EXAMINING SPATIAL RESOLUTION, STIMULUS PERCEPTION AND RELATIVE CONTRIBUTIONS OF THE OCTAVOLATERALIS SUB-SYSTEMS OF THE GOLDFISH (CARASSIUS AURATUS)

by

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Abstract

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In two separate series of behavioral experiments, spatial resolution and perceptual dimensions corresponding to physical stimulus attributes (frequency, amplitude and position) of a vibratory dipole source were assessed using classically conditioned respiratory suppression in goldfish (*Carassius auratus*). In study 1 detection thresholds (at 40 Hz) were compared across distances of 1.5 – 24 cm, 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 by four distance doublings. 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 $\leq$ 6 cm (< 1 body length). Gas bladder deflation resulted in a large increase in threshold (17 dB), demonstrating that the inner ear alone is capable of detecting a low frequency vibratory source without the benefit of pressure detection enhancement and that the gas bladder contributes to audition at a lower frequency limit than previously predicted. Study 1 confirms an auditory role for the gas bladder enhanced inner ear of goldfish in the detection of a low frequency (40Hz) vibratory source. 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.

In study 2 behavioral measures of generalization to a vibratory stimulus were obtained for frequency, stimulus amplitude and position in the goldfish. Goldfish completely failed to generalize to octave interval changes in frequency, both lesser and greater than the CS. However, they did appear to generalize weakly to a loudspeaker stimulus of the same frequency (40 Hz) and repetition rate as the CS. Animals also generalized to the vibratory source presented at a range of source positions. As source distance increased, animals appeared to generalize more broadly to changes in position. Goldfish also appeared to generalize to stimulus amplitude. Response decrements, between amplitude test stimuli and the CS, ranged between 10-40% but were not statistically different.

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Chapter 1

Introduction

The perceptual world of organisms is a construction of their sensory systems. It is an ever-changing product of a riotous barrage of stimuli constantly impinging on sensory receptors. Animals rarely, if ever, experience the world one stimulus at a time or through one sensory channel at any given time. The stimuli that define an organism’s environment are perceived simultaneously, filtered and processed on multiple sensory channels, resulting in a functional representation of the animal’s world; it’s umwelt. Even within a single sensory channel multiple stimuli simultaneously impinge upon the receptors in that single channel. It follows that the ability to distinguish salient biological stimuli (e.g. prey, predators, potential mates) in the environment from noise is essential to survival at the most basic levels (Fay & Popper, 2000). Virtually all vertebrate classes appear to share this functional capacity (Fay, 1988).

Vertebrate Audition

The demands of an animal’s natural environment and evolution shape sensory systems by selecting for those abilities and traits that are most adaptive for a particular environment (Bass, Bodnar & Marchaterre, 1999; Fay & Popper, 2000). In the case of vertebrate audition these selective pressures have resulted in a wide array of mechanistically diverse auditory structures, especially in lower vertebrates (e.g. fish, amphibians, and reptiles), yet with fundamental functional similarities common to virtually all vertebrates. These functions include sound detection, the ability to localize sound sources, frequency analysis (Fay, 1998), acoustic feature discrimination (Fay, 2000; Popper & Coombs, 1980) and more arguably auditory scene analysis (Bregman, 1990; Fay, 1998; Fay & Popper, 2000). Although all vertebrates studied perform similar auditory tasks, it is in the degree of acuity associated with each task where there may be great variability between species (Fay & Popper, 2000).

Behavioral studies of sensory systems supply the necessary context for evaluating the vast and growing body of neurophysiological literature. Many of these physiological studies are conducted by isolating individual subsystems that serve a particular sensory system (e.g. the octavolateralis system of fishes). These studies contribute to a better understanding of the capabilities and limitations of each subsystem. However, to determine how information is integrated within and across sensory modalities and influences behavior, whole behaviors of interest (e.g. responses associated with detection of specific stimuli) must be quantitatively evaluated. These same behaviors must then be examined in situations where the contributing sensory channels are isolated; as well as investigating various combinations of the relevant or influencing sensory channels. An ideal vertebrate candidate for this type of study is the goldfish (*Carassius auratus*). The subsystems of the goldfish octavolateralis system have been isolated and exhaustively characterized at the physiological level making this otophysan fish an opportune model for vertebrate auditory function as part of a multichannel sensory system.

Otophysan Auditory Mechanisms

All fish have an octavolateralis system, consisting of an auditory / vestibular system and a lateral line (Fay & Edds-Walton, 2008; Schellart & Wubbels, 1998). Otophysans are a representative group of fish that are considered “hearing specialists”, referring to the fact that when compared to other fish, they have a relatively large audible frequency range and greater sensitivity than many other fish (Braun & Grande, 2008; Ladich & Popper, 2004). These enhanced abilities of hearing specialists are due, at least in part, to additional structures, all of which include air-filled cavities, known to improve the detection of sound pressure (Popper & Schilt, 2008; Yan, Fine, Horn, & Colón, 2000). In otophysan species, such as *Carassius,* this involves specialization of the anterior vertebrae, inner ear and gas bladder; collectively recognized as the Weberian apparatus. In other non-otophysan species sound pressure detection is enhanced by structures such as prootic auditory bullae (otic gas bladder) (von Frisch, 1938) or suprabranchial chambers (Yan, 1998, 2001). All of which are specialized structures intimately associated with the inner ear and consequently with hearing enhancement via pressure transduction. In the case of the Weberian apparatus there is a direct mechanical pressure-to-motion transduction mechanism involved (Popper & Fay, 1993; Yan, 2003; Yan, Fine, Horn, & Colón, 2000).

Octavolateralis System

The term octavolateralis system refers to the inner ear and the lateral line complex common to all bony and cartilaginous fish (McCormick, 1982; Popper & Fay, 1999). Hair cells are the common primary mechanism responsible for sensory signal transduction in both of these subsystems (Coffin, Kelley, Manley & Popper, 2004; Popper & Platt, 1993).

Hair Cells

Stereocilia (specialized microvilli) (Flock & Cheung, 1977) are located at the apex of the hair cell and surround a single long true cilium, the kinocilium. Mechanical deflection of hair cell stereocilia either results in depolarization (deflections toward the kinocilium) or hyperpolarization (deflections away from the kinocilium) of the cell, resulting in a receptor potential (Hudspeth & Corey, 1977). Even though the ultimate stimulus for hair cells in both the inner ear and lateral line is deflection of the apical ciliary bundle, these cells respond to very specific stimuli. Specificity for a particular stimulus is primarily determined by the hair cell endorgans and accessory structures providing a mechanical link between the animal and its environment (particle motion or pressure) to which they are sensitive (Kalmijn, 1988). Furthermore, in addition to neuromast morphology, location and organization also contribute to response specificity. For example groups of hair cells in the inner ear are sensitive to inertial forces while those of the lateral line exhibit sensitivity to both velocity and acceleration components of the sound field (Popper & Platt, 1993).

Lateral Line

The lateral line is both velocity sensitive and acceleration sensitive (Coombs & Janssen, 1990; Denton & Gray, 1983; Kalmijn, 1989; Kroese & Schellart, 1992). Two types of sensory receptors are found in this system, superficial neuromasts and canal neuromasts, with both receptor types having oppositely oriented groups of hair cells (Flock & Wersall, 1962; Munz, 1989). Superficial neuromasts are located on the surface of the skin and function to detect water motion relative to the animal (velocity and direction). Canal neuromasts are located between pores in a canal under the skin and respond proportionately to differences in pressure between pores, serving as differential particle acceleration detectors (Kalmijn,1988), or more simply pressure gradient detectors (Coombs & Montgomery, 1999). These neuromasts are responsive to the direct stimulus of water moving over the skin (particle motion), and it is particle motion that is dominant in the area of the sound field closest to the stimulus source known as the nearfield (Bleckmann, 2004; Kalmijn, 1988). Sensitivity to particle motion makes the lateral line particularly useful in supplying information about nearby animate and inanimate objects as well as local water currents (Coombs, New & Nelson, 2002).

Inner Ear

The Otophysan inner ear is a set of symmetrically paired structures, having both vestibular (detection of body accelerations and orientation) and auditory functions (detection of fluctuating pressure waves). Each structure has three endolymph filled semicircular canals**,** one horizontally oriented (sensitive to postural pitch), and two vertically oriented (one anterior and one posterior), all of which are sensitive to inertial movement of the hair cell cupula with respect to the crista surface. Hair cell stimulation in these canals provides sensory information necessary for locomotor and visual stabilization, both static and dynamic (Popper & Platt, 1993).

