dramatically in Fig. 5 where representative responses of Subject 2 in the pilot study were traced from the original records. Respiration was measured, in this case, by recording the output of a phonograph cartidge that was mechanically attached to the fish's lower lip. For the frequencies of 20, 25, 60, and 70 Hz, there is no indication that the onset of the stimulus elicited any respiratory

suppression. The stimuli at 30, 35, 45, and 50 Hz all elicit varying degrees of respiratory suppression, in terms of both amplitude and rate. In no case, however, is the suppression as immediate or complete as it is to the 40-Hz training tone.

The sharp generalization decrements would not be any more surprising than the fact that goldfish can be trained to make fine auditory frequency discriminations (Fay, 1970a, b) were it not for the differences between the procedures used to study generalization and discrimination. The present training procedures consisted of signalling shock by a change from some minimal level of ambient noise to a tonal stimulus 30 dB above threshold. The subjects had no prior experience with pure tones of any frequency and the ambient noise level was, at the very most, 30 dB below the signal intensity. In the psychophysical discrimination experiment (Fay, 1970a, b) the shock was signalled by a change from a series of tone bursts at one frequency to a brief series of bursts at a slightly different frequency. In both cases, the fish is conditioned to discriminate one sensory pattern from another. How-

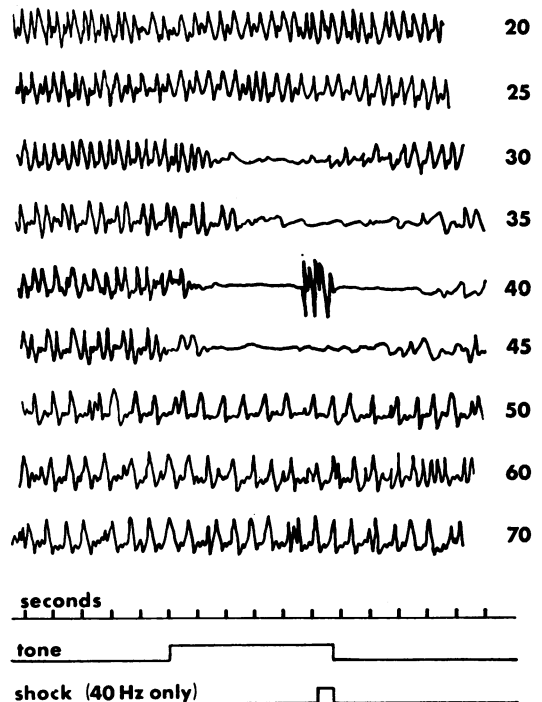


Fig. 5. Representative respiratory suppression responses from Subject 2 (Pilot) during tests for generalization.

ever, the precise nature of the discrimination differs considerably for the two conditioning paradigms. In the psychophysical procedure, the total stimulus energy remained the same since all stimuli were presented 30 dB above threshold, and only the frequency changed. Difference thresholds were determined by gradually reducing the frequency difference between the two tones until the conditioned response disappeared. In the generalization experiment, on the other hand, not only do relative frequency changes take place as a pure tone is added to the ambient noise, but its major change is one of intensity. That is, in physical terms, the conditioned stimulus is an abrupt increase in the signal-to-noise ratio. It is thus surprising that the goldfish responded equivalently in these two situations. That is, both conditioning procedures resulted in the control of the response by stimulus frequency.

As Guttman (1956) has pointed out in regard to light wavelength generalization in the pigeon, steep generalization decrements are less surprising when the sensory system involved contains a topographical representation of the relevant stimulus dimension, as is probably the case with tonal representation in most terrestrial vertebrates. Thus, to identify the response with the activity of particular sensory neurons, and not with others, is a simple conceptual matter and may be adequately explained by Pavlov's well known irradiation hypothesis. This kind of conception fits well with Thompson's (1965) work in which the frequency generalization behavior of cats was successfully predicted from measurements of spatial and functional relations among frequency sensitive neurons in the auditory cortex.

In the goldfish, however, the auditory system is far more undifferentiated and there is no evidence for spatial tonal representation either in the ear (Furukawa and Ishii, 1967) or in the mid-brain (Page, 1970). The afferent neural code for frequency in the goldfish has thus been assumed to be a purely temporal one and this has been supported by the observations of Furukawa and Ishii (1967) that spikes in single saccular nerve fibers are phase-locked to stimulus cycles. Thus, the problem of auditory frequency generalization in the goldfish is similar to the problems raised by metronome frequency generalization, tactile vibratory frequency generalization (Konorski, 1948), and

visual flicker frequency generalization (John, Shimokochi, and Bartlett, 1969). In the more physiological language of the classical conditioning literature, the overlapping neuronal centers constituting the engram may be temporal as well as spatial.

At a more behavioral level, Brown (1965) suggested that stimulus generalization can be viewed much like transfer of training, so that generalization occurs to the extent that two stimuli contain components that are identical, and that lead to equivalent neural events just before the final common pathway. Again, the interesting aspect of the present generalization decrements for the goldfish is that tones of different frequency do not lead to equivalent neural events just before the final common pathway, and that this is true whether or not explicit differential conditioning along the frequency dimension occurred. In Sutherland's (1969) language, the goldfish very readily switches in the frequency analyzer. In fact, the goldfish might be termed rigid in this respect. Switching to Baron's (1965) terminology, auditory frequency appears to be a dimension high in the attending hierarchy for the goldfish. Whatever vocabulary one chooses to deal with the failure to generalize, it is clear that auditory frequency is a stimulus dimension of major importance and substantial informational content for the goldfish. These results suggest, further, that auditory frequency differentiation in the fish may be a rich area of study for other allied disciplines such as neurophysiology, ethology, and ecology.

The additional finding that the relation between frequency and the amount of generalization (in Hz) approximates a power function is difficult to deal with at this point. It is interesting, however, that the psychophysical frequency difference limens (in Hz) as determined by the method described above, approximate two power functions of frequency (Fay, 1970a, b). From 50 to 400 Hz, the exponent of the power function is about 0.7 while between 400 and 1000 Hz, the exponent becomes about 1.35. The difference limens are, however, about 10 times smaller than the amount of generalization as measured in the present experiment. In any case, a power function relationship seems to be a general rule for frequency differentiation in the goldfish, and is most probably due to receptor characteristics and innervation patterns. These phy-

biological problems are treated in more detail elsewhere (Fay, 1970a, b). These basic similarities between the results of the discrimination and generalization experiments further indicate that, in both situations, the goldfish learns essentially the same thing. Of course, the generalization procedures do not require that the goldfish respond differentially to the different stimulus frequencies. Thus, the near equivalence of the two procedures is a psychological one, and this alone is an important datum for the comparative analysis of sensory behavior.

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