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The Detection of Pressure Fluctuations, Sonic Audition, Is the Dominant Mode of Dipole-Source Detection in Goldfish (*Carassius auratus*)

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Abstract

Behavioral detection of a low-frequency (40 Hz) vibratory dipole at source distances of 1.5–24 cm was measured by classically conditioned respiratory suppression in goldfish (*Carassius auratus*). Detection thresholds were compared across distances and before and after ablation of individual octavolateralis sensory channels. Detection thresholds, expressed in units of pressure (SPL), remained roughly constant as distance between the stimulus source and animal increased. Lateral line inactivation, using CoCl_2 , had no measurable effect on sensitivity, although some other results can be construed as weak evidence for a small contribution of the lateral line to dipole detection when source distances are ≤ 6 cm (< 1 body length). Gas bladder deflation resulted in a large increase in threshold (17 dB), demonstrating that the gas bladder contributes to audition at low frequencies. The present study confirms an auditory role for the gas bladder-enhanced inner ear of goldfish in the detection of low-frequency vibratory sources. Sonic audition (detection of pressure fluctuations) appears to be the dominant mode of dipole-source detection for goldfish when measured by conditioned behaviors in psychophysical experiments.

Keywords

audition; sonic; dipole detection; goldfish; lateral line

The hydroacoustic world of fishes is shaped by multiple forms of energy associated with sound sources in their environment. In aquatic environments, pressure waves or sonic cues are often associated with substantial hydrodynamic structures and pressure gradients. Sound sources in water produce highly nonuniform fields, dominated by bulk flow and hydrodynamic forces close to the source and more uniform spatial distributions of pressure fluctuations at greater distances (Kalmijn, 1988). True propagating sound waves are not able to form in shallow water (Rogers & Cox, 1988), particularly at low frequencies. It has been convincingly argued that low-frequency, primarily hydrodynamic, sources (dominated by fluid motion rather than pressure) are the primitive stimuli to the inner ear (Kalmijn, 1989). Yet multiple groups of fishes, generally referred to as hearing specialists, have evolved new mechanisms to detect the pressure field surrounding sound sources, by transducing pressure fluctuations to mechanical stimulation of the inner ear. This ability to detect pressure specifically enables more sensitive hearing at all frequencies and an extension of the upper-frequency range (Braun & Grande, 2008). Still, it is unclear how those specialized fishes with high sensitivity to pressure

fluctuations detect low-frequency vibratory sources that present both fluctuating pressure fields and substantial hydrodynamic disturbances.

Although the mechanosensory lateral line and otolithic inner ear share displacement sensitive hair cells, each type of sensor is maximally sensitive to different physical dimensions of the stimulus field surrounding a moving or sound-producing object (Braun, Coombs, & Fay, 2002; Platt, Popper, & Fay, 1989). The otolithic inner ear is stimulated by relative motion between the otolith and the underlying sensory macula. This is believed to occur when the body of the fish is displaced and the more dense otolith lags behind (Denton & Gray, 1983). This has been described as the *direct path* of inner ear stimulation (Fay & Popper, 1975). Whole body accelerations of the fish can, in principal, occur even in the acoustic farfield, if the particle accelerations that compose the propagating pressure wave are large enough. Most likely, this direct path of inner ear stimulation, which we will call *inertial audition*, is of greatest importance in the hydrodynamic nearfield, where large pressure gradients and bulk movement of fluid can impose substantial whole body displacements to fishes (Denton & Gray, 1983).

In otophysan fishes such as goldfish, the saccule is also mechanically linked to the gas bladder via the Weberian apparatus (Rosen & Greenwood, 1970; Von Frisch, 1938). Pressure fluctuations cause volume changes in the gas bladder, and the movement of the bladder wall is conducted to the endolymph of the saccule. This so-called “indirect path” of inner ear stimulation is well known to provide otophysans the ability to detect pressure (e.g., Coombs & Popper, 1979; Kleerekoper, Petronella, & Roggencamp, 1959; Popper & Fay, 1973; Popper & Platt, 1993; van Bergeijk, 1967; Yan, Fine, Horn, & Colón, 2000). Although this sense, which we term *sonic audition*, is clearly of importance in the acoustic farfield, it is also stimulated by the high pressure fluctuations found within the nearfield. Because low-frequency sources impart greater whole body accelerations at equivalent distances, it has been suggested that the importance of sonic audition (pressure sensitivity) is reduced at low frequencies and within the nearfield (Sand & Karlsen, 2000), but most measures of hearing sensitivity have used sources of 100 Hz or higher.

In the earlier literature (e.g., Cahn, 1967), it was assumed that the lateral line played an accessory role to the inner ear, perhaps aiding localization and detection of low-frequency sources at close distances. A substantial body of literature has now established that the lateral line is not an accessory hearing organ and does not directly contribute to the detection of sounds, in the sense of fluctuating pressure fields (e.g., Denton & Gray, 1983; Dijkgraaf, 1963; Enger, Kalmijn, & Sand, 1989; Sand, 1984). Nonetheless, many stimuli that produce pressure fields also produce steep gradients of pressure close to the source. At low frequencies these may extend to distances of several fish body lengths (Kalmijn, 1988), and these gradients are potentially very salient lateral line stimuli. Thus although a “sound” source simultaneously provides appropriate stimulation to the auditory and lateral line system, we currently have very few data on how these sensory modalities might interact to control behavior (Braun et al., 2002). Coombs (1994) and Fay (1969) both examined behavioral responses to low-frequency dipole sources, and Coombs (1994) showed that the lateral line is likely not involved in detection responses, but did not evaluate the relative contributions of sonic or inertial auditory submodalities.