Additionally the inner ear contains three sensory otolith endorgans; these are the saccule, which is somewhat frequency selective (Fay & Edds-Walton, 1997a, 1997b; Lanford, Platt & Popper, 2000; Sugihara & Furukawa, 1989), the lagena and the utricle. Each endorgan contains a solid calcium carbonate otolith overlaying and mechanically linked to hair cells, located on the endorgan sensory epithelium. This linkage between the hair cells and otolith is achieved by a gelatinous membrane (the cupula). The hair cells of each endorgan are positioned with the axis of optimum sensitivity located in different orientations (Popper & Fay, 1993; Popper & Platt, 1993) and the particle motion associated with hydrodynamic flow is transduced as the result of the relative difference in inertial movement between the otolith and cupula (Popper & Platt, 1993). In short, all of these endorgans are acceleration (inertial) sensitive and oriented in different planes, coding head motion, possibly in three dimensions (Fay, 1984; Fay & Edds-Walton, 1997b), as well as providing a mechanism for directional hearing (Popper & Fay, 1993; Popper & Platt, 1993).

Weberian Apparatus

The Weberian apparatus is a pressure-to-motion transduction mechanism consisting of the inner ear, gas bladder and Weberian ossicles (von Frisch, 1938). The Weberian ossicles (four ossicles in goldfish) are derived from modified vertebra elements and collectively form a structure which acoustically links the gas bladder with the saccule (Rosen & Greenwood, 1970; Ladich & Wysocki, 2003).

Gas bladder (swimbladder)

The gas bladder is a gas filled structure that is compressible and linked to the inner ear by the Weberian ossicles. Changes in ambient pressure cause volume changes in the gas bladder. As the gas bladder expands and contracts, it causes the Weberian ossicles to move, producing movement in the endolymph of the inner ear. Movement of endolymph stimulates hair cells, resulting in transduction of pressure changes to mechanical energy and ultimately resulting in relatively improved auditory sensitivity (Ladich & Wysocki, 2003). With regard to hearing specialists, deflating the gas bladder is known to increase auditory thresholds (Yan, 2003).

Sound in Aquatic Environments

Any understanding of how the octavolateralis system and gas bladder function with regard to the detection of sound, necessitates familiarity with the nature of sound as it travels through water. Sound waves are characterized by propagating compressions and rarefactions of mass through a medium. They are defined by the physical properties of pressure, velocity, acceleration and displacement. Moreover, water is a more efficient medium for conducting sound than air due to the fact that it is much more dense; virtually incompressible when compared to air. The result is that sound travels approximately 4.5X faster in water (Cutnell & Johnson, 1998) and attenuates relatively little over distance, less than any other naturally occurring type of energy propagating in an aqueous environment. Knowing this, it follows that in environments where animals are not usually found in relatively close proximity to one another and where visibility is constrained, audition has a distinct sensory advantage over chemo-sense, vision (Popper & Coombs, 1980) and electric sense (Coombs, New & Nelson, 2002). The problem is that some environments, including those that are aquatic, are potentially more noisy than terrestrial environments because sound attenuates less over distance in more dense media.

When an object or organism moves through or perturbs some medium there is a net movement of mass as well as particle displacement with no net movement of mass (characterized by particles oscillating such that they move closer together and then apart). Net particle movement and particle oscillation are different aspects of the same sound field. Nearfield energy is characterized by a net movement of mass (particle movement) and farfield energy by energy transfer without net mass displacement. In the nearfield, of a sound field, particle displacement (hydrodynamic flow in the case of aqueous environments) predominates in the farfield propagating pressure (sound) waves predominate.

Stimulus frequency is an important factor influencing the size of the nearfield. The separation of the propagating wave and the relative rate of nearfield attenuation are wavelength dependent, as frequency increases the size of the nearfield decreases. Therefore lower frequency signals result in larger nearfield size than higher frequency signals.

Nearfield energy dominates closest to the sound source and diminishes as distance from the source increases, meaning that the designations “near and “far” are more descriptive of the prevailing energy components in a given sound field than of bounded zones or physical regions (Kalmijn, 1988). Nearfield and farfield energy components overlap completely, they are present concurrently everywhere within a sound field. However, because sound pressure attenuates at a slower rate over distance than particle displacement, in any given sound field there is an “intermediate” or “transitional” zone where neither energy component is negligible (Kalmijn, 1988). It is the area in a sound field where particle flow does not prevail over sound pressure; an area where both components are potentially important.

Much can be learned about the stimuli that have the potential to influence an animal’s behavior by examining the limits of an animal’s sensory abilities. However if we are interested in determining how a particular sense functions in that animal’s perception of it’s environment, it is equally important to examine behavioral responses to stimuli that approximate those found in an animal’s natural environment.

Biological Sound Sources

Animal activity in aquatic environments (e.g. movement through the water and vocal communication), cause mechanical disturbances resulting in both hydrodynamic flow (particle displacement) and propagating (acoustic) pressure waves (particle oscillation) (Kalmijn, 1988). Most aquatic biological sound sources are best characterized by dipole or higher order multipolar motion (e.g. quadrupole or octupole) (Kalmijn, 1988). Higher order motions attenuate much more rapidly than monopole or dipole motion. For this reason in the area some distance from a multipolar stimulus source dipole motion tends to dominate. Monopole motion is relatively less complex than dipole motion and attenuates less rapidly than dipole motion but it is characterized by volume changes associated with a pulsating sphere (van Bergeijk, 1967; Kalmijn, 1988). Although some biological stimuli may be characterized by changes in volume (e.g. a gas bladder pulsated to produce sound for the purpose of communication) the vast majority are not characterized by changes in volume (Kalmijn, 1988). A dipole is a more relevant representation of naturally occurring stimuli because aquatic stimuli that oscillate along a single axis (such as the limb motion associated with a swimming organism) occur with much greater frequency than those that produce changes in volume.

Dipole stimuli produce relatively complex sound fields. However, stimulus sources that generate less complicated sound fields (predominantly loudspeakers and surface wave generators) have been used historically to study auditory sensory processes in fish (Fay, 1988). Currently there is a growing body of research investigating more biologically relevant and acoustically complex stimuli. Such stimuli include dipoles that result in hydrodynamic motion as well as pressure waves (e.g. Fay, 1969; Coombs, 1994; Braun, Coombs & Fay, 2002) and shaker table systems (Fay, 1984) that can simulate hydrodynamic (particle) motion along multiple axes (e.g. Fay & Edds-Walton, 1997a; Lu, Xu & Buchser, 2003; Horowitz, Tanyu, & Simmons, 2007).

Particle accelerations associated with nearfield acoustic energy can be detected by both the inertial inner ear and the pressure gradient sensitive lateral line (Kalmijn, 1988, 1989). Although many fish species seem to respond solely to hydrodynamic (particle) motion, specializations have evolved in some fish (e.g. Otophysans) that make it possible to detect pressure as well as hydrodynamic motion and pressure gradient changes (Braun & Grande, 2008).

Audition in Otophysan Fish

Otophysan fish, such as goldfish (*Carassius auratus*), have evolved to use the array of mechanisms commonly found in fish (an inertial inner ear and the lateral line) and a specialized structure not found in all fish to aid in the task of sound source detection, stimulus source localization and frequency analysis in an aquatic environment. In these animals the gas bladder is mechanically coupled to the inner ear enabling the detection and transduction of pressure fluctuations. Because they possess this accessory sensory structure (Weberian apparatus), goldfish are able to detect and respond to both particle motion as well as acoustic pressure, possibly contributing to the ability to localize sound sources (Fay & Popper, 2000; Popper & Fay, 1997).

Goldfish are native to Asia. They were introduced into North America about 300 years ago and are commonly found in shallow, murky, slow moving, freshwater; such as ponds, lakes, streams and rivers (Gilbert & Williams, 2002; Page & Burr, 1991). They are known to school and are omnivorous, feeding on plants, small crustaceans, insects and zooplankton. In these shallow and visually challenging environments the octavolateralis system can provide the animal with a rich auditory vista of its environment. The lateral line is capable of providing the animal with information concerning other fish or organisms and obstacles in close proximity as well as information about local water currents; contributing to the animal's ability to school, avoid predators and to detect possible food items. Additionally, the inner ear and gas bladder complex may provide the animal with information about both proximate and more distant acoustic stimulus sources.

