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Perception of Frequency, Amplitude and Azimuth of a Vibratory Dipole-Source by the Octavolateralis System of Goldfish (*Carassius auratus*)

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Abstract

Goldfish (*Carassius auratus*) were conditioned to suppress respiration to a 40 Hz vibratory source and subsequently tested for stimulus generalization to frequency, stimulus amplitude and position (azimuth). Animals completely failed to generalize to frequencies separated by octave intervals, both lesser and greater than the CS. However they did appear to generalize weakly to an aerial loudspeaker stimulus of the same frequency (40 Hz) after conditioning with an underwater vibratory source. Animals had a gradually decreasing amount of generalization to amplitude changes, suggesting a perceptual dimension of loudness. Animals generalized largely or completely to the same underwater source presented at a range of source azimuths. When these azimuths were presented at a transect of 3 cm, some animals did show decrements in generalization, while others did not. This suggests that although azimuth may be perceived more saliently at distances closer to a dipole source, perception of position is not immediately salient in conditioned vibratory source detection. Differential responding to test stimuli located towards the head or tail suggests the presence of perceptual differences between sources that are rostral or caudal with respect to the position of the animal or perhaps the head.

Keywords

lateral line; auditory; octave generalization; dipole

Vibratory dipole sources in aquatic environments are inherently multidimensional stimuli (Braun, Coombs & Fay, 2002). Sinusoidal displacements of an object produce both hydrodynamic flow (spatial patterns of fluid motion or potential motion) and oscillating fields of acoustic pressure (Kalmijn, 1988). These sources thus provide proximate stimuli for both lateral line and inner ear sensory channels, respectively (Coombs, 1994; Dailey & Braun, 2009), but it is never clear *a priori* which channel controls behavior when animals

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respond to dipole sources (Braun, Coombs, & Fay, 2002). Further, it is clear that animals may selectively attend to individual aspects of a multi-modal stimulus in different behavioral contexts (Braun & Coombs, 2010).

The proximate stimulus fields for the lateral line and the inner ear differ greatly in terms of spatial properties. With increasing distance from the source, hydrodynamic flow attenuates much more rapidly than pressure (Harris & van Bergeijk, 1962). Thus, close to the source, the hydrodynamic field contains steeper spatial gradients in amplitude and this might allow the lateral line to contribute more information about the position of the source. In contrast, the gas bladder-coupled inner ear depends on a point source that is actually within the body and spatial changes with distance may be less pronounced. The exquisite pressure sensitivity of the goldfish auditory system may instead be put to use determining source amplitude, frequency or temporal envelope. The experiments described below use stimulus generalization techniques to determine what stimulus parameters are most salient to goldfish, to illuminate which stimulus features subjects use in their perception of vibratory sources. In other words, do they attend to location or frequency, or both? Does the perception of azimuth change as a function of distance, with decreasing lateral line stimulation? Is the perception of amplitude distinct from that of distance? Do goldfish possess perceptual dimensions of pitch, loudness, or location in space for this type of source?

The inner ear of goldfish (*Carassius auratus*), like all otophysans, is adapted to detect pressure via mechanical coupling to the gas bladder. This allows for more sensitive hearing (relative to fish without a mechanical linkage) at all frequencies and may also enhance spatial hearing as well if pressure inputs are compared with inertial inputs (Braun & Grande, 2008). Mechanical coupling to a tympanic gas cavity also generally increases the upper frequency limit of fish hearing, but it has recently been demonstrated that pressure sensitivity is also important for conditioned detection of low frequency sources (Dailey & Braun, 2009; Nauroth & Mogdans, 2009).

The mechanosensory lateral line, like the inner ear, is also sensitive to low frequency vibratory sources. Behavioral and physiological measures have shown that the sensitive bandwidth of the goldfish lateral line ranges between < 1 and ~ 200 Hz (Coombs, 1994; Plachta, Mogdans, & Bleckmann, 1999; Weiss & Martini, 1970; reviewed in Coombs & Montgomery, 1999). Kalmijn has also argued forcefully (1988) for the importance of low frequency stimuli for the lateral line. The lateral line, unlike the inner ear, detects vibratory sources by the hydrodynamic flow they create within a relatively small spatial field surrounding the source (Dijkgraaf, 1963; Harris & van Bergeijk, 1962; Kalmijn, 1988, 1989). It is most sensitive to sources within no more than one or two body lengths of the animal (Denton & Gray, 1983; Dijkgraaf, 1963; Gray, 1984; Kalmijn, 1988, 1989). The hydrodynamic fields created by vibratory sources (see below) are spatially complex, and the lateral line is capable of analyzing this spatial (and temporal) pattern of water motions to determine source properties, particularly location in space (Coombs & Conley, 1997a, 1997b; Coombs, Finneran & Conley, 2000; Coombs, Hastings, & Finneran, 1996; Coombs & Patton, 2009; Ćurčić-Blake & van Netten, 2006; Goulet, Engelmann, Chagnaud, Franosch, Suttner, & vanHemmen, 2008; Nelson, MacIver, & Coombs, 2002).

The majority of behavioral studies on the octavolateralis systems have focused on auditory stimulation channels and loudspeaker stimuli. Classical conditioning and stimulus generalization paradigms have been used effectively to demonstrate that goldfish experience perceptual auditory dimensions corresponding to stimulus dimensions of amplitude modulation (Fay, 1972; Fay, Chronopoulos, & Patterson, 1996) and frequency (Fay, 1969, 1970, 1992) as well as complex waveform qualities such as temporal pattern (periodicity-

pitch) (Fay, 1972, 1994, 1995a) and spectral profile (amplitude spectrum shape) (Fay, 1995a, 1998, 2005). These studies have revealed that the sense of hearing in goldfish is qualitatively similar in many ways to that of other high acuity hearing species, including humans. In all of these perceptual studies, stimuli were of relatively high frequencies and delivered with aerial loudspeakers. Thus the hydrodynamic flow was presumably minimal and it may be assumed that the demonstrated perceptual qualities rely on inner ear sensory channels.