This study measures behavioral detection thresholds in goldfish, a hearing specialist, to a 40-Hz vibratory dipole stimulus. We manipulated both source distance and available sensory channels to determine how goldfish detect a low-frequency vibratory source using multiple transmission pathways and sense organs. These senses include inertial audition (the detection of particle motion as in linear acceleration of the animal imposed by surrounding fluid motions), sonic audition (the detection of pressure fluctuations), and hydrodynamic sensation by the lateral line (via the detection of spatial and temporal gradients in pressure). Detection thresholds

were measured before and after gas bladder deflation, as well as before and after lateral line inactivation, effectively testing the contribution of sonic audition (pressure sensitivity) and hydrodynamic sensation (changes in pressure gradients) to the detection of low frequency sources within the hydroacoustic nearfield. The possibility of range fractionation or differential contributions of hydrodynamic inputs was explored using a wide range of source distances that present either strongly hydrodynamic stimuli (close to the source) or more spatially uniform acoustic pressure fluctuations (farther from the source).

General Method

Subjects

The subjects were Goldfish (*Carassius auratus*) obtained from a commercial fish dealer. Animals were of unknown sex and age (7 to 10 cm standard length [SL]). They were communally housed in 151-L and 208-L tanks, fed commercial fish food, and maintained at a water temperature of 24.5–26.0 °C. All experiments were conducted within the parameters for animal care and use set forth by the Hunter College Institutional Animal Care and Use Committee.

Materials

All experiments were conducted in a clear acrylic tank, 39.5 cm × 44.5 cm × 15 cm (see Figure 1), located in a sound-dampening booth (single-wall Industrial Acoustics Corporation, New York, NY) and positioned on a vibration isolation table (Nano-k, Minus k Technology, Inglewood, CA). The subject was restrained in a cage constructed to accommodate the size and body profile of each animal. These cages were constructed of balsa wood and 0.64-cm nylon mesh attached to the wood frame in a manner permitting secure positioning of the animal by the nylon mesh while precluding contact with the wooden frame. The cages permitted unencumbered respiration and pectoral fin movement, while limiting overall body movement and permitting controlled positioning of the fish with respect to the source. Water depth was 10 cm and the cage was suspended from the sides of the arena such that the bottom of the animal was 2.5 cm from the arena floor. Given these dimensions, the entire arena may be considered within the nearfield, although theory (Kalmijn, 1988) and measurement (see below) both indicate that only the region very close to the source (<10 cm) is dominated by highly structured spatial differences in hydrodynamic flow.

Procedure

The present report is based on three experiments wherein the minimum vibratory amplitude required for dipole detection was measured in 11 groups of subjects. In each group, a vibrating bead, (see *Stimulus Generation and Measurement* section) was positioned at one of five distances (1.5, 3, 6, 12, and 24 cm) from the lateral surface of the animal's body along a transect 1 cm caudal to the operculum, with the elevation of the bead adjusted to the eye level of the animal (see Figure 1). The axis of bead motion was vertical, parallel to the midsagittal plane of fish and perpendicular to the bottom of the testing arena. All sessions were conducted in darkness. Some animals were used in multiple groups and most animals required multiple testing sessions to generate sufficient data to estimate threshold. It is important to note that although animals were restricted to a specific position in the arena, they were not firmly wrapped or held by the mesh netting. Nonetheless, damage to the lateral line superficial (surface) neuromasts may have resulted from contact with the nylon mesh or during the transfer from the home aquarium. Superficial neuromasts are sensitive to water velocity with respect to the surface of the animal, and damage to these neuromasts could result in a potential loss of sensitivity to this physical aspect of the stimulus. However, the most salient lateral line cues produced in the nearfield by a vibratory dipole source are pressure gradients that stimulate canal neuromasts (Coombs, Braun, & Donovan, 2001;Engelmann, Hank, & Bleckman,

2002;Kalmijn, 1989). For this reason we felt that any lateral line damage caused by the cage would be relatively inconsequential. It is also possible that the cage alters the dipole flow field, but this effect should be small and relatively equal for all subjects and conditions.

Stimulus Generation and Measurement

The conditioned stimulus (CS) consisted of a 40-Hz pulsed dipole signal, produced and amplified using a Tucker Davis Technologies (TDT, Alachua, FL) System 3 digital/analog converter, a Crown (Crown Audio Inc., Elkhart, IN) D-45 power amplifier, and a Brüel & Kjær (Brüel & Kjær, Norcross, GA) Model 4810 minishaker. Stimulus amplitude was controlled by a TDT System 3 programmable attenuator (PA5). The dipole source was a polyvinyl sphere 8 mm in diameter (“the bead”) inserted onto a 15-cm shaft (a 16-gauge Hamilton syringe needle) and attached to the minishaker suspended above the arena on a motorized positioning system (Uni-slide, Velmex, Bloomfield, NY). The CS consisted of a train of five 500-ms pulses of 40-Hz sinusoidal vibration presented once per second over 5 s. Each pulse was shaped by 10-ms cosine-squared gates to reduce transients and preserve spectral integrity. The unconditioned stimulus (US) was a single 100-ms AC (60 Hz) electric pulse ($3\text{--}9 V_{\text{pk-pk}}$ from an isolated variable transformer), delivered through wire mesh electrodes (see Figure 1) immediately after the fifth pulse. The unconditioned response (UR) was suppression of respiration after the electric pulse was presented (see Figure 2). Stimulus amplitude and power spectrum were calibrated in the absence of the fish (insertion calibration). Experimental pressure levels in the location of the animal were determined using a miniature hydrophone (Model 8103 Brüel & Kjær). Stimulus amplitude was expressed as sound pressure level (SPL; dB re: $1 \mu\text{Pa}_{\text{RMS}}$) at the animal.