There is an extensive modern literature on fish hearing and the octavolateralis system spanning a broad range of related areas such as inner ear and lateral line physiology and function (e.g. Chagnaud, Hofmann & Mogdans, 2007; Coombs & Montgomery, 1999; Edds-Walton & Popper, 2000; Coombs, Hastings & Finneran, 1996; Engelmann, Hanke & Bleckman, 2002; van Netten, 2006), accessory auditory structures (e.g. Lechner & Ladich, 2008), detection of vibratory sources (e.g., Braun & Coombs, 2000; Coombs, 1994), sound perception (e.g. Fay, 1972, 1995a), stimulus source location (e.g. Ćurčić-Blake & van Netten, 2006), hearing loss and the effects of noise on hearing (e.g. Amoser

& Ladich, 2003, 2005; Smith, Kane & Popper, 2004), multisensory interaction (e.g. Braun, Coombs & Fay, 2002; New & Kang, 2000), and evolution of the inner ear and lateral line (e.g. Braun & Grande, 2008; Kalmijn, 1989) (also see reviews by Parker, 1918; Popper & Fay, 1973; and Popper & Fay, 1993). Goldfish have frequently been the subjects in a significant number of these physiological (e.g. Coombs & Fay, 1987; Furukawa & Ishii, 1967; Ladich & Wysocki, 2003; Schmitz, Bleckmann & Mogdans, 2008) and behavioral studies (e.g. Coombs, 1994; Fay, 2005) (also see reviews by Bigelow, 1904; Fay 1995a).

Objectives

The purpose of this research is to extend existing research concerning questions about audition and multisensory interactions by focusing on the multichannel octavolateralis system. Spatial resolution and the relative contribution of the auditory system, lateral line and gas bladder are examined, as they relate to detection of external environmental signals and auditory perception in the goldfish (*Carassius auratus*).

One of the primary questions to be addressed within this thesis is how information from each of these goldfish auditory subsystems contributes to the task of audition? Increased response speed and superior stimulus discrimination are examples of benefits often associated with multisensory integration (Stein & Meredith, 1993). This suggests that a consequence of blocking a sensory channel or channels associated with a multisensory process may be deficits in response behaviors. It is reasonable to hypothesize then that integration of two or more sensory channels can make the difference between what is adequate and what is most advantageous to guide or elicit a behavior. Although, over time organisms may compensate (partially or completely) for this loss by using one or more remaining intact channels (New, 2002; New & Kang, 2000; Rojas & Moller, 2002). Each of the auditory sensory mechanisms previously described (inner ear and lateral line) is capable of detecting some aspect or aspects, of the energy in a sound field from a single, proximately located, vibrating stimulus, thus providing a set of structures in which to tease apart and assess the multisensory contributions of a related group of subsystems (Braun, Coombs & Fay, 2002).

The second major question addressed in the present research is one of stimulus perception. How will the animal judge a vibrating stimulus if it is changed along the dimensions of frequency, signal amplitude or location? Fay (1969, 1970, 1972, 1992, 1994, 1995a, 1998, 2000, 2005) and others (e.g. Fay, Chronopoulos & Patterson, 1996) have provided evidence that the hearing sense in goldfish is perceptually comparable to the hearing sense in humans. A logical hypothesis based on these previous studies would be that goldfish have perceptual dimensions similar to those of pitch, loudness and stimulus location. Generalization paradigms can be used to ascertain which stimulus features are attended to by the animal and the degree of perceptual similarity between stimuli (Guttman & Kalish, 1956).

General Method and Approach

The present research consists of two behavioral studies, one study consisting of a series of detection experiments and the other a series of generalization experiments. In the first series of experiments (Study 1) individual sensory channels were manipulated (inactivated) to aid in assessing the sensory abilities and sensitivity of the remaining intact systems as they relate to the behavior of interest. Detection thresholds were determined for animals with an intact octavolateralis system as well as animals in which the lateral line and or gas bladder was temporarily inactivated. These detection thresholds were compared before and after manipulation of the animal’s sensory capabilities and or the stimulus. This method made it possible to determine the relative contributions of the inner ear, lateral line and Weberian apparatus, with respect to the animal’s perception of its environment, and to explore how receptivity changes at different locations within a sensory zone around the animal. Changes in behavioral responses associated with changes in receptivity were used to quantify this organism’s ability to detect a 40 Hz dipole stimulus. Detection (stimulus amplitude) thresholds were determined and used as a measure of the effect of stimulus location on detection. The expected outcome when comparing intact animals to those with manipulation of one or more auditory sensory structures, was that if the inner ear, lateral line and the Weberian apparatus operate independently, there should be little or no difference in responses. However, if there is integration between one or more of these systems then graded responses and or complete decrements in responding were expected to occur in manipulated animals.

A second series of experiments was designed to determine the perceptual dimensions that correspond to physical stimulus attributes (frequency, amplitude and position) of the same vibratory dipole. These were assessed in the context of a generalization paradigm (see Study 2). Differences in behavioral responses between the CS and test stimuli, were used to quantify the animal’s perceptual experience with the stimulus on a continuum of similarity. The anticipated outcome when manipulating stimulus frequency, amplitude and position, was that goldfish would be found to possess perceptual dimensions corresponding to pitch, loudness, and location in space for this type of vibratory source.

Chapter 2

Study 1

The Detection of Pressure Fluctuations, Sonic Audition, Is the Dominant Mode of Dipole-Source Detection in Goldfish (*Carassius auratus*) (Dailey & Braun, 2009 [1])

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 non-uniform 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

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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 comprise 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 like goldfish, the saccule is also mechanically linked to the gas bladder via the Weberian apparatus (von Frisch, 1938; Rosen & Greenwood, 1970). 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., Kleerekoper, Petronella, & Roggencamp, 1959; van Bergeijk, 1967; Popper & Fay, 1973; Coombs & Popper, 1979; Popper & Platt, 1993; 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., Dijkgraaf, 1963; Denton & Gray, 1983; Sand, 1984; Enger, Kalmijn, & Sand, 1989). 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 little data on how these sensory modalities might interact to control behavior (Braun, Coombs, & Fay, 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 and 208-liter tanks, fed commercial fish food and maintained at a water temperature of 24.5-26.0ºC. 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 x 44.5 cm x 15 cm (see Figure 2.1), located in a sound dampening booth (single wall Industrial Acoustics Corporation, New York, NY, USA) and positioned on a vibration isolation table (Nano-k, Minus k Technology, Inglewood, CA, USA). 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 .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.

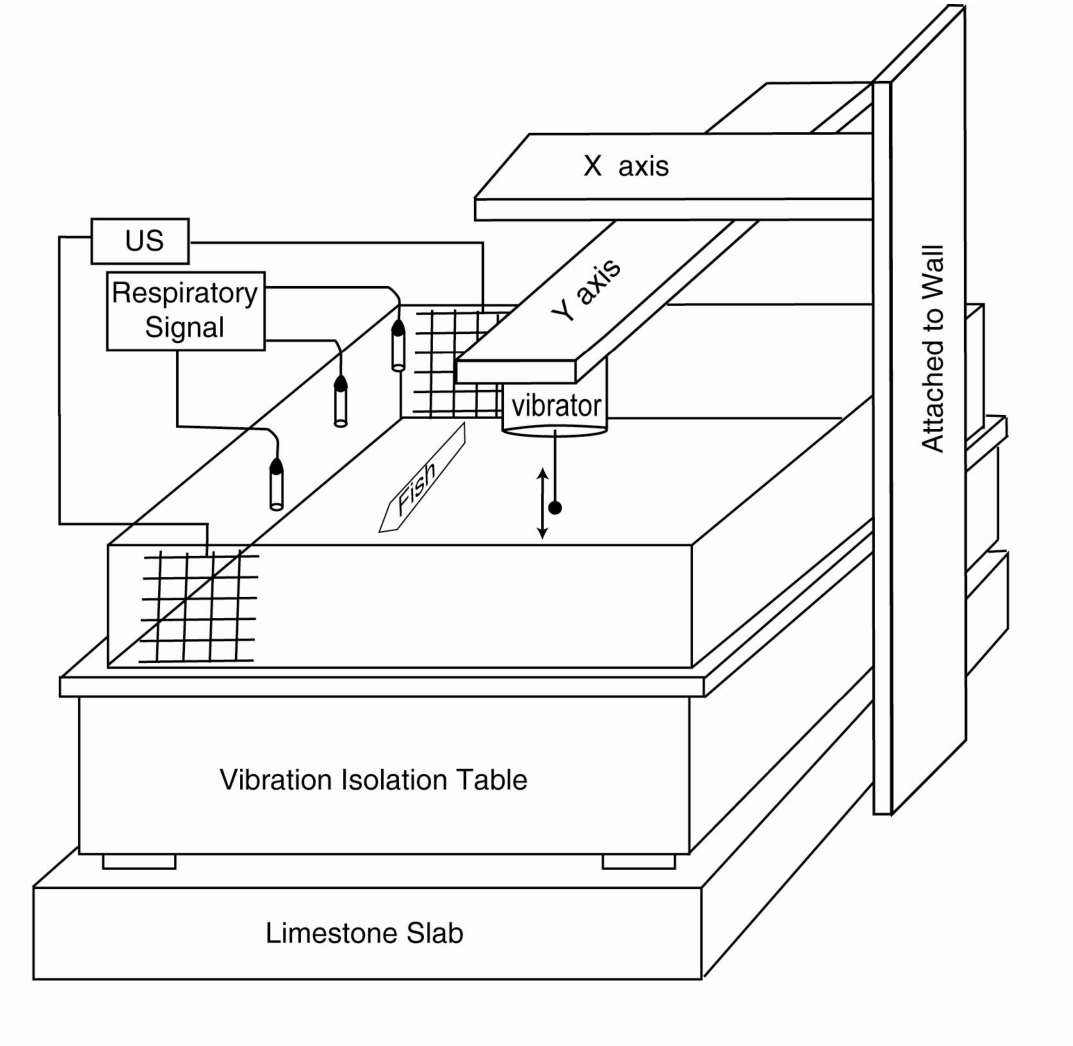

Figure 2.1. Schematic diagram of the experimental arena, vibration isolation table, positioning system slides (X and Y axes) and the vibrator (minishaker) / dipole complex. Also depicted are the wire mesh electrodes used to deliver the US and the carbon-rod electrodes used to monitor the animal's respiration