In this study, we measured response generalization to determine if goldfish possess perceptual dimensions of pitch, loudness, or location in space for a vibratory dipole source. We used a low frequency underwater dipole source to present potent stimulus fields to both the inner ear and the lateral line systems. The present report describes three generalization experiments. Test stimuli differed from a conditioned stimulus along a single physical dimension; either frequency, stimulus amplitude or position (azimuth). We presented training and test stimuli at a range of azimuthal positions along three different distance transects, using both randomized and fixed signal amplitudes to control for position-dependent differences in amplitude.

General Method

Subjects

All goldfish (*Carassius auratus*) were procured from a commercial fish dealer. Animals were 9 to 11 cm standard length and of unknown sex and age. All animals were housed communally in 151 and 208-liter tanks at a water temperature of 24.5–26.0°C and maintained on a diet of commercial fish food. Experiments were conducted in adherence to the guidelines for animal care and use as prescribed by the Hunter College Institutional Animal Care and Use Committee (Protocol # CBB 1/08-T3, valid February 2002 to January 2009).

Materials

Experiments were performed in a 39.5 cm × 44.5 cm × 15 cm test tank, constructed of .64 cm clear acrylic. This tank rested on a vibration isolation table (Nano-k, Minus k Technology, Inglewood, CA, USA), located in a single wall sound attenuating booth (Industrial Acoustics Corporation, New York, NY, USA) and filled to a water depth of 10 cm. Subjects were restrained in cages to restrict body movement and maintain the location of the animal in a fixed position within the sound field produced by the stimulus. Each cage was constructed of balsa wood, .64 cm nylon mesh and customized to accommodate the body profile of individual animals, permitting movement of the pectoral fins and unrestricted respiration.

Procedure

The cage was attached to the sides of the test tank and suspended 2.5 cm above the arena floor, as measured from the bottom of the cage. The top of the cage was generally 1.5–2.5 cm below the water surface, depending on cage size. Six groups of five animals each were used in three experiments. All animals were tested in darkness and allowed at least 30 minutes to dark-adapt prior to experiments. All animals were naïve with regard to the vibratory dipole source, and each was used in only a single experiment.

Stimulus generation and measurement—A dipole source was constructed by inserting one end of a 15 cm shaft (a 16 gauge Hamilton syringe needle) into an 8mm (in diameter) polyvinyl sphere (the “bead”) and attaching the other end of the shaft to a Brüel & Kjær (Brüel & Kjær, Norcross, GA, USA) minishaker (model 4810). The stimulus signal

was produced by a Tucker Davis Technologies (TDT, Alachua, FL, USA) System 3 digital / analog converter, amplified by a Crown (Crown Audio Inc., Elkhart, IN, USA) D-45 power amplifier. Control of the signal amplitude was achieved using a programmable attenuator (TDT System 3 PA5). The minishaker was situated over the arena and attached to a Uni-slide (Velmex, Bloomfield, NY, USA) motorized positioning system. Training and test stimuli were presented along transects parallel to the lateral surface of the animal's body, with the dipole bead at three transect distances (see Figure 1). In all cases, the elevation of the bead was adjusted to the eye level of the animal. The axis of bead vibration was vertical, perpendicular with respect to the floor of the test tank and parallel to the dorsoventral plane of the animal. In experiment 1, a loudspeaker (RCA PRO 880AV), suspended ~1m above the water surface was also used to present one test stimulus. All conditioning and test stimuli consisted of a five second pulse train, composed of five 500 ms pulses of sinusoidal vibration presented once per second. Pulses were shaped using a 10 ms cosine-squared gate designed to maintain spectral integrity. Stimulus presentation and response measurement were controlled by custom-written scripts using MATLAB R2006a (Mathworks Inc., Natick, MA).

Stimulus amplitude was expressed as SPL (dB re: 1 μ Pa_{RMS}) at the animal. Calibration of stimulus amplitude was via insertion calibration; measured in the absence of the animal. A miniature hydrophone (model 8103 Brüel & Kjær) was used to measure pressure levels in the location of the animal for all stimuli.

Behavioral measures—Responses to the CS were quantified by measuring classically conditioned suppression of respiration. Respiration was measured as a voltage potential between 2 carbon-rod electrodes. One carbon rod was positioned within 1cm of the fish's mouth and another located approximately 10 cm behind the caudal fin. A third (ground) electrode was positioned behind the animal in the near rear corner of the tank. This respiratory potential was digitized and amplified using a TDT Bio-amp (DB4 / HS4). After low pass filtering (10Hz), using a Model 852 Rockland Filter (Victoria, BC, Canada), the signal was digitized, using a TDT RP2.1 at 580 samples / second. Quantifying respiratory activity was accomplished by using the Pythagorean Theorem to estimate the length of this digitized waveform (Fay, 1995b).

Following Fay (1995b), changes in respiration were quantified as a ratio of respiratory activity occurring in the 5 s interval of CS presentation (time B), divided by the sum of respiratory activity in the 5 s interval prior to stimulus presentation (time A) and the measured respiration during time B. [Suppression ratio, $SR = B / (A + B)$]. Values equal to 0.5 indicate unchanged respiration between times A and B. Values less than 0.50 indicate a decrease (suppression) of respiration during the CS and values greater than 0.50 signify increased respiration (see Figure 2).

Respiratory suppression is a graded response allowing the animal's SR to be used as a confidence rating of stimulus similarity (e.g. Fay, 1969, 1970, 1992 1995a, 2005). Low SR values in response to test stimuli represented the subject's estimate of similarity between the CS and test stimuli. The degree of generalization to test stimuli was expressed by dividing the median response to each test stimulus (SR_{TEST}) by the response for the CS presented during the test session. These normalized measures were expressed as percent generalization.