Detection Measures

Detection was measured by classically conditioned suppression of respiration. Respiration was monitored by measuring the potential between two carbon-rod electrodes; one placed within 1 cm of the animal's mouth and the other positioned approximately 10 cm behind the caudal fin. A third (ground) electrode was placed in the rear corner of the tank closest to the animal (see Figure 1). A TDT Bio-amp (DB4/HS4) was used to digitize and amplify the respiratory potential. The amplified signal was low-pass-filtered (10 Hz) using a Model 852 Rockland Filter (Victoria, BC, Canada). The signal was then digitized at 580 samples per second using a TDT RP2.1. The amount of respiration was quantified by estimating the length of the digitized waveform, using the Pythagorean Theorem, after the method described by Fay (1995). This measure of respiration quantifies the intensity and rate of breathing cycles in a single measure.

Measures of respiratory suppression (see Figure 2) are represented as a ratio of the amount of respiration during the 5-s interval of CS presentation (Time B), divided by the amount of respiration 5 s prior to stimulus presentation (Time A) plus the amount during Time B; suppression ratio (SR) = $B/(A + B)$. A value of 0.5 reflects unchanged respiration when comparing Time A to Time B. Therefore values >0.50 indicate an increase in respiration during Time B in relation to Time A, and values <0.50 indicate a decrease or suppression of respiration during Time B (Fay, 1995). The SR was used as the behavioral indicator of stimulus detection (e.g., Fay, 1969,1995;Fay & Coombs, 1992).

Nonstimulus trials were collected during a 10-s interval prior to every training or test trial. This interval consisted of two contiguous 5-s periods for which a suppression ratio was calculated and used to ensure that the animal was respiring normally prior to test trials. A trial was initiated only if the animal's SR during these nonstimulus trials was $0.5 (\pm 0.1)$. If the animal was breathing erratically, the experiment paused for 30 s before checking for normal respiration again, at which point a trial began. The nonstimulus trial data set used to estimate normal respiratory variability included only the first such “pretest” respiratory sample.

Training Procedure

A criterion of $SR < 0.40$ was used to indicate that the stimulus was detected (YES responses). Suppression ratio values of ≥ 0.40 were considered as a failure to detect the stimulus (NO responses). This value was adopted from previous studies (e.g., Fay & Coombs, 1983), as well as preliminary data collected for the present study, which all indicate that unaltered respiration has an SR of 0.5 and a standard deviation of 0.1 (Fay & Coombs, 1983, 1992). Therefore, respiratory suppressions of < 0.40 would be expected to occur by chance in approximately 17% of the nonstimulus trials.

The US voltage was adjusted to maintain the UR (Fay, 1992). Intertrial intervals averaged 180 s. Training consisted of a maximum of 40 presentations of the CS and US. The criteria for completion of training (CR acquisition) were 8 suppressions in any 10 trials or 16 suppressions in any 20 trials. Figure 3 illustrates the results of a successful training session.

Testing Procedure and Threshold Determination

Test sessions were conducted within a week after training. A pretest screening was conducted prior to each test wherein subjects were required to display three consecutive suppressions ($SR < 0.40$) in response to the training stimulus before continuing to threshold testing. If an animal failed to display three consecutive suppressions in 20 trials, an attempt was made on the same day to retrain that animal.

Detection thresholds were determined using a transformed tracking method, with a 2 up/1 down rule (Niemiec & Moody, 1995). Two consecutive detections ($SR < .40$ responses) were required before the source level was decreased by an increment of 3 dB. A NO response (a failure to indicate that the stimulus was detected) resulted in an increase in level of 3 dB (see Figure 4). A reversal was defined as a change in responding (from yes-to-no or vice versa), and the median stimulus amplitude of 20 reversals was used as the estimated threshold for each animal. Multiple test sessions were necessary to accumulate 20 reversals for most animals. Subsequent tests began at a level of 12 dB above the lowest stimulus amplitude achieved by that animal during the previous test session. The first two reversals in each test session were discarded, and data for any given test session were included only if at least five response reversals occurred. The detection threshold for each group of animals was calculated as the mean of the individual thresholds in each group of four.

Training and Testing Conditions

Eleven groups ($n = 4$ per group) of animals were trained and then tested in four different experiments. In eight groups, the subjects were conditioned to a stimulus identical to the testing stimulus. In the remaining three groups, subjects were trained with the dipole bead, and subsequently tested with the minishaker alone (without the shaft or bead; see below). Following Fay (1995), testing was conducted with constant reinforcement to avoid extinction.

Data Analysis

Detection thresholds expressed in decibels, although they are the result of a logarithmic transformation of a ratio, are appropriate for use with parametric statistical tests because it is reasonable to expect that sensory responses are themselves logarithmically distributed (Stevens, 1957). In all cases, we tested for parametric distribution of the data prior to hypothesis testing using Shapiro-Wilk tests. In Experiments 3 and 4, in which the data for all groups were normally distributed (Shapiro-Wilk $p > .05$), parametric tests were used to analyze the data; otherwise nonparametric tests were used (Experiments 1 and 2). Data for each experiment were analyzed as indicated below. Statistical analysis was conducted using SPSS 13.0.1 for Windows with a significance criterion of $p < .05$. This significance criterion was adjusted for

multiple comparisons by Bonferroni correction where appropriate. A subset of these data was presented previously in abstract form (Dailey & Braun, 2005).