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 eleven groups of subjects. In each group, a vibrating bead, (see *Stimulus Generation and Measurement*) 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 2.1). 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 which stimulate canal neuromasts (Kalmijn, 1989; Coombs, Braun, & Donovan, 2001; Engelmann, Hank, & Bleckman, 2002). 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, USA) System 3 digital / analog converter, a Crown (Crown Audio Inc., Elkhart, IN, USA) D-45 power amplifier and a Brüel & Kjær (Brüel & Kjær, Norcross, GA, USA) model 4810 minishaker. Stimulus amplitude was controlled by a TDT System 3 programmable attenuator (PA5). The dipole source was a polyvinyl sphere 8mm 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, USA). The CS consisted of a train of five 500 ms pulses of 40 Hz sinusoidal vibration presented once per second over five seconds. Each pulse was shaped by 10 ms cosine-squared gate to reduce transients and preserve spectral integrity. The unconditioned stimulus (US) was a single 100 ms AC (60 Hz) electric pulse (3 - $9V_{pk\text{-}pk}$ from an isolated variable transformer), delivered through wire mesh electrodes (see Figure2.1) immediately after the fifth pulse. The unconditioned response (UR) was suppression of respiration after the electric pulse was presented (see Figure 2.2). 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 SPL (dB re: $1\mu Pa_{RMS}$) at the animal.

Detection Measures

Detection was measured by classically conditioned suppression of respiration. Respiration was monitored by measuring the potential between 2 carbon-rod electrodes; one placed within 1cm 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 2.1). A TDT Bio-amp (DB4 / HS4) was used to digitize and amplify the respiratory potential. The amplified signal was low pass filtered (10Hz) using a Model 852 Rockland Filter (Victoria, BC, Canada). The signal was then digitized at 580 samples / second using a TDT RP2.1.

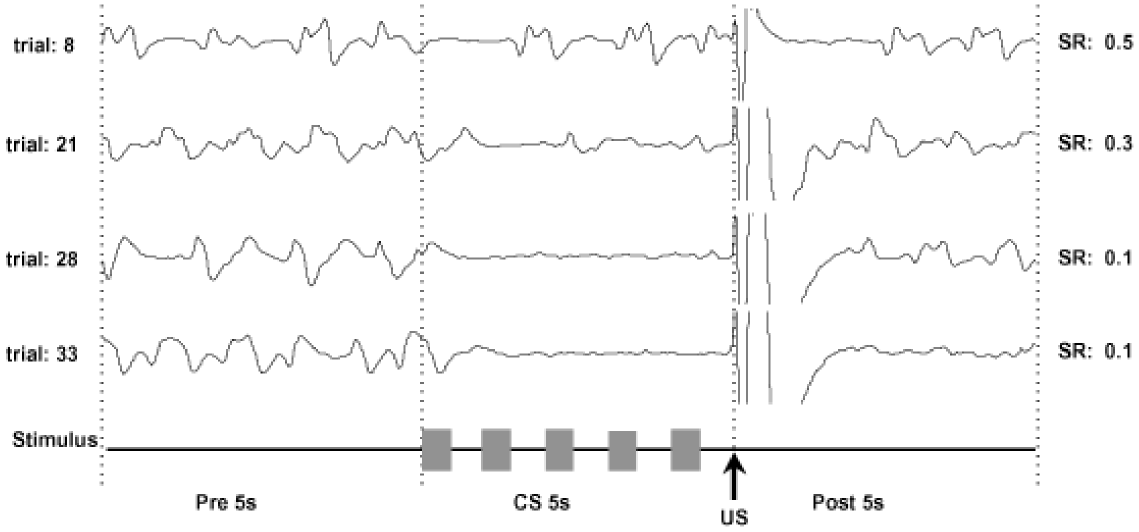

Figure 2.2. Respiratory waveforms of a single animal during four stimulus presentations. Each trace represents the respiration occurring during a single trial, indicated at the left of the figure. The first 5 s of each trial is designated as the “Pre” period. The CS is presented during the next 5 s period, as shown schematically at the bottom. The last CS tone burst is immediately followed by the 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.

The amount of respiration was quantified by estimating the length of the digitized waveform, using the Pythagorean Theorem, after the method described in Fay (1995b). This measure of respiration quantifies the intensity and rate of breathing cycles in a single measure.

Measures of respiratory suppression (see Figure 2.2) 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 relative to time A and values < 0.50 a decrease or suppression of respiration during time B (Fay, 1995b). The SR was used as the behavioral indicator of stimulus detection (e.g., Fay, 1969; Fay, 1995a & b; Fay & Coombs, 1992).

Non-stimulus 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 non-stimulus trials was 0.5 (+/- 0.1). If the animal was breathing erratically, the experiment paused for 30 s before checking for normal respiration again, at which point at trial began. The non-stimulus trial data set used to estimate normal respiratory variability only included the first such “pre-test” respiratory sample.

Training Procedure

A criterion of SR < 0.40 was used to indicate that the stimulus was detected (YES responses). Suppression ratio values ≥ 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 non-stimulus trials.

The US voltage was adjusted to maintain the UR (Fay, 1992). Inter-trial 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 2.3 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.

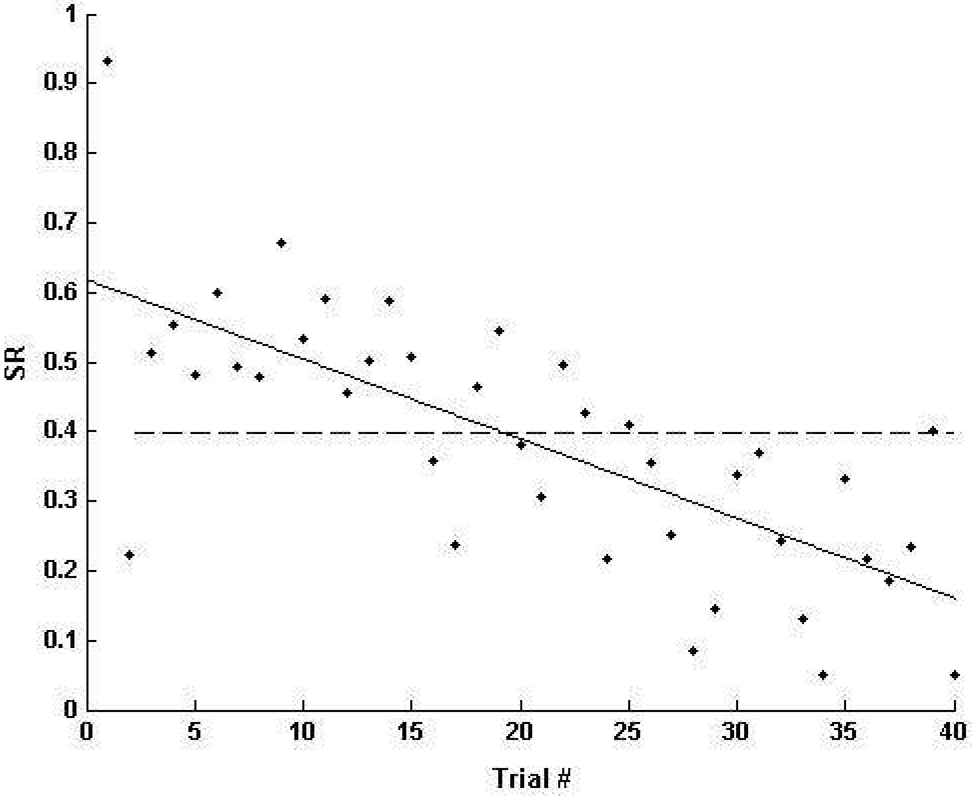

Figure 2.3. Suppression ratios from a single animal plotted as a function of trial number, over the course of a single training session. Each data marker represents the animal's suppression ratio for a given trial. The horizontal dashed line represents the .40 SR criteria for detecting and responding to the CS. The sloped line is the regression line (y = -.011(x) + .62) calculated for the 40 data points.