Estimates of ongoing respiratory variability were collected during a catch trial composed of two contiguous 5 s intervals immediately preceding each training or test trial. The SR for each catch trial was used to ensure normal respiration (an SR of 0.5 $\pm$ 0.1) immediately

prior to the trial. If respiration was unstable, the program paused for 30 s and then repeated the catch trial until breathing became more regular before commencing the next test trial.

Training procedure—Animals were conditioned to suppress respiration in response to a fixed-position, fixed-frequency dipole source (the CS). The CS was located at the 0 position (see Figure 1) along one of the three distance transects. This training position (1 cm tailward of the caudal edge of the animal's operculum) corresponds to the location of the anterior chamber of the animal's bipartite gas bladder (Coombs, 1994). In one experiment (see following section), the vibration displacement was randomly varied, but in all other cases, the CS was presented at a single amplitude during training.

The unconditioned stimulus (US) was a single 100 ms AC (60 Hz) electric pulse (3 - 9V_{pk-pk} at the source), generated by an isolated variable transformer, delivered via wire screen electrodes on the tank walls opposite the head and tail of the fish. The US was delivered immediately following the fifth tone pulse of each training trial. Suppression of respiration following the electric pulse was the unconditioned response (UR). The UR was maintained by adjusting US voltage as needed (Fay, 1992). Inter-trial intervals (ITI) averaged 120 s. The criteria for successful acquisition of the CR were 10 consecutive suppressions (i.e. $SR \leq 0.40$) or 16 suppressions in any 20 training trials, whichever came first.

Training and testing conditions—Test sessions were conducted within six days after training. A pretest, using the specific CS, was conducted prior to each session. Subjects were required to show three consecutive presentations of the CS eliciting $SR's \leq 0.40$ to continue to testing. The mean number of trials necessary to meet this criterion was five.

In each experiment, stimulus presentations were organized into blocks of either five or six stimuli. Each block consisted of a series of test stimuli differing along a single dimension, presented in random order. The last stimulus in each block was identical to the CS and delivered with reinforcement. Test stimuli were not reinforced. Test sessions consisted of eight complete blocks of trials with each stimulus presented once per block.

Data Analysis

Raw data (mean absolute SRs) for each experiment were tested for normal distribution using Shapiro-Wilk tests. Differences in response strength between stimuli, within experimental conditions, were then analyzed as appropriate using repeated measures ANOVAs with post hoc Holm-Bonferroni corrected (Aickin & Gensler 1996) paired samples t-tests (experiments 1 and 2) and Friedman's ANOVAs with post hoc corrected Wilcoxon Signed Ranks (experiment 3). All data analysis was conducted using SPSS 13.0.1 for Windows.

Experiment 1: Generalization to Frequency

Method

One group of five animals was trained with the dipole bead located at a distance of 3 cm (see Figure 1), using a fixed stimulus amplitude of 98 dB_{RMS} re 1μPa (as measured at the animal). This corresponds to 22 dB SL (sensation level, i.e. dB above threshold) for the 40 Hz stimulus (Dailey & Braun, 2009) at this distance.

Test sessions were conducted with the stimulus in the training position (see Figure 1). The test stimuli (10, 20, 80, and 160 Hz) were presented in random order and with equal sound pressure levels at the animal (as previously determined by insertion calibration). In addition to these four test stimuli, a fifth test stimulus was presented by loudspeaker, at 40 Hz. The loudspeaker was mounted above the surface of the water. The CS, repeated as the final stimulus of every test block, was delivered by the dipole source. The signal amplitude was

adjusted so that all stimuli were 98 dB_{RMS} re 1 μPa at the position of the subject. The sixth stimulus of every test block was the CS, a 40 Hz dipole stimulus, delivered with reinforcement.

Results

Animals showed a complete failure to generalize to any of the novel test frequencies when delivered by the dipole source (see Figure 3). Animals did not respond or responded only weakly to dipole stimuli at other frequencies. This difference in response strength was significant when comparing the CS and all novel test stimuli (10, 20, 80, 160 Hz dipoles, 40 Hz loudspeaker) ($F(5, 20) = 23.45, p < .001, \eta_p^2 = .85$). Post hoc analysis using paired samples *t*-tests confirmed this difference in response strength between the CS and the novel stimuli (adjusted $p < 0.05, r > .92$ for all test stimuli). Animals exhibited more generalization to the 40 Hz aerial loudspeaker stimulus, showing about 40% of the response strength shown to the CS (see Figure 3).

Experiment 2: Generalization to Stimulus Amplitude

Method

A group of five animals was also conditioned to the dipole bead located at a distance of 3 cm (see Figure 1), using a fixed stimulus amplitude of 98 dB_{RMS} re 1 μPa (~22 dB SL) at the animal's position. This group of animals was tested for generalization to the amplitude of a 40 Hz vibratory dipole. Four test stimuli were used, differing in 3 dB increments around the CS amplitude (i.e. 92, 95, 98, 101 and 104 dB SPL (~16–28 dB SL) (see Table 1). Test stimuli were presented in the same location as the CS.

Results

Relative response strength decreased as a function of increasing difference in amplitude between the CS and test stimuli (see Figure 4). That is, subjects exhibited a modest generalization gradient corresponding to source amplitude. The slope of the generalization gradient was an approximately linear function of the difference in amplitude from the CS. This generalization gradient was approximately symmetrical, in that response strength declined when presented with both higher and lower amplitude test stimuli. Decreases in stimulus amplitude of 3 dB and 6 dB resulted in mean response decrements of 20% and 40% respectively. Increases in stimulus amplitude of 3 dB and 6 dB resulted in response decrements of 10% and 30% respectively. A repeated measures ANOVA indicated that there was a significant effect of stimulus amplitude ($F(4, 16) = 3.49, p = .031, \eta_p^2 = .47$), although an adjusted paired samples *t*-test post hoc analysis failed to detect significant differences between mean absolute SR's for individual test stimuli (i.e. 92, 95, 101, 104 dB SPL) and the training condition.