Experiment 1: Detection Across a Range of Source Distances

Method

Subjects—Five groups of 4 animals each were used in this experiment.

Procedure—Animals were trained and tested at one of five different source distances. Each group was trained with the vibratory source (consisting of the shaft and bead, which together we refer to as the *dipole bead*) located at one of five positions: 1.5, 3, 6, 12, or 24 cm from the animal (all subjects were located at the same position in the tank, >5 cm from the nearest wall and 2.5 cm above the tank floor). These groups were each subsequently tested using the training source distance and with constant reinforcement.

Data analysis—Comparisons between samples were conducted using Kruskal-Wallis analyses of variance (ANOVAs) and Bonferroni adjusted Mann-Whitney *U* tests.

Results

Detection thresholds, when expressed in units of pressure, did not change significantly as a function of distance (see Table 1). The average detection threshold was 79 dB (re: 1 $\mu\text{Pa}_{\text{RMS}}$). The individual variance in threshold was large, particularly in the group tested close to the source, but was much lower for groups tested with sources located 24 cm away (see Figure 5). Threshold levels for sources 1.5 and 3 cm were very nearly equal and then began to increase by 3 dB per distance doubling between 3 and 12 cm. The difference in threshold between 12 and 24 cm was again very small, <1 dB (see Figure 5). This overall pattern was not statistically significant, however, because an ANOVA revealed no significant differences in threshold as a result of distance condition (Kruskal-Wallis $\chi^2 = 3.9$, $p > .05$).

Experiment 2: Comparison of Detection Thresholds for Hydrodynamic Versus Aerial Sound Sources

Method

Subjects—Three groups of 4 subjects each (as described in the General Method section) were used in this experiment. The results are compared with the results of the three groups of animals tested at equivalent distances in Experiment 1.

Procedure—Each group of animals was trained with the dipole bead located at 3, 6, or 12 cm. These same three groups were subsequently tested using the source in their respective training positions, but with the shaft and bead removed (minishaker-alone source). The minishaker-alone source (without a shaft or vibratory object within the water) was essentially a small aerial loudspeaker. The pressure waveform recorded in the location of the fish (see Figure 6) was greatly distorted at high amplitudes (see Figure 6, C-F), most likely due to the large increase in signal amplitude required to power the greater displacement of the aerial source. At a distance of 3 cm, where the difference between minishaker alone and dipole bead sources was greatest, generating equivalent pressures at the fish required approximately 7.5 times the voltage without the dipole. At lower amplitudes, the aerial source produced a 40-Hz pressure variation that more closely resembled the dipole source, but some spectral differences were apparent at higher amplitudes (compare Figure 6, panels B and H).

Data analysis—Comparisons between samples were conducted using Kruskal-Wallis ANOVAs and Bonferroni-adjusted Mann-Whitney *U* tests.

Results

As can be seen in Figure 7, the pressure measured at the animal at threshold was higher for minishaker-alone sources than that measured at threshold (Experiment 1) for hydroacoustic sources (vibrating bead in the water at all distances). Bonferroni-corrected Mann–Whitney *U* tests revealed that, with $\alpha = .025$, minishaker-alone thresholds were significantly greater than were thresholds to the dipole bead at distances of 3 and 6 cm ($p = .02$ for both), but not at a distance of 12 cm. Unlike thresholds to dipole stimuli, there was a significant effect of distance on threshold to the aerial source (Kruskal–Wallis $\chi^2 = 6.57$, $p = .04$). However, post hoc analysis (Bonferroni-corrected Mann–Whitney *U* tests) failed to confirm any significant change in threshold between the specific locations. Across two distance doublings (3 to 12 cm), the threshold increased by less than 3 dB.

Experiment 3: Lateral Line Contribution to Source Detection

Method

Subjects—Two groups of 4 subjects each were used in this experiment. These animals were trained with the vibratory stimulus (dipole bead) located in either the 3-cm or 6-cm position, where each would subsequently be retested after inactivating the lateral line, using the same source and with constant reinforcement.

Procedure—Lateral line inactivation was achieved by treatment with a 0.1 mmol l^{-1} solution of CoCl_2 in calcium-free artificial freshwater ($1.78 \text{ mmol l}^{-1} \text{ KCl}$, $3.57 \text{ mmol l}^{-1} \text{ KNO}_3$, $3.57 \text{ mmol l}^{-1} \text{ NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, $7.14 \text{ mmol l}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, and $14.28 \text{ mmol l}^{-1} \text{ NaCl}$). The artificial freshwater stock solution was added to de-ionized water until conductivity was equal to that of the animal's home tank and pH adjusted to home tank levels by adding NaHCO_3 . Animals were treated in a holding tank for 18 hr and returned to their home tank 24 hr before testing. This procedure results in temporary lateral line inactivation lasting 14–21 days, in water containing $1 \text{ mmol l}^{-1} \text{ Ca}$, according to Karlson and Sand (1987). All testing was completed within 4 days of treatment, and the calcium concentration in the animals' home tank was measured using atomic absorption spectrometry, by an independent water quality testing service (Robertson Microlit Laboratories, Madison, NJ) at 0.1 mmol l^{-1} . This is well below the Ca^{2+} concentration used by Karlson and Sand (1987).

Data analysis—Independent-samples *t* tests were used to compare the data for both conditions.

Results

Figure 8 presents the effects of CoCl_2 treatment in comparison to data replotted from Experiment 1 at the same source distances. After lateral line inactivation, thresholds measured at 3 and 6 cm were very nearly identical in these two groups. At 6 cm, the thresholds were also very similar to those measured with the lateral line intact (Experiment 1). At 3 cm, thresholds were approximately 4 dB higher after lateral line inactivation, but this difference was not significant, $t(6) = .72$, $p = .50$, adjusted $\alpha = .025$.