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 2.4). 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 12dB 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 5 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 (1995b), testing was conducted with constant reinforcement to avoid extinction.

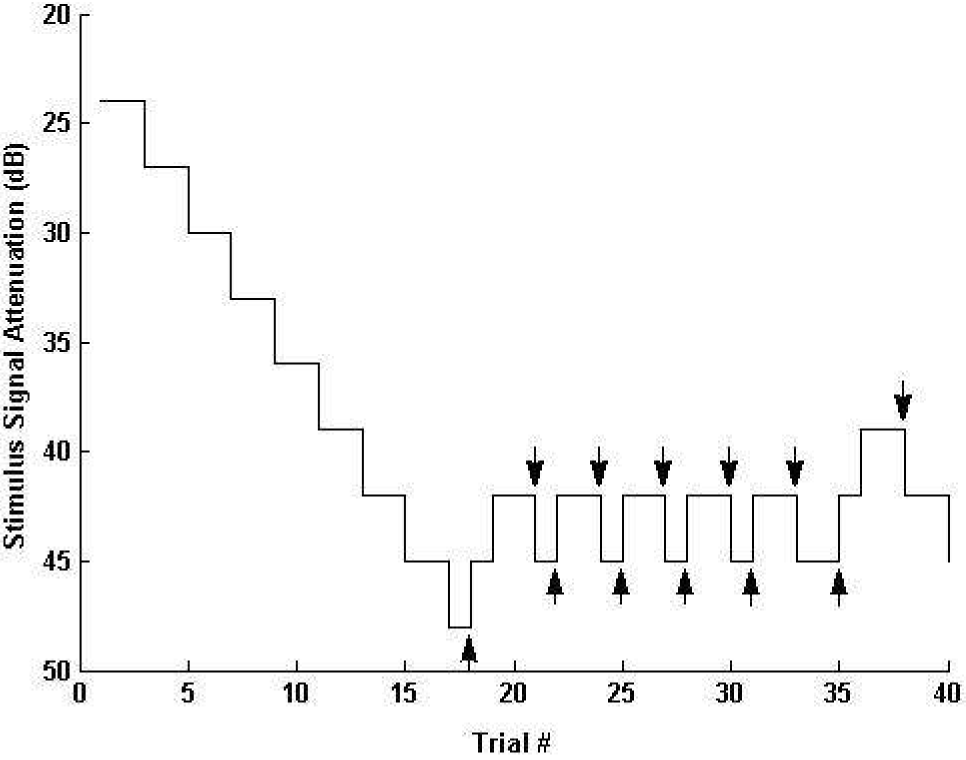

Figure 2.4. Behavioral responses plotted as a function of signal attenuation and trial number during the first detection test session for a representative animal. Two successive correct detections of the same test stimulus resulted in a 3 dB decrease of signal strength for the next stimulus presentation. 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.

Data analysis

Detection thresholds expressed in dB, 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 where the data for all groups was normally distributed (Shapiro-Wilk $p > .05$) parametric tests were used to analyze the data, otherwise non-parametric tests were employed (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 were presented previously in abstract form (Dailey & Braun, 2005).

Experiment 1

Detection across a range of source distances

Method

Subjects.

Five groups of four 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 will 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 ANOVAs and 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 2.1). The average detection threshold was 79 dB (re: 1μPa$_{RMS}$). 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 2.5). 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 2.5). This overall pattern was not statistically significant however, as an analysis of variance revealed no significant differences in threshold as a result of distance condition (Kruskal-Wallis $\chi^2 = 3.9$, $p > .05$).

Table 2.1

Summary of Thresholds By Condition

Distance (cm)	1.5	3	6	12	24
	Mean (S. D.)	Mean (S. D.)	Mean (S. D.)	Mean (S. D.)	Mean (S. D.)
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 1)		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).

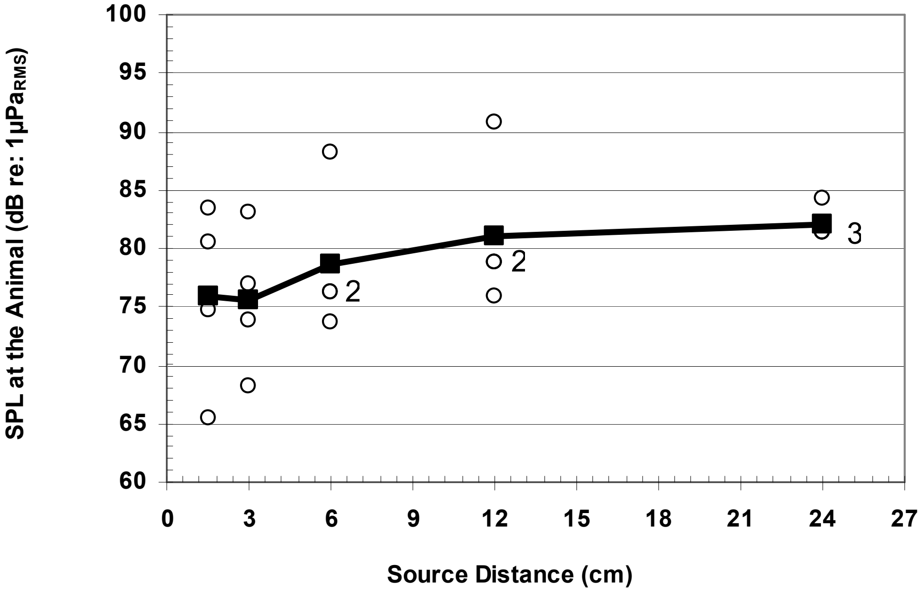

Figure 2.5. Sound pressure level (SPL) at the threshold of detection using a pulsed 40 Hz vibratory stimulus, plotted as a function of distance from the animal. The bold line and solid black markers represent the average for groups of animals tested at 1.5, 3, 6, 12, and 24 cm. Open circles represent 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.

Experiment 2

Comparison of detection thresholds for hydrodynamic versus aerial sound sources

Method

Subjects.

Three groups of four 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 either 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 2.6) was greatly distorted at high amplitudes (see Figure 2.6, 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.

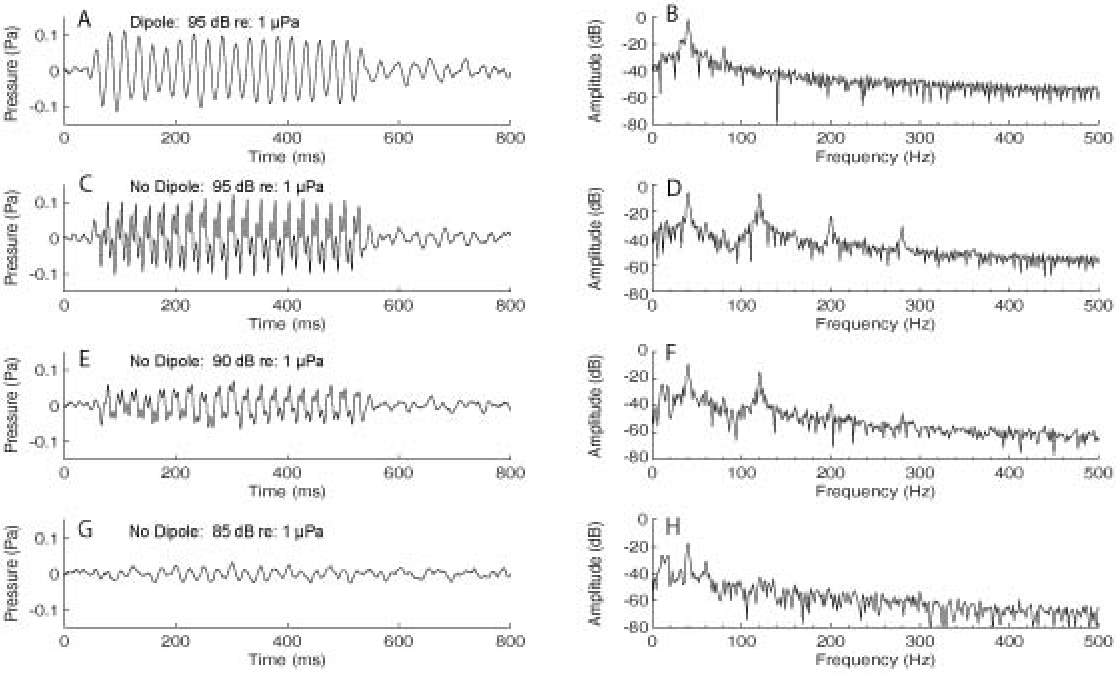

Figure 2.6. Hydrophone records (at the position of the fish) for (A and B) both the 95 dB dipole stimulus (polyvinyl bead and shaft) and the (C-H) minishaker alone source (with the shaft and bead removed) at three source levels (85-95 dB). The corresponding amplitude spectra are shown to the right of each hydrophone recording. The minishaker was positioned in the 3 cm position.