Experiment 3: Generalization of Source Azimuth

Method

This experiment used four separate groups of five animals to examine the effect of source azimuth when presented at a range of distances (3, 6, or 12 cm). One group, group A was trained and tested with a source at a single fixed amplitude at a transect 3 cm from the lateral body surface. Because the source was vibrated with a fixed amplitude, the pressure magnitude at the fish varied systematically with source azimuth (varying by a similar range as the stimuli in experiment 2). The other three groups were trained with a range of stimulus amplitudes at a fixed location and then tested over a range of source azimuths such that pressure magnitude at the fish was uncorrelated to azimuth. This range of pressure

magnitude at the fish was also similar to the amplitude variation of experiment 2 (see Table 1 and Figure 1).

Group A—Animals in group A were trained using a fixed amplitude source (104 dB SPL, or 28 dB SL at the animal's position) and a transect distance of 3 cm. In this case, stimulus amplitude varied systematically with azimuth (when measured at the approximate position of the gas bladder), decreasing by approximately 4 dB as azimuth is varied from the training position in either direction (see Table 1).

Groups B – D—In groups B – D, the signal amplitude was varied randomly during training to eliminate the use of amplitude cues that accompany changes in azimuth (see Table 1 and Figure 1). Each animal was trained with CS amplitudes varied over twice the range presented during testing. Consequently, we trained the 3 cm group (B) by presenting a CS randomly varying between 92 and 104 dB SPL (16–28 dB SL), the 6 cm group (C) CS varying between 99 and 104 dB SPL (20–25 dB SL), and the 12 cm group (D) CS varying between 100 and 104 dB SPL (19–23 dB SL).

Test sessions were conducted with signal amplitude at the stimulus source held constant (at the midpoint of the training range). During testing the dipole position varied in 2 cm increments along a single transect over an 8 cm range (± 4 cm) centered on and including the training (0) position (see Figure 1). The rationale for this was that animals would be 'instructed to ignore' amplitude differences and thus response differences might be based entirely on perceived position differences. Each transect (B, C, and D) was tested individually to examine the possibility that perception of azimuth differed as a function of distance.

Results

When stimulus amplitude was allowed to vary systematically with azimuth (group A), responses to test stimuli (i.e. source locations -4 , -2 , $+2$, $+4$; see Table 1 and Figure 1) differed significantly from the conditioned stimulus ($\chi^2(4, n=5) = 11.20, p = .024$, Kendall's $W = .56$) (see Figure 5A). However, none of the pairwise comparisons (adjusted Wilcoxon Signed Ranks) were significant. Qualitatively, as the position of the source moved rostral to the gas bladder, three subjects did show a decrement in generalization, but the other two subjects actually responded more strongly than to the CS. Dramatically, positions caudal to the gas bladder resulted in more consistent responding and less generalization than rostral-ward positions or similar amplitude changes.

In contrast, when source amplitude was varied during training and the test stimuli amplitudes adjusted to the middle of that range, no clear gradients in responding were noted (see Figure 5). In all three of these groups, there were no statistically significant differences between test stimuli [3 cm transect ($\chi^2(4, n=5) = 5.60, p > .05$), 6 cm transect ($\chi^2(4, n=5) = 8.48, p > .05$), 12 cm transect ($\chi^2(4, n=5) = 2.72, p > .05$)], indicating that animals generalized completely to all test stimuli. Qualitatively, the 3 cm transect did have the least variability and 3 or 4 animals appear to have slight generalization decrements that correspond with increasing changes in azimuth. But animals largely generalized to all azimuths at all transect distances.

Discussion

These experiments reveal what aspects of a multi-dimensional and potentially multi-modal stimulus are salient to naïve goldfish with a full complement of sensory receptors. Future ablation experiments with these and other stimuli will also be needed to determine how each of these multiple modalities contribute to vibratory source perception. The present results

suggest that the non-spatial stimulus features dominate the conditioned perception of vibratory sources in goldfish (in this experimental setting). In our experiments, goldfish displayed a perceptual dimension of amplitude and were responding was greatly sensitive to stimulus frequency, but changes in azimuth had little salience. The spatial pattern of lateral line excitation is vastly altered by changes in source azimuth (Coombs, Hastings & Finneran 1996), yet animals did not respond differentially to multiple source azimuths when amplitude was removed as a cue. This does not mean that the lateral line does not contribute to dipole source perception, but it suggests that positional information supplied by lateral line inputs are not salient in this behavioral task. This is consistent with the finding that conditioned detection of dipoles depends only on the pressure sensitive inner ear and is unaffected by lateral line inactivation (Dailey and Braun, 2009).

Response similarity and stimulus perception

When an animal's response to the new stimulus is similar to one evoked by a familiar stimulus, the animal may be said to have generalized to the new stimulus (Pavlov, 1927; Mackintosh, 1974). Learned responses are usually characterized by a flexibility that permits the influence of previous experience (Kimble, 1961) because recurring natural events tend to be similar and thus generalization is essential to various forms of perceptual grouping. Further, as sources move through the environment, the physical stimulus at the receiver may be systematically altered and accurate generalization must be important for the consistent perception of sources as they move or otherwise change (i.e. Auditory streams *sensu* Bregman, 1990).

The phenomenon of stimulus generalization can be used to determine what aspects of a stimulus field contribute to perception. A comparison of response magnitude for test versus conditioned stimuli may reveal total generalization to test stimuli (equal response magnitudes), a failure to generalize (no responses to the novel stimulus), or expose a gradient in responding (Guttman & Kalish, 1956; Kimble, 1961; Mackintosh, 1974; Pavlov, 1927). Graded responses to test stimuli can be interpreted as graded confidence levels in the animal's judgment of similarity between test stimuli and a CS (e.g. Fay, 1995a). Response gradients are generally very orderly or systematic in appearance, often characterized by monotonic decrements in responding corresponding to increasing difference(s) between some physical element(s) of the test stimulus and the CS. This has been taken as evidence that the subject experiences a perceptual dimension corresponding to that physical dimension (Guttman & Kalish, 1956; Guttman, 1963; Mackintosh, 1974). Complete generalization is more difficult to interpret without further discrimination testing. Complete generalization (as in the azimuth experiments) does not mean that an animal is incapable of discriminating between the test and conditioned stimulus (Brown, 1965; Mackintosh, 1974; Shepard, 1987), rather that without further instruction, the subject treats these stimuli as equivalent (Kimble, 1961). That is, the similarities of the stimuli are more salient than the differences. On the other hand, a complete absence of generalization (as in the frequency experiment) suggests that the stimuli are perceived as unrelated, but does not demonstrate that the subject cannot perceive any similarities. The ability to discriminate the stimuli however may be assumed.