Experiment 4: Gas Bladder Contribution to Source Detection

Method

Subjects—One group of 4 animals was used in this final experiment.

Procedure—This group was trained with the dipole bead located 6 cm from the right lateral side of the subject. They were subsequently tested three times, once after gas bladder deflation, a second time after reinflation (~2 weeks later), and a third time after sham bladder deflation.

All testing used the dipole stimulus and constant reinforcement. Prior to the first test the subjects were anesthetized in a buffered solution of 100 mg MS222 (methanesulfonate salt, Sigma Chemicals) l^{-1} H_2O . One scale was then removed from the lateral side of the animal over the area of the anterior chamber of the gas bladder. The gas bladder was punctured and air removed using a 25-gauge needle connected to a 29-cm length tubing (0.397 cm interior diameter) and a 5-mL syringe. The syringe was drawn back as resistance allowed (typically 0.8–1.4 mL). The animal was then placed in a 19-L bucket of fresh water and revived. These animals no longer appeared to be neutrally buoyant in water, as indicated by the fact that they sank to the bottom of the bucket before being revived.

All of the animals in this group were tested a second time after waiting 11–20 days, when the gas bladder has presumably refilled. Finally the animals were tested a third time after an additional 7–12 days, following a sham bladder deflation. Sham deflations included anesthesia and were identical to the true deflations except that the syringe was simply touched to the area where the scale was removed, without insertion of a needle. These animals floated more slowly to the bottom of the bucket before being revived. Threshold data collected in Experiment 1 for 2 of these 4 animals was used as a control for comparison, after recovering from gas bladder deflation.

Data analysis—A repeated-measures ANOVA and Bonferroni-corrected paired samples t tests were used to analyze the three conditions.

Results

Deflation of the gas bladder significantly decreased sensitivity in relation to the results of Experiment 1 and in comparison to recovery measures (see Figure 9). After allowing 11–21 days for the gas to be replenished, thresholds decreased by approximately 17 dB. A repeated-measures ANOVA revealed a significant difference in threshold between deflated, recovered, and sham-deflated conditions, $F(1, 3) = 42.27, p = .007$. Post hoc tests (paired-samples t tests with Bonferroni correction) confirmed that the threshold in the deflated gas bladder condition was significantly higher ($p = .007$, adjusted $\alpha = .0125$) than that in the sham and recovered conditions. Thresholds measured after sham deflation and after bladder deflation (10–21 days recovery) were nearly identical and not statistically different from each other ($p = .39$).

Discussion

We manipulated stimulus type (vibratory dipole bead vs. minishaker alone), source distance, and availability of peripheral sensory channels (gas bladder deflation and lateral line inactivation) and subsequently measured detection thresholds.

Source Calibration and Measurement of Stimulus Attributes

Since the octavolateralis system responds to so many different aspects of a hydroacoustic field, specifying the appropriate stimulus attribute and amplitude is a challenge. The appropriate stimulus attribute for sonic audition is acoustic pressure and is relatively easy to measure with miniature hydrophones (as in the current study). It should be noted however that the spatial distribution of pressure can be discontinuous over very small spatial scales, particularly close to the source (Kalmijn, 1988). Coombs (1994) already noted that the distribution of pressure within the small area of the hydrophone face can vary by as much as 24 dB. The hydrophone used in the present study was smaller than that used by Coombs, but if we assume that the point pressure at the center of the hydrophone face ($\sim 9 \text{ mm}^2$) is 75 dB at 1.5 cm from the source, then the pressure at the edge of the hydrophone closest to the source will be over 80 dB and the opposite edge will be just under 71 dB (following an orthogonal transect to the flow contours). It should be pointed out that the pressure receiver of goldfish, the anterior chamber

of the gas bladder, is similar in size to that of miniature hydrophones. It may be safe to assume that hydrophones and fish pressure receivers integrate over space in similar ways, but point hydrophone pressures greatly simplify the complexity of the pressure distribution within the nearfield and are not appropriate for measuring the incredibly steep pressure gradients within a few centimeters of the source.

The inertial auditory sense responds not to pressure but to displacement of the otolith, which is thought to be caused by the bodily acceleration of the fish imposed by sound or hydrodynamic particle motion (Denton & Gray, 1983). Although direct acceleration of the head has been widely used as a stimulus for inertial audition (Fay, 1984; Lu, Popper, & Fay, 1996), there have been no direct measures of the acceleration of a fish in a free sound field. It is possible to model (Kalmijn, 1988) or measure (Coombs, Hastings, & Finneran, 1996) the hydrodynamic flow field of a dipole source. Such descriptions are reasonable depictions of the stimulus to the lateral line, which is directly responsive to the distribution of fluid motions (although knowledge of the fish's motion is also needed to determine the net flow across the body surface, as cited by Denton and Gray, 1982). With respect to inertial audition, however, it is not entirely clear how to predict the acceleration of the fish from the knowledge of pressure differences across the body surface. Presumably the rigid body of the fish integrates over its volume and accelerates with a single magnitude and direction (Denton & Gray, 1983). Ultimately we will need to directly measure the movements of a fish in a sound field, which may be feasible using laser Doppler technologies.