At lower amplitudes, the aerial source produced a 40-Hz pressure variation that more closely resembles the dipole source but some spectral differences were apparent at higher amplitudes (compare Figure 2.6, panels B and H).

Data analysis.

Comparisons between samples were conducted using Kruskal-Wallis ANOVAs and adjusted Mann-Whitney U tests.

Results

As can be seen in Figure 2.7, 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 α= .025, minishaker alone thresholds were significantly greater than 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 χ^2 = 6.57, *p*=.04). However, post hoc analysis (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.

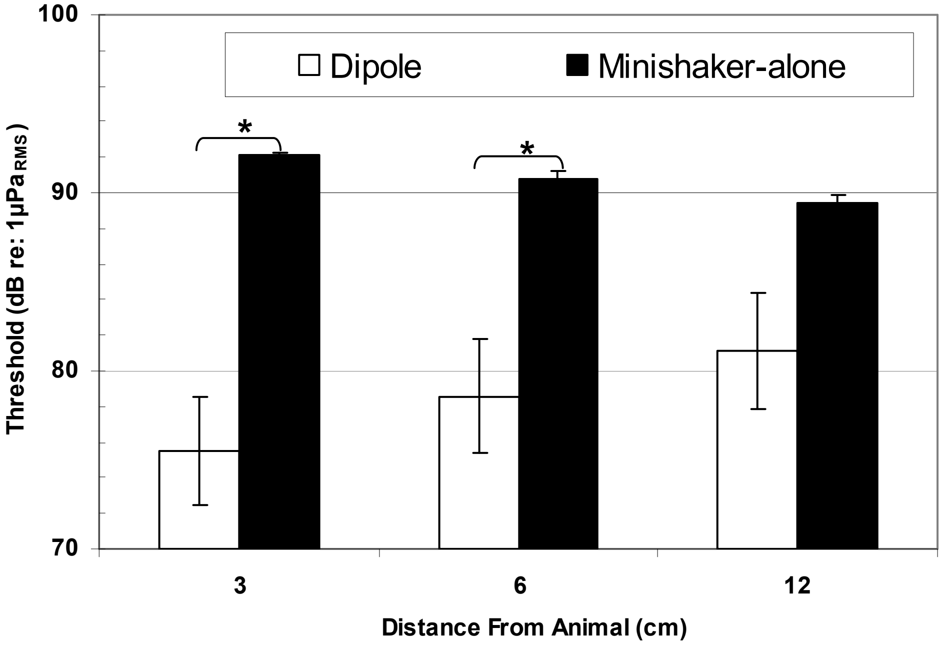

Figure 2.7. Mean detection thresholds for a 40 Hz pulsed dipole source consisting of a minishaker attached to a shaft and bead within the water (white bars) compared to thresholds for a source that consisted of the minishaker alone, without a shaft in the water (black bars). At the 3 cm and 6 cm distance there was a significant difference (*) between these conditions. There was no significant difference between positions in the minishaker-alone condition. Error bars represent +/- 1 s.e.m. Note that responses to the in-water source (white bars) are replotted from Figure 2.5.

Experiment 3

Lateral line contribution to source detection

Method

Subjects.

Two groups of four subjects each were used in this experiment. These animals were trained with the vibratory stimulus (dipole bead) located in either the 3 or 6 cm position, where each would subsequently be re-tested 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 mmol l^{-1} KCL, 3.57 mmol l^{-1} KNO_3, 3.57 mmol l^{-1} NaH_2PO_4 H_2O, 7.14 mmol l^{-1} $MgSO_4$ $7H_2O$, 14.28 mmol l^{-1} 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 hours and returned to their home tank 24 hours before testing. This procedure results in temporary lateral line inactivation lasting 14-21 days, in water containing 1mmol l^{-1} Ca, according to Karlsen 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 .1 mmol l^{-1}. This is well below the Ca2+ concentration used by Karlsen and Sand (1987).

Data Analysis.

Independent samples t-Tests were used to compare the data for both conditions.

Results

Figure 2.8 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 $\alpha = .025$).

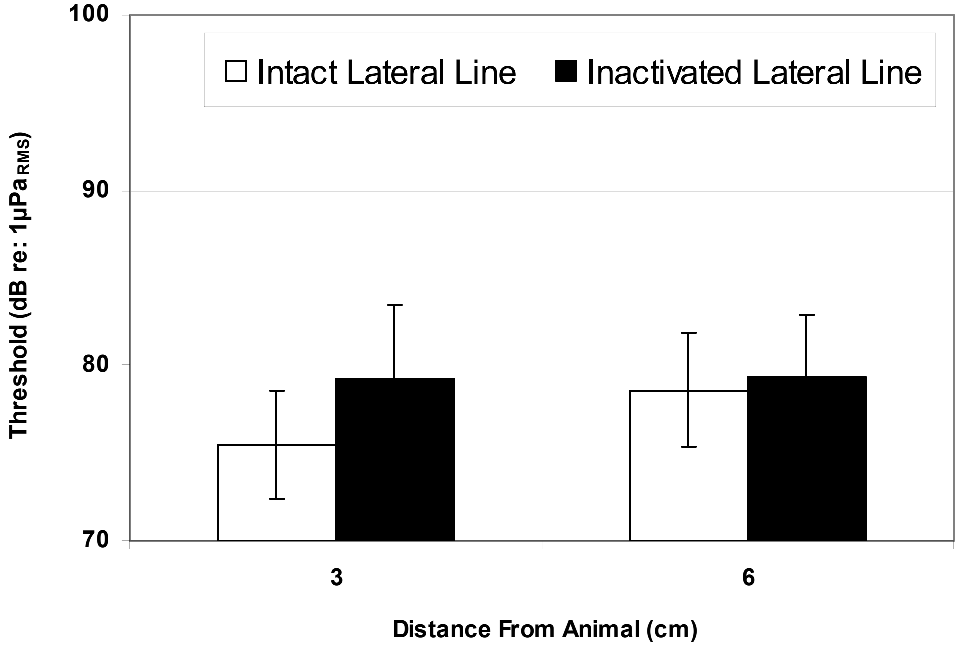

Figure 2.8. Sound pressure level (SPL) thresholds, for detection of a pulsed 40 Hz vibratory stimulus, plotted as a function of distance from the animal and lateral line condition. N = 4. Error bars represent +/- 1 s.e.m.

Experiment 4

Gas bladder contribution to source detection

Method

Subjects.

One group of four 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 re-inflation (~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 (.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 liter 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 re-filled. 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 that the syringe simply touched 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 one for two of these four animals was used as a control for comparison, after recovering from gas bladder deflation.

Data Analysis.

A repeated measures ANOVA and corrected paired samples *t*-Tests were used to analyze the three conditions.

Results

Deflation of the gas bladder significantly decreased sensitivity relative to the results of experiment 1 and in comparison to recovery measures (see Figure 2.9). 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 correction) confirmed that the threshold in the deflated gas bladder condition was significantly higher (p = .007, adjusted α = .0125) than 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).