Fay (1970, 1992) used a stimulus generalization paradigm to explore the perceptual dimension of pitch in goldfish. The resulting response curves in both experiments clearly show gradients in responding characterized by uniform decrements in response strength with increasing differences in frequency between the CS and novel stimuli (using more closely spaced test stimuli than in the present experiments). Fay (1995a) also demonstrated a timbre-like perceptual dimension in goldfish using stimuli of pure tones, harmonic complexes and pulsed signals, delivered by loudspeaker. Both stimulus pulse repetition rate and spectral envelope were salient to the subjects.. As differences between the CS and the

test stimuli increased, response strength decreased. These experiments demonstrate that fish have perceptual dimensions of pitch that relate both to physical frequency and other waveform periodicities (e.g. periodicity pitch).

Goldfish do not generalize to frequencies separated by octave intervals

Test frequencies located at full octave intervals from the CS failed to elicit any notable response from the animals in our experiments. The lack of response to the dipole test stimuli suggests that animals experienced perceptual differences between the CS and the test stimuli. Conversely, responses evoked by the loudspeaker stimulus could be interpreted to indicate that the CS and loudspeaker were perceived as being similar along some dimension; the only invariant in this case being frequency (and pressure amplitude). These two sources have completely different pressure-velocity relationships and spatial properties, which suggests that frequency is a highly salient aspect of octavolateralis perception, even more so than the nature of the source itself (dipole versus loudspeaker) or the relative contributions of multiple submodalities.

The complete failure of goldfish to generalize to frequency in the present experiment appears at first glance to differ from the findings of previous studies that showed evidence for a perceptual dimension similar to human pitch perception in goldfish (Fay, 1969, 1970, 1994, 1995a, 2005). Fay (1969, 1970) showed that goldfish exhibit symmetrical generalization gradients within a single octave. However, the dipole stimuli in the present frequency experiment were separated by one octave intervals, and Fay's test stimuli were located almost exclusively within 1 octave of the CS. His results show that for test stimuli < 30 Hz or > 65 Hz, relative response strength was $\leq 15\%$, and consistent with the present results, test stimuli more than 1 octave away did not elicit responses at all. Fay's (1969, 1970, 1972) finding that goldfish failed (nearly completely) to generalize to frequencies one octave below or above the CS, taken together with our results, suggests that fish frequency perception is similar to humans within an octave (Fay, 1969, 2005), but unlike humans, fish do not generalize across octaves.

Pitch perception and octave generalization in vertebrates

The relative importance of frequency in goldfish octavolateralis function is intriguing because they have relatively few differently tuned channels contributing to peripheral frequency analysis (Furukawa & Ishii, 1967; Fay, 1997). A perceptual dimension corresponding to stimulus frequency is present in many widely-disparate groups [fishes (Fay, 1969, 1970, 1994, 1995a, 2005), birds (MacDougall-Shackleton & Hulse, 1996; Njegovan, Ito, Mewhort, & Weisman, 1995; Cynx, 1993; Page, Hulse, & Cynx, 1989) and non-human primates (Brosch, Selezneva, Bucks, & Scheich, 2004)], it is likely a primitive vertebrate sensory ability (Fay, 1998; Popper & Fay, 1999). Although frequency selectivity is common to all vertebrates studied to date, only one common mechanism has been identified in all of these groups, except mammals (Ashmore, 1991), electrochemical hair cell resonance (Fay & Popper, 2000). Thus if pitch perception is truly a primitive perceptual ability, it may be based on non-homologous peripheral mechanisms or on shared primitive temporal processing mechanisms. An exception to the apparent functional similarity in pitch perception across vertebrates may be the perception of octave equivalence. Some mammalian listeners generalize robustly to frequencies separated by octaves [humans (e.g. Humphreys, 1939; Deutsch, 1972), Rhesus Monkeys (Wright, Rivera, Hulse, Shyan, & Neiworth, 2000; Wright, 2007), rats (Blackwell & Schlosberg, 1943) and dolphins (Richards, Wolz, & Herman, 1984; Ralston, Herman, Williams, Gory, & Jerger, 1988)]. But octave generalization does not appear to be a shared trait among vertebrates. For example, European starlings were found to possess a perceptual dimension corresponding to stimulus frequency but they did not generalize to frequencies that were harmonically related by full

octave intervals (Cynx, 1993). The present results indicate that goldfish also do not generalize in this way. The perception of octave equivalence, the similarity of tones separated by an octave interval, may be a more recent evolutionary feature in the history of vertebrate audition.

Goldfish generalize to stimulus amplitude and azimuth

In the present study, when stimulus frequency and location in space were held constant, and test stimuli varied in amplitude, relative response strength appeared to diminish as a roughly monotonic function of the difference between test stimulus amplitude and that of the CS (see Figure 4). This suggests that all of the test stimuli were perceived as similar to the CS but to varying degrees which depended on their similarity in amplitude. Response decrements between test stimuli and the CS, ranged between 10 – 40% but they were not statistically different. Nonetheless, our results suggest the presence of a loudness-like dimension in the perception of dipole sources by goldfish. Previous studies (Dailey & Braun, 2009) have shown that the inner ear is the primary channel for the detection of dipole sources in goldfish.