Since the point pressure measures can be used to ensure the consistency of source amplitude, one might assume that these measures could easily be used as a stand-in for the other stimulus attributes as well. Within the hydrodynamic nearfield, the particle velocity has a predictable relationship with pressure, but the boundaries of small behavioral tanks, including the walls and water surface, can cause great deviations from the predictions based on unbounded conditions (Parvulescu, 1967). Still, many authors have used hydrophone readings to calibrate source amplitude and ensure experimental consistency of the stimulus at a fixed location within the behavioral arena. The precise particle motion at that location may be wildly different from ideal predictions, but it should be relatively consistent from trial to trial, if all spatial relationships and acoustic properties are held constant.

The bottom line for the current study is that we cannot know the magnitude of inertial auditory stimulus for any of our source configurations. We can, however, make some assumptions about their relative amplitude. Imposed accelerations of the fish's body should decline rapidly with increasing source distance (assuming that all positions are equally free of artifacts caused by tank walls). The dipole bead within the water also presumably presents a much greater inertial auditory stimulus than does the aerial minishaker-alone condition. The aerial minishaker alone probably also creates a relatively more homogenous spatial field (without complex three-dimensional patterns); thus it is likely to be an ineffective stimulus to the lateral line and inertial auditory systems. We use these assumptions in the analysis of the present results.

Detection Thresholds: Context and Interpretation

As source distance increased, the minimally detectable pressure to the subjects increased by less than 7 dB (just over twofold) over a distance range of 1.5 to 24 cm (four distance doublings), and the bulk (~ 3 dB) of this decrement in sensitivity occurred between 3 and 6 cm. These sensitivity differences in threshold were not statistically significant, which may be due to high individual variability in estimated thresholds. Nonetheless, we find the pattern of results to be quite informative. We interpret this pattern (and the lack of overall statistical difference) as evidence that the responses we measured were generally proportional to the animal's detection of pressure. The increase in sensitivity below 12 cm, if it in fact is not a random result, may reflect input from other sensory modalities, including inertial audition or lateral line systems.

Inactivation of the lateral line did not result in statistically different thresholds in groups tested at the same source distances. However inactivated groups tested at 3 and 6 cm had nearly equal thresholds, both 3 dB higher than did intact fish tested at 3 cm, suggesting that the lateral line could enhance sensitivity at distances less than 6 cm. It should be stated however that the present data do not provide statistical support for any involvement of the lateral line in the conditioned detection of this source. At distances of 6, 12, and 24 cm, pressure alone is sufficient to explain all responses. Over this distance range, pressure thresholds were very nearly independent of distance, and the displacement required to produce these pressures increased by 12 dB per distance doubling, exactly as predicted by dipole field equations (Kalmijn, 1988).

Inertial audition cannot be eliminated without also eliminating sonic audition (pressure sensitivity), so it is more difficult to speculate on its role. If the threshold measured following gas bladder deflation is taken as an inertial hearing threshold, the relatively low sensitivity (with respect to sound pressure at 6 cm) could reflect the fact that without a pressure transduction mechanism, the appropriate stimulus is fish displacement, rather than pressure. Very high stimulus pressures are required to produce suprathreshold particle motion at the fish. If this interpretation of the results is correct, then low-frequency sources can often be detected by their pressure component alone at a level below that which would stimulate inertial audition. Inertial audition might still somehow contribute to auditory behaviors, but not at near-threshold levels.

Coombs (1994) also measured behavioral thresholds to vibratory dipole sources (50 Hz) as a function of distances between 1.5 and 6 cm. As in the present study, lateral line inactivation was found to have little or no demonstrable effect on conditioned responses. Fay (1969) also reported conditioned responses to a large 40-Hz dipole source and found that pressure thresholds changed modestly (<6 dB) over a range of 30 cm. These reports, together with the present data, strongly support the conclusion that vibratory dipoles are detectable by sonic audition (detection of pressure fluctuations) alone and that under simple conditioning regimes, goldfish attend to the pressure component of a dipole source.

In both studies however, pressure thresholds increased as a function of distance, particularly close to the source (within 12 cm). This suggests that steep pressure gradients close to the source somehow contribute to increased detection sensitivity. In Coombs (1994), pressure sensitivity declined by 7 dB from 1.5 to 6 cm from the source, in contrast to the 3 dB reported in the present study over the same distance. In the present study, a similar increase in threshold occurred between 3 and 12 cm.

Data from Experiment 2 clearly demonstrate that goldfish are much more sensitive to a 40-Hz hydroacoustic (dipole bead) stimulus than to an aerial (minishaker alone) sound source presented at the same frequency and located at distances ranging from 3 to 12 cm from the animal. Possibly, this difference in sensitivity is due to salient hydrodynamic features of the dipole field that are absent in the aerial condition, and which could potentially be detected by other sensory channels. Hydrophone records show that the minishaker-alone source can be driven to create equal pressure levels (see Figure 6) at the location of the fish, but absent the local field (and bulk water movements) surrounding the dipole bead, the fish do not respond at equivalent pressure levels. This result could be interpreted as a failure to generalize to the testing stimuli, as the training CS always consisted of the dipole bead stimulus (no-bead sources were not effective as conditioning stimuli, perhaps due to low saliency). However, all test stimuli, including minishaker alone, were delivered with reinforcement, and there was no evidence of retraining (increasing response strength with repetition) during test sessions.

There was also less variability in individual thresholds between animals tested in the minishaker-alone condition when compared to those tested in the dipole bead condition. This was true of animals tested with dipole stimuli at 24 cm as well, suggesting that both stimulus conditions presented highly uniform stimulus fields when compared to other source distances. It is unclear why thresholds to the pressure source located above the tank were approximately 10 dB higher than the distant (24 cm) dipole condition, but it suggests that hydrodynamic stimuli (i.e., particle motion or incompressible flow) may enhance pressure-detection performance, even for stimuli at distances as great as 24 cm. If the steep pressure gradients closer to a vibrating source necessarily contribute to detection at threshold via the lateral line, then selectively blocking it while leaving the inner ear and gas bladder complex intact should have resulted in a decrement in sensitivity, yet evidence for lateral line contributions at any distance is still lacking.