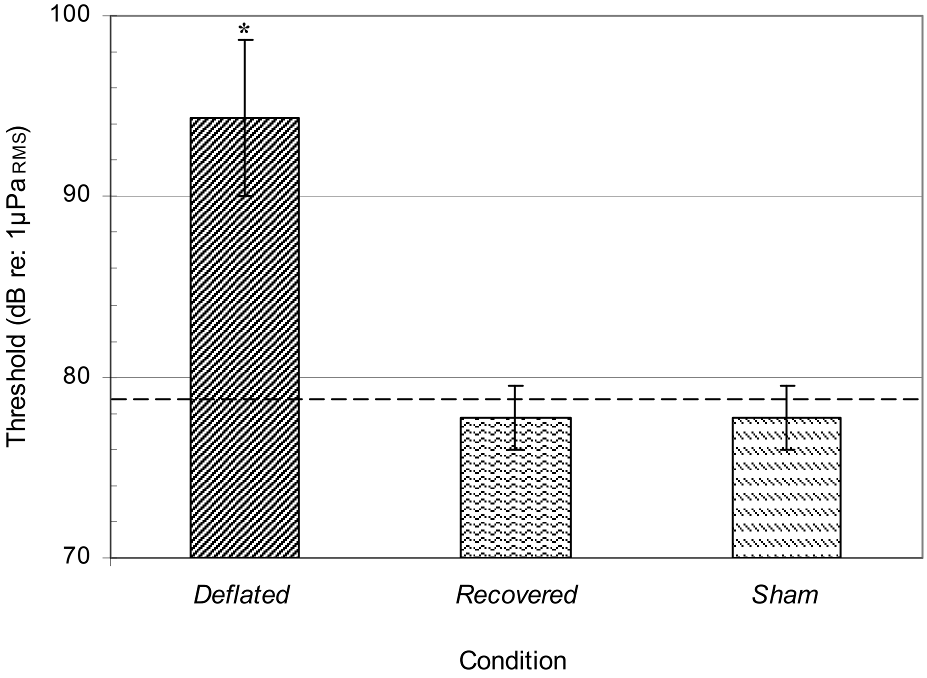

Figure 2.9. Behaviorally determined detection thresholds for a 40 Hz vibratory stimulus situated 6 cm from the animal. The same group of animals was used in each condition. N=4 for all groups shown. The dotted line represents the previously obtained detection threshold for animals used in experiment 1. Error bars represent +/- 1 s.e.m.

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 (~9 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 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 in 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, 1993). 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 (Parvlescu, 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 the aerial minishaker-alone condition. The aerial minishaker-alone probably also creates a relatively more homogenous spatial field (without complex three-dimensional patterns), thus is likely to be an ineffective stimulus to the lateral line and inertial auditory systems. We will 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 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 two clearly demonstrate that goldfish are much more sensitive to a 40 Hz hydroacoustic (dipole bead) stimulus than an aerial (minishaker-alone) sound source presented at the same frequency and located at distances ranging 3–12 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 2.6) 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 / 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 vs. 6 cm (~3 dB) but not in treated animals tested at 3 vs. 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 (Sand & Hawkins, 1973; Sand & Enger, 1973; Popper, Fay, Platt, & Sand, 2003), primarily because the particle motions accompanying low frequency sources are so large as to rival the re-radiant motions of the gas bladder (van Bergeijk, 1967; Harris 1964) 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 < 100 Hz. More recent studies (Fay, Coombs, & Elepfandt, 2001; Coombs & Fay, 1997) 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 (von Frisch, 1938; Fay, 1969; Fay & Popper, 1974, 1975; 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 insure 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/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 room of Chicago’s Green Mill Jazz Club states: “$E=MC^2$ (± 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 1.5X 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 confirms 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 (under-water sources) are detected at lower pressure levels than 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 non-pressure 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 one 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 non-otophysan 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.

Chapter 3

Study 2

The Perception of Frequency, Amplitude and Position of a Vibratory Dipole Stimulus by the Octavolateralis System of Goldfish (*Carassius auratus*)

The octavolateralis systems of the goldfish (*Carassius auratus*), consisting of the inner ear and mechanosensory lateral line systems, are capable of detecting vibratory stimuli that are close to the animal as well as relatively distant stimuli. The inner ear of goldfish, 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 (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 detecting 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. Known behavioral and physiological stimulus response frequencies, for the goldfish lateral line, range 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 frequencies for lateral line stimuli. 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 (Dijkgraff, 1963; Harris & van Bergeijk, 1962; Kalmijn, 1988, 1989). It is most sensitive to stimuli within no more than one or two body lengths of the animal (Denton & Gray, 1983; Dijkgraff, 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 (Coombs & Conley, 1997a, 1997b; Coombs, Finneran & Conley, 2000; Coombs, Hastings & Finneran, 1996; Coombs & Patton, 2009; Ćurčić-Blake & van Netten, 2006; Goulet et al., 2008; Nelson, MacIver & Coombs, 2002) to determine source properties.

Vibratory (e.g. dipole) stimuli in aquatic environments produce both hydrodynamic flow (particle motion) and oscillating fields of acoustic pressure. Both types of stimulus fields may be characterized along multiple dimensions, such as amplitude or frequency. For a dipole stimulus location in space might be a salient physical dimension as well. Multiple octavolateralis modalities are most likely stimulated by the complex stimulus fields presented by dipoles (Coombs, 1994; Braun, Coombs & Fay, 2002; Dailey & Braun, 2009; Braun & Coombs, 2009) but the individual contribution of the inner ear and lateral line systems to the perception of these sources is unknown. The 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 and van Bergeijk, 1962). Thus, close to the source, the hydrodynamic field contains steeper spatial gradients and this might allow the lateral line to contribute more information about the position of the source.

Behavioral studies employing both 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 stimulus 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 vertebrate animals, including humans.

In many situations, animals encounter novel stimuli that are similar in some way, to a previously encountered stimulus. When the animal’s response to the new stimulus is similar to the one evoked by the familiar one, 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) during encounters with novel stimuli, so the ability to generalize is adaptive because recurring natural events tend to be similar, along one or more physical dimensions. Thus generalization is essential to category formation and other 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 (Bregman, 1990).

In an experimental setting, the phenomenon of stimulus generalization can be used to determine what aspects of a source contribute to perception and how animals judge similarity of sources. Stimulus generalization experiments entail conditioning a subject to a particular stimulus and then probing with novel (test) stimuli that vary along a single physical dimension. A comparison of response magnitude (for example) for test versus conditioned stimuli may reveal total generalization to test stimuli (equal response magnitude), a failure to generalize (no response), or expose a gradient in responding (Guttman & Kalish, 1956; Kimble, 1961; Mackintosh, 1974; Pavlov, 1927). Graded responses to test stimuli in generalization experiments have been interpreted as indicating graded confidence levels in the animal's judgment of similarity between test stimuli and a CS (e.g. Fay, 1995a). When gradients in responding occur they are generally very orderly or systematic in appearance, often characterized by monotonic decrements in responding that correspond to an increasing difference(s) between some element(s) of the test stimulus and the CS (Mackintosh, 1974). If graded responses correspond to the physical dimension varied during testing, this suggests that the subject experiences a perceptual dimension corresponding to a specific physical dimension of a stimulus (Guttman, 1963; Mackintosh, 1974). Complete generalization is more difficult to interpret without further discrimination testing. Complete generalization 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 both 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 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.

Generalization experiments have been employed to examine the perceptual capacities (Anderson, 2000) of a number of diverse species, including rats (e.g. Blackwell & Schlosberg, 1943; Pierrel, 1958), pigeons (e.g. Guttman & Kalish, 1956), rabbits (e.g. Siegel, Hearst, George & O'Neil, 1968), goldfish (e.g. Fay, 1970, Fay, 1992, 1998 & 2005) and humans (e.g. Artigas, Aznar-Casanova & Chamizo, 2005; Kalish, 1958; Thomas & Jones, 1962). These types of experiments identify stimulus dimensions to which the animal is attending (e.g. auditory frequency, auditory intensity, visual frequency). They also provide clues indicating which stimulus dimensions may be salient for the animal in a more natural environment and reveal corresponding perceptual dimensions (e.g. pitch, loudness, hue).

In this study, we measured response generalization to a vibratory dipole source to test the hypothesis that goldfish possess perceptual dimensions of pitch, loudness, or location in space for this type of source. 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. We presented training and test stimuli at a range of positions along three different distance transects, using both randomized and fixed signal amplitudes during training. Interpretation of the data presented in the present study is based on the commonly accepted theory that generalization gradients can be used as a measure of perceptual similarity (Guttman & Kalish, 1956).

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 24.5-26.0ºC and maintained on a diet of commercial fish food. Experiments were executed in adherence to the guidelines for animal care and use as prescribed by the Hunter College Institutional Animal Care and Use Committee.

Materials

Experiments were performed in a 39.5 cm x 44.5 cm x 15 cm test tank, constructed of .64 cm clear acrylic. This tank was situated 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, and output from the minishaker. 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 distances (see Figure 3.1).