Stimuli presented at varying azimuths to animals trained with a CS of randomly varied intensity evoked relatively uniform responses along each of the three transects (3, 6, and 12 cm), indicating that the novel vibratory stimuli were perceived as being very similar to the CS and that source position was not a salient feature of the prior conditioning. When source amplitude was fixed (allowing for differences in perceived loudness), responses could not be explained by loudness differences alone. Rather, responses appeared to be somewhat dichotomous. More rostral test positions evoked responses that were either greater or lesser than the CS, but the response of some animals could be interpreted as a weak gradient of generalization. This gradient was not inconsistent with likely perceived loudness differences. In contrast, as the source moved caudal to the gas bladder, all subjects showed a sharper decrement in generalization without a clear relationship to increasing change in position. This could indicate a perceptual difference between sources rostral or caudal to the fish that is stronger than any corresponding differences in perceived loudness.

When animals were trained with randomly varying stimulus intensity, increasing change in source azimuth elicited slightly weaker responses both for sources that were rostral (headward) and caudal (tailward) to the training position. There was a marginally more obvious gradient in responses when tested along the 3 cm transect (see Figure 5A) as compared to the 6 or 12 cm transects (see Figure 5C). Along the 3 cm transect as the source position was moved towards the tail (–2, –4 cm positions), response strength declined nearly linearly to approximately 70% of the response to the CS. Responses for the group tested at 12 cm (slightly more than one body length from the animal) were generally above 80%. This trend in relative response strength suggests that animals may perceive azimuth more saliently at close range, which is consistent with both stimulus hydrodynamics (which become more uniform at greater distances) and with concepts of minimal audible angles (Mills, 1958). The hydrodynamic fields that stimulate the lateral line system are, of course, also more spatially heterogeneous at close range. In principle, this might allow for more precise perception of space if the lateral line were engaged in this sensory task, but the present results do not show a dramatic effect of distance on the perception of azimuth. More emphatically, there is no statistical difference in the response to any test stimulus in these experiments. That is, goldfish generalized completely (or nearly so) to dipole sources presented over a wide range of azimuths and perceived loudness.

Summary

Goldfish did not generalize to stimulus frequencies separated by octave intervals from the training stimulus. They did generalize to stimuli of the same frequency presented by sources (i.e. dipole and loudspeaker) that create dissimilar stimulus fields, indicating that frequency is a salient octavolateralis stimulus. Additionally, we found that these animals seemed to perceive stimuli of the same frequency that vary along the dimensions of intensity and azimuth to be qualitatively similar, although there is good support for a perceptual dimension of loudness. Azimuth may be perceived more saliently at close range, but is generally not of paramount salience in octavolateralis detection of dipole sources. Experiments such as these may provide useful information concerning how perception of particular stimuli (e.g. vibratory sources) changes with increasing physical distance and which dimensions of a stimulusfield animals attend as location in space changes relative to the animal.

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References

- Aickin M, Gensler H. Adjusting for multiple testing when reporting research results: The Bonferroni vs Holm methods. *American Journal of Public Health*. 1996; 86:726–728. [PubMed: 8629727]
- Ashmore JF. The electrophysiology of hair cells. *Annual Review of Physiology*. 1991; 53:465–476.
- Blackwell HR, Schlosberg H. Octave generalization, pitch discrimination, and loudness thresholds in the white rat. *Journal of Experimental Psychology*. 1943; 33:407–19.
- Braun CB, Coombs S. Vibratory sources as compound stimuli for the octavolateralis systems: Dissection of specific stimulation channels using multiple behavioral approaches. *Journal of Experimental Psychology: Animal Behavior Processes*. 2010; 36:243–257. [PubMed: 20384404]
- Braun CB, Coombs S, Fay RR. What is the nature of multisensory interaction between octavolateralis sub-systems? *Brain, Behavior and Evolution*. 2002; 59:162–176.
- Braun, CB.; Grande, T. Evolution of peripheral mechanisms for the enhancement of sound reception. In: Webb, JA.; Popper, AN.; Fay, RR., editors. *Fish bioacoustics*, Springer handbook of auditory research. Springer-Verlag; New York: 2008. p. 99-144.
- Bregman, AS. *Auditory scene analysis: The perceptual organization of sound*. MIT Press; Cambridge, MA: 1990.
- Brosch M, Selezneva E, Bucks C, Scheich H. Macaque monkeys discriminate pitch relationships. *Cognition*. 2004; 91:259–272. [PubMed: 15168897]
- Brown, JS. Generalization and discrimination. In: Mostofsky, DI., editor. *Stimulus Generalization*. Stanford University Press; Stanford, CA: 1965. p. 7-23.
- Coombs S. Nearfield detection of dipole sources by the goldfish (*Carassius auratus*) and the mottled Sculpin (*Cottus bairdi*). *Journal of Experimental Biology*. 1994; 190:109–129. [PubMed: 7964388]
- Coombs S, Conley RA. Dipole source localization by mottled sculpin. I. Approach strategies. *Journal of Comparative Physiology A*. 1997a; 180:387–399.
- Coombs S, Conley RA. Dipole source localization by the mottled sculpin II. The role of lateral line excitation patterns. *Journal of Comparative Physiology A*. 1997b; 180:401–415.
- Coombs, S.; Montgomery, JC. The enigmatic lateral line system. In: Popper, AN.; Fay, RR., editors. *Comparative hearing: Fish and amphibians*, Springer handbook of auditory research. Springer-Verlag; New York: 1999. p. 319-362.