Inactivating the lateral line (Experiment 3) had no measurable effect on threshold at distances of 3 cm or 6 cm, indicating that the lateral line does not contribute to low-frequency dipole stimulus detection at these distances. Although no statistical effect of CoCl_2 treatment was found, it is interesting to note that there was a difference in pressure sensitivity in intact animals tested at 3 versus 6 cm (~ 3 dB) but not in treated animals tested at 3 versus 6 cm. This could be interpreted as a slight increase in sensitivity provided by the lateral line at 3 cm relative to 6 cm in intact subjects. It bears repeating, however that this very small effect of lateral line inactivation was not statistically significant. Unfortunately, cytochemical or physiological confirmation of CoCl_2 treatment efficacy was not available, so it is also possible that we simply had not truly inactivated the lateral line. It is likely, however, that the drug had some effect at the concentrations used, but there was no observable effect on behavior.

Hearing enhancement via the gas bladder is considered to be frequency-dependent, with auditory gain diminishing at low frequencies (Sand & Hawkins, 1973). It has been argued that the gas bladder may not contribute to sensitivity at low frequencies (Popper, Fay, Platt, & Sand, 2003; Sand & Enger, 1973; Sand & Hawkins, 1973), primarily because the particle motions accompanying low-frequency sources are so large as to rival the reradiant motions of the gas bladder (Harris, 1964; van Bergeijk, 1967) even at reasonably large source distances. The experimental evidence is equivocal. Fay and Popper (1974, 1975), for instance, found no effect of gas bladder deflation on microphonic potentials recorded from the ears when stimulated using standing waves (produced by a loud speaker) or direct vibration of the head at stimulus frequencies of <100 Hz. More recent studies (Coombs & Fay, 1997; Fay, Coombs, & Elepfandt, 2001) have shown physiologically that eighth-nerve units respond proportionately to the pressure component of a 50-Hz dipole source and that these responses are dramatically reduced upon gas bladder deflation. In the present study deflation of the gas bladder (Experiment 4) resulted in a dramatic decrease in sensitivity to the dipole stimulus located at a distance of 6 cm from the animal, on average by approximately 17 dB. Our finding is consistent with the now large body of evidence that the gas bladder Weberian ossicle complex improves sensitivity by 20–40 dB when tested using airborne sounds greater than 300 Hz (Fay, 1969; Fay & Popper, 1974, 1975; Von Frisch, 1938; Yan et al., 2000; Zeddies & Fay, 2005). We suggest that this increase in sensitivity also extends to low-frequency sources, although the gain afforded by the gas bladder for lower frequencies may be less than that at higher frequencies.

Thresholds obtained with the dipole bead were much more variable than those obtained in the minishaker-alone condition, and variance decreased with increasing distance from the source. This variation between animals may, in part, be the product of difficulties encountered in calibration within the spatially very discontinuous inner nearfield. Although great care was exercised in attempting to ensure that each animal, within a group, was placed in exactly the same position with respect to the dipole bead, there is always the possibility for human error in placement. The same holds true for placement of the hydrophone during insertion calibration

(see also Coombs, 1994). Moreover, once behaving animals were placed into position they may have shifted position (± 1 cm) within the restraining device. Any of these factors could have influenced the trend of diminishing variability in threshold as a function of increasing distance between the animal and the stimulus. Pressure attenuates at a rate of $1/\text{distance}^2$ for dipole sources (Kalmijn, 1988), which closely matches the 12-dB increase in source displacement per distance doubling required to produce equal pressures as the source distance increased. However, closer to the source, this rate of attenuation differs greatly depending on the precise orientation of the distance transect and its relationship to the source center (Coombs, 1994). Coombs (1994) found that a 10-mm change in source elevation resulted in a 6-dB change in source threshold at a distance of 1.5 cm from the source and a change of 3 dB at 6 cm. This means that any error in the position of the animal with respect to the dipole would have contributed to the variability between animals, especially at the positions closest to the dipole.

On the Meaning of a 3 dB Difference and Statistical Testing

A long-standing graffito in the men's restroom of Chicago's Green Mill Jazz Club states "E = MC² (± 3 dB)." The message implied is that on a grand scale, measurements that differ by less than 3 dB are somehow trivial. In the present study, several groups could be distinguished by small differences (< 3 dB) in sensitivity. In some cases, this difference was significant, in other cases not. The statistical significance of such a finding, of course, only reflects the variability of responses and the power of the test, not the "importance" of such a difference. A 3-dB difference in pressure amounts to a $1.5\times$ difference in amplitude. Whether such a difference is found to be significant or not, such a small difference in sensitivity is difficult to interpret, as the difference could easily be negated by very small differences in ambient noise, position within the sound field, and the vagaries of small-tank acoustics. In the preceding discussion we have described which differences are significant and which are not, and tried to interpret group differences in light of both the statistical significance and the magnitude of the difference. Small differences could reflect the differential participation of multiple sensory systems, or they may just reflect a lack of precision in our measurement or the testing conditions.

Conclusions

Behavioral data collected in the present study undoubtedly confirm an auditory role for the gas bladder-enhanced inner ear of goldfish in the detection of low-frequency vibratory sources over a wide range of distances. The lateral line does not appear to provide substantial input to this behavioral task, but there are some suggestive data that we interpret as possible lateral line input at distances of 6 cm or less. The role of inertial audition is less clear. Sources that create hydrodynamic stimuli (underwater sources) are detected at lower pressure levels than are purely acoustic sources (aerial loudspeakers), which suggests that the detection of sources in water could involve both pressure and detection of particle acceleration (inertial audition) at a distance of 6 cm. The near identity of pressure thresholds for a hydrodynamic source across the distance range of 1.5–24 cm suggests otherwise, however. If nonpressure cues were contributing to detection at close range, one might expect these cues to decrease in amplitude with distance, yet the threshold is unchanged. While we provided evidence that hydrodynamic stimuli are much more detectable than equal-pressure airborne stimuli, we cannot explain this difference on the basis of specific sensory channels. Experiment 1 clearly shows that pressure alone is sufficient to explain the response to hydrodynamic sources presented at distances from 1.5 cm to 24 cm. Thus we conclude that sonic audition (detection of pressure fluctuations) is the dominant mode of detection of low-frequency dipole sources in water for goldfish and, most likely, all otophysans. It is not clear if this finding would also extend to species with other kinds of hearing specializations (Braun & Grande, 2008), but future studies with nonotophysan hearing specialists may examine that question. Although our findings do not preclude

contributions by other detection channels, if other channels are involved at threshold, their contribution is limited in goldfish.

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Figure 1.

Schematic diagram of the experimental arena, vibration isolation table, positioning system slides (x and y axes) and the vibrator (minishaker)/dipole complex. Also depicted are the wire mesh electrodes used to deliver the unconditioned stimulus and the carbon-rod electrodes used to monitor the animal's respiration.

Figure 2.

Respiratory waveforms of a single animal during four stimulus presentations. Each trace represents the respiration occurring during a single trial, indicated at the left of the figure. The first 5 s of each trial is designated as the “Pre” period. The conditioned stimulus (CS) is presented during the next 5-s period, as shown schematically at the bottom. The last CS tone burst is immediately followed by the unconditioned stimulus (US; shock), indicated by the upward pointing arrow. The corresponding suppression ratio (SR) for each trial is shown on the right. The large oscillations during the first second following the US (shock) are artifacts of the relay protecting the amplifier.

Figure 3.

Suppression ratios from a single animal plotted as a function of trial number, over the course of a single training session. Each data marker represents the animal's suppression ratio for a given trial. The horizontal dashed line represents the .40 suppression ratio criterion for detecting and responding to the conditioned stimulus. The sloped line is the regression line, $y = -.011(x) + .62$, calculated for the 40 data points.

Figure 4.

Behavioral responses plotted as a function of signal attenuation and trial number during the first detection test session for a representative animal. Two successive correct detections of the same test stimulus resulted in a 3-dB decrease of signal strength for the next stimulus presentation. Any failure to detect a single test stimulus resulted in an increase of 3 dB for the next stimulus presentation. Reversals in the trend of responding and signal amplitude are marked by arrows. Twelve reversals are shown in this test session.

Figure 5. Sound pressure level at the threshold of detection using a pulsed 40-Hz vibratory stimulus, plotted as a function of distance from the animal. The bold line and solid black markers represent the average for groups of animals tested at 1.5, 3, 6, 12, and 24 cm. Open circles represent thresholds for individual animals tested at each location. Some markers represent multiple animals with identical thresholds and these are indicated with the numbers of individuals to the right of each marker.

Figure 6.

Hydrophone records (at the position of the fish) for (panels A and B) both the 95- dB dipole stimulus (polyvinyl bead and shaft) and for (panels C–H) the minishaker- alone source (with the shaft and bead removed) at three source levels (85–95 dB). The corresponding amplitude spectra are shown to the right of each hydrophone recording. The minishaker was positioned in the 3-cm position.

Figure 7.

Mean detection thresholds for a 40-Hz pulsed dipole source consisting of a minishaker attached to a shaft and bead within the water (open bars) in comparison with thresholds for a source that consisted of the minishaker alone, without a shaft in the water (solid bars). At the 3-cm and 6-cm distances there was a significant difference (asterisk) between these conditions. There was no significant difference between positions in the minishaker-alone condition. Error bars represent ± 1 SEM. Note that responses to the in-water source (open bars) are replotted from Figure 5.

Figure 8. Sound pressure level thresholds, for detection of a pulsed 40-Hz vibratory stimulus, plotted as a function of distance from the animal and lateral line condition. $N = 4$. Error bars represent ± 1 SEM.

Figure 9.

Behaviorally determined detection thresholds for a 40-Hz vibratory stimulus situated 6 cm from the animal. The same group of animals was used in each condition. $N = 4$ for all groups shown. The dotted line represents the previously obtained detection threshold for animals used in Experiment 1. Error bars represent ± 1 SEM.

Table 1
Summary of Thresholds by Condition

Distance (cm)	1.5		3		6		12		24	
	M	SD	M	SD	M	SD	M	SD	M	SD
Dipole (Experiment 1)	76.0	7.9	75.5	6.2	78.6	6.5	81.1	6.6	82.0	1.5
Minishaker alone (Experiment 2)			92.1	0.3	90.8	0.8	89.4	1.0		
Lateral line inactivation (Experiment 3)			79.3	8.4	79.4	6.9				
Gas bladder deflation (Experiment 4)					94.4	8.7				
Gas bladder recovered (Experiment 4)					77.8	3.6				

Note. Mean refers to mean thresholds expressed as sound pressure level (SPL; dB RMS re: 1μPa).