- Coombs S, Finneran JJ, Conley RA. Hydrodynamic image formation by the peripheral lateral line system of the Lake Michigan mottled sculpin, *Cottus bairdi*. Philosophical Transactions of the Royal Society of London B. 2000; 355:1111–1114.
- Coombs S, Hastings M, Finneran J. Measuring and modeling lateral line excitation patterns to changing dipole source locations. Journal of Comparative Physiology A. 1996; 178:359–371.
- Coombs S, Patton P. Lateral line stimulation patterns and prey orienting behavior in the Lake Michigan mottled sculpin (*Cottus bairdi*). Journal of Comparative Physiology A. 2009; 195:279–297.
- Ćurčić-Blake B, van Netten SM. Source location encoding in the fish lateral line canal. Journal of Experimental Biology. 2006; 209:1548–1559. [PubMed: 16574811]
- Cynx J. Auditory frequency generalization and a failure to find octave generalization in a songbird, the European starling (*Sturnus vulgaris*). Journal of Comparative Psychology. 1993; 107:140–146. [PubMed: 8370267]
- Dailey DD, Braun CB. The detection of pressure fluctuations, sonic audition, is the dominant mode of dipole-source detection in goldfish (*Carassius auratus*). Journal of Experimental Psychology: Animal Behavior Processes. 2009; 35:212–223. [PubMed: 19364230]
- Denton, EJ.; Gray, J. Proceedings of the Royal Society of London. Series B. Vol. 218. 1983. Mechanical factors in the excitation of Clupied lateral lines; p. 1-26.
- Deutsch D. Octave generalization and tune recognition. Perception & Psychophysics. 1972; 11:411–412.
- Dijkgraaf S. The functioning and significance of the lateral-line organs. Biological Reviews. 1963; 38:51–105. [PubMed: 14027866]
- Fay, RR. Auditory sensitivity of the goldfish within the near acoustic field. U.S. Naval Submarine Medical Center, Submarine Base; Groton, CT: 1969. Report No. 605 (1-11)
- Fay RR. Auditory frequency generalization in the goldfish (*Carassius auratus*). Journal of Experimental Analysis of Behavior. 1970; 14:353–360.
- Fay RR. Perception of amplitude-modulated auditory signals by the goldfish. Journal of Acoustical Society of America. 1972; 52:660–666.
- Fay RR. Analytic listening by the goldfish. Hearing Research. 1992; 59:101–107. [PubMed: 1629039]
- Fay RR. Perception of temporal acoustic patterns by the goldfish (*Carassius auratus*). Hearing Research. 1994; 76:158–172. [PubMed: 7928708]
- Fay RR. Perception of spectrally and temporally complex sounds by the goldfish (*Carassius auratus*). Hearing Research. 1995a; 89:146–154. [PubMed: 8600120]
- Fay, RR. Psychoacoustical studies of the sense of hearing in the goldfish using conditioned respiratory suppression. In: Klump, GM.; Dooling, RJ.; Fay, RR.; Stebbins, WC., editors. Methods in comparative psychoacoustics. Birkhäuser Verlag; Basel: 1995b. p. 249-262.
- Fay, RR. Frequency selectivity of saccular afferents of the goldfish revealed by revcor analysis. In: Lewis, ER., et al., editors. Diversity in Auditory Mechanics. World Scientific; Singapore: 1997. p. 69-82.
- Fay RR. Auditory stream segregation in goldfish (*Carassius auratus*). Hearing Research. 1998; 120:69–76. [PubMed: 9667432]
- Fay RR. Perception of pitch by goldfish. Hearing Research. 2005; 205:7–20. [PubMed: 15953511]
- Fay RR, Chronopoulos M, Patterson RD. The sound of a sinusoid: Perception and neural representations in the Goldfish (*Carassius auratus*). Auditory Neuroscience. 1996; 2:377–392.
- Fay RR, Popper AN. Evolution of hearing in vertebrates: The inner ears and processing. Hearing Research. 2000; 149:1–10. [PubMed: 11033242]
- Furukawa T, Ishii Y. Neurophysiological studies on hearing in goldfish. Journal of Neurophysiology. 1967; 30:1377–1403. [PubMed: 6066445]
- Gray J. Interaction of sound pressure and particle acceleration in the excitation of the lateral-line neuromasts of sprats. Proceedings of the Royal Society of London B. 1984; 220:299–325.
- Goulet J, Engelmann J, Chagnaud BP, Franosch JP, Suttner MD, vanHemmen JL. Object localization through the lateral line system of fish: theory and experiment. Journal of Comparative Physiology A. 2008; 194:1–17.

- Guttman, N. Laws of behavior and facts of perception. In: Koch, S., editor. *Psychology: A Study of a Science. Vol 5: The process areas, the person, and some applied fields: Their place in psychology and science.* McGraw-Hill; New York: 1963. p. 114-178.
- Guttman N, Kalish HI. Discriminability and stimulus generalization. *Journal of Experimental Psychology.* 1956; 51:79–88. [PubMed: 13286444]
- Harris GG, van Bergeijk WA. Evidence that the lateral line organ responds to near-field displacements of sound sources in water. *Journal of the Acoustical Society of America.* 1962; 34:1831–1841.
- Humphreys LG. Generalization as a function of method of reinforcement. *Journal of Experimental Psychology.* 1939; 25:361–372.
- Kalish HI. The relationship between discriminability and generalization: A reevaluation. *Journal of Experimental Psychology.* 1958; 55:637–644. [PubMed: 13563782]
- Kalmijn, AJ. Hydrodynamic and acoustic field detection. In: Atema, J.; Fay, RR.; Popper, AN.; Tavolga, WN., editors. *Sensory biology of aquatic animals.* Springer-Verlag; New York: 1988. p. 83-130.
- Kalmijn, AJ. Functional evolution of lateral line and inner ear sensory systems. In: Coombs, S.; Görner, P.; Münz, H., editors. *The mechanosensory lateral line: Neurobiology and evolution.* Springer-Verlag; New York: 1989. p. 197-215.
- Kimble, GA. *Hilgard and Marquis conditioning and learning.* Appleton-Century-Crofts; New York: 1961.
- MacDougall-Shackleton SA, Hulse SH. Concurrent absolute and relative pitch processing by European Starlings (*Sturnus vulgaris*). *Journal of Comparative Psychology.* 1996; 110:139–146.
- Mackintosh, NJ. *The psychology of animal learning.* Academic Press; New York: 1974. p. 484-542.
- Mills AW. On the minimum audible angle. *Journal of the Acoustical Society of America.* 1958; 30:237–246.
- Nauroth I, Mogdans J. Goldfish and Oscars have comparable responsiveness to dipole stimuli. *Naturwissenschaften.* 2009 in press.
- Nelson ME, MacIver MA, Coombs S. Modeling electrosensory and mechanosensory images during the predatory behavior of weakly electric fish. *Brain, Behavior and Evolution.* 2002; 59:199–210.
- Njegovan M, Ito S, Mewhort D, Weisman R. Classification of frequencies into ranges by songbirds and humans. *Journal of Experimental Psychology.* 1995; 21:33–42. [PubMed: 7844505]
- Page SC, Hulse SH, Cynx J. Relative pitch perception in the European starling (*Sturnus vulgaris*): Further evidence for an elusive phenomenon. *Journal of Experimental Psychology: Animal Behavior Processes.* 1989; 15:137–146. [PubMed: 2708939]
- Pavlov, IP. *Conditioned reflexes.* Oxford University Press; Oxford: 1927. p. 113-119.
- Plachta D, Mogdans J, Bleckmann H. Responses of midbrain lateral line units of the goldfish, *Carassius auratus*, to constant-amplitude and amplitude-modulated water wave stimuli. *Journal of Comparative Physiology A.* 1999; 185:405–417.
- Popper, AN.; Fay, RR. The auditory periphery in fishes. In: Fay, RR.; Popper, AN., editors. *Comparative hearing: Fish and amphibians.* Springer-Verlag; New York: 1999. p. 43-100.
- Ralston JV, Herman LM, Williams HNS, Gory JD, Jerger KK. Melody recognition by an Atlantic Bottlenose dolphin. *Journal of the Acoustical Society of America.* 1988; S77
- Richards DG, Wolz JP, Herman LM. Vocal mimicry of computer-generated sounds and vocal labeling of objects by a Bottlenosed dolphin, *Tursiops truncatus*. *Journal of Comparative Psychology.* 1984; 98:10–28. [PubMed: 6705501]
- Shepard RN. Toward a universal law of generalization for psychological science. *Science.* 1987; 237:1317–1323. [PubMed: 3629243]
- Weiss BA, Martini JL. Lateral-line sensitivity in the blind cavefish (*Anoptichthys jordani*). *Journal of Comparative and Physiological Psychology.* 1970; 71:34–37.
- Wright AA. An experimental analysis of memory processing. *Journal of Experimental Analysis of Behavior.* 2007; 88:405–433.
- Wright AA, Rivera JJ, Hulse SH, Shyan M, Neiwirth JJ. Music perception and octave generalization in rhesus monkeys. *Journal of Experimental Psychology: General.* 2000; 129:291–307. [PubMed: 11006902]

Figure 1.

Partial view of the experimental tank (not to scale) illustrating the stimulus (bead and shaft) position relative to the animal. Concentric circles mark the training (or zero position) position for each respective transect. Each circle in the diagram indicates a test stimulus position. See table 1 for relative pressure measured at the animal for each of these stimulus positions in each experiment. Frequency (experiment 1) and amplitude (experiment 2) tests were conducted at a single location, the 0 (training) position, on the 3 cm transect. Location tests (experiment 3) were conducted individually along each of the three transects (3, 6, and 12 cm). Each circle also represents the pressure levels at the fish schematized as the circle diameter for each test stimulus in experiment 3. The inner circle shows the smallest CS amplitude during training.

Figure 2.

Five respiratory waveforms, for each of two animals, representing the range of responses to the CS and the test stimuli, during the course of an azimuth test. Individual traces represent respiration for single trials during testing in experiment three. The stimulus type is indicated at the left of each trace and the suppression ratio to the right. The first 5 s of each trial is designated as the “Pre” period, as indicated at the bottom of the figure. In the 5s period immediately following the “Pre” period the stimulus was presented. The suppression ratio (SR) for each trial appears to the right of the corresponding trace. The block of five waveforms in the top portion of the figure are representative of an animal (from experiment 3 group B) generalizing almost completely to all test stimuli. Those waveforms in the bottom part of the figure are representative of a single animal (from experiment 3 group A) showing a response gradient. For this particular animal an increasing decrement in responding can be seen for tailward stimuli, with responses falling to the level of chance ($SR \geq .50$) for the most distant tailward stimulus. A similar gradient in responding by this animal to headward stimuli did not occur, but rather the animal weakly generalized across both test stimuli ($SR \sim 0.3$ or 50% generalization).

Figure 3.

Normalized responses (error bars show 95% confidence intervals) as a function of frequency for dipole and loudspeaker sources. Training stimulus (CS) is indicated by the upward facing arrow. In this Figure and Figures 4 and 5, the mean for dipole stimuli is indicated by the heavy black line and individual median responses by the thinner lines. The group mean response for the loudspeaker stimulus is indicated by the solid black square and individual median responses by white squares.

Figure 4. Normalized responses (with 95% confidence intervals) as a function of dipole vibration amplitude (re: training amplitude). The CS is indicated by the upward facing arrow.

Figure 5. Normalized responses (with 95% confidence intervals) as a function of source location.. Training stimulus position is indicated by upward facing arrows. Figure A (3 cm transect) show responses for animals trained using a fixed amplitude. Figures B (3 cm transect), C (6 cm transect) and D (12 cm transect) contain data for animals trained using a range of amplitudes.

Table 1

Pressure (dB re: 1uPa) measured at position of the animal for all test stimuli

Experiment	#1 Frequency		#2 Amplitude		#3 Location		
Transect	3cm	loudspeaker	3cm	3cm	3cm	6cm	12cm
<u>Source Location</u>							
-4				99	102	102	
-2				102	104	103	
0	98	98	92-104	104	104	104	104
2				102	104	104	
4				100	103	103	