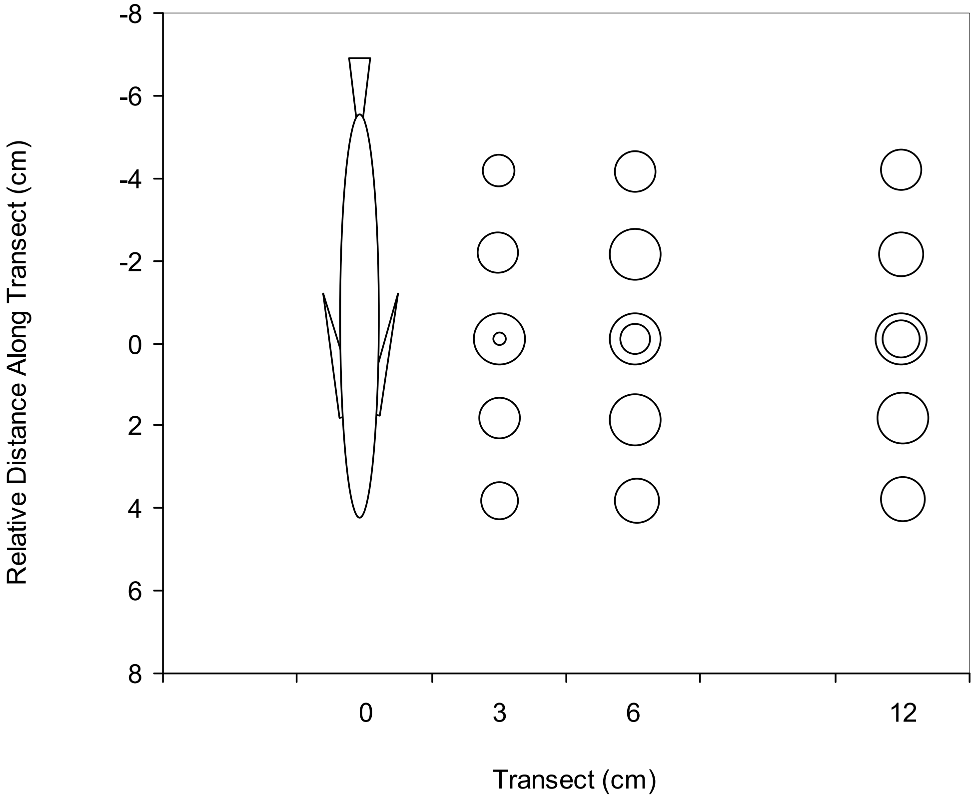

Figure 3.1. Partial view of the experimental tank (not to scale) illustrating the stimulus (bead and shaft) position relative to the animal. Concentric circles mark the training position for each respective transect. Each circle in the diagram indicates a test stimulus position. See Table 3.1 for relative 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.

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 oriented in parallel with 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: 1μPa $_{RMS}$) at the animal. Calibration of stimulus amplitude and spectral power were achieved via insertion calibration; measured in the absence of the animal. A miniature hydrophone (model 8103 Brüel & Kjær) was used to ascertain 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 3.2).

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 category of test stimulus (SR_{TEST}) by the median response for the CS during the test session. These normalized measures were expressed as percent generalization.

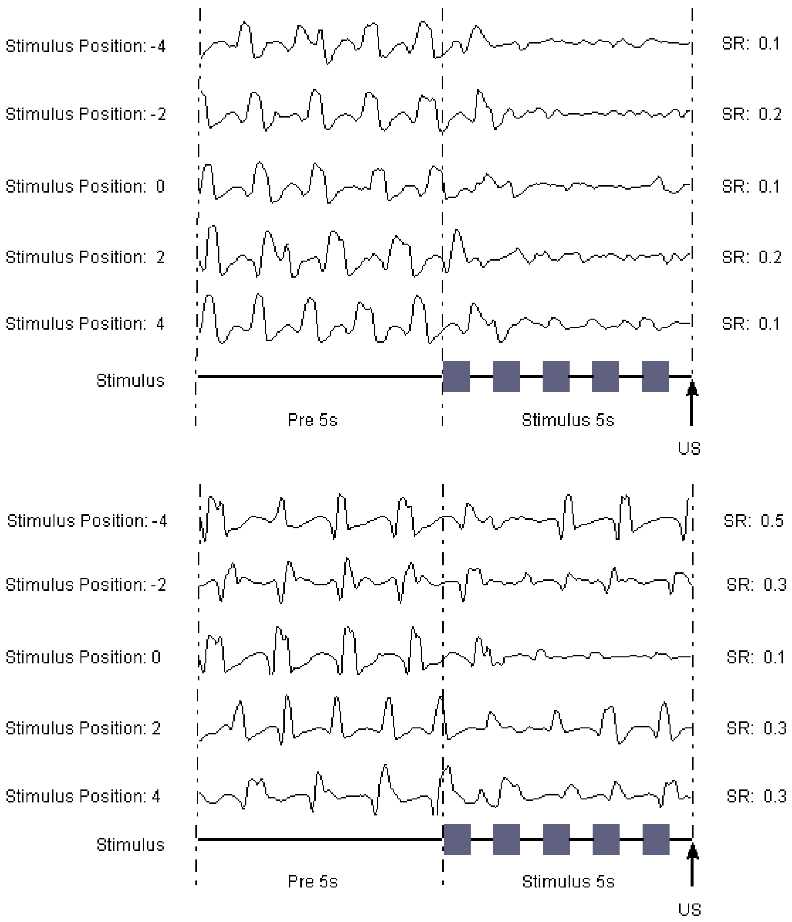

Figure 3.2. Respiratory waveforms, for two animals, representing the range of responses to the CS and the test stimuli, during the course of a position test. Individual traces represent respiration for single trials during testing in experiment three. The stimulus type is indicated at the left of each 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 the test stimuli. Those waveforms in the bottom part of the figure are representative of a single animal (from experiment 3 group A) showing a gradient in responding. For this particular animal an increasing decrement (gradient) in responding can be seen for tailward stimuli, responding 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 neither did the animal demonstrate complete generalization (SR = ~.1).

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 +/- 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). 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 - $9V_{pk\text{-}pk}$), 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 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 the 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 of trials.

Experiment 1: Generalization to Frequency

One group of five animals was trained with the dipole bead located at a distance of 3 cm (see Figure 3.1), using a fixed stimulus amplitude of 98 dB_{RMS} re 1µPa (as measured at the animal). This corresponds to 22 dB SL for the 40 Hz stimulus (Dailey & Braun, 2009) at this distance.

Test sessions were conducted with the stimulus in the training position (see Figure 3.1). The test stimuli (10, 20, 40 (CS), 80, and 160 Hz) were presented in random order and presented with equal pressure at the animal (as previously determined by insertion calibration). In addition to these four test stimuli, a fifth 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 of all stimuli were adjusted to reach 98 dB_{RMS} re 1μPa at the position of the subject.

Experiment 2: Generalization to Stimulus Amplitude

A group of five animals was also conditioned to the dipole bead located at a distance of 3 cm (see Figure 3.1), using a fixed stimulus amplitude of 98 dB_{RMS} re 1µPa (~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 (~16-26 dB SL) see Table 3.1). Test stimuli were presented in the same location as the CS.

Table 3.1

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

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

Experiment 3: Generalization of Source Position

This experiment used four separate groups of five animals (groups A, B, C and D) each trained using a fixed position CS either randomly varied in amplitude (groups A-C) or of fixed amplitude (group D). All groups were trained and tested with stimuli presented along one of three different transects; either 3, 6, or 12 cm. Groups A and D were trained and tested at the 3 cm source transect and groups B and C at the 6 and 12 cm transects respectively (see Figure 3.1). We measured stimulus generalization as a function of source position in each group of animals.

Groups A-C

In groups A-C, the signal amplitude was varied randomly during training to eliminate the use of amplitude cues that occur during testing (see Table 3.1 and Figure 3.1). Each animal was trained with CS amplitudes varied over twice the range (up to ± 6 dB) presented during testing (< 3 dB). Consequently, we trained the 3 cm group (A) by presenting a CS randomly varying between 92 and104 dB SPL (16-28 dB SL), the 6 cm group (B) CS varying between 99 and 104 dB SPL (20-25 dB SL), and the 12 cm group (C) 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. 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 3.1).

Group D

Group D was trained using a fixed amplitude source. Animals in group D were trained at the 3 cm position using a fixed signal amplitude of 104 dB SPL (28 dB SL) at the animal’s position. These animals were tested using the same method as described for groups A-C; stimuli were of fixed amplitude at the source and varied in 2 cm increments along a transect parallel to the animal and centered on the CS position (see Figure 3.1).

All Groups (A-D)

Immediately after each training trial the bead was removed from the water and then returned to the training position. This was done for the purpose of permitting the animal to acclimate to the sound of the computer controlled motorized positioning system (used to move the bead) and any sensory cues associated with the bead penetrating the water during testing. In all cases, the test stimuli were presented at a fixed signal amplitude but varied in spatial location (which caused amplitude differences at the position of the fish; see Table 3.1 and Figure 3.1). The range of CS amplitudes for each group was well above the detection threshold of the animal (~16-28 dB SL), as previously determined for each training position (Dailey & Braun, 2009).

Data Analysis

Test data for individual animals were normalized for analysis. The median SR for each test stimulus was corrected normalized using the formula $((.5-SR_{TEST\ STIMULUS})/(.5-SR_{CS}))*100$. Normalized values greater than 100% resulted when the response to a novel stimulus was greater than the response to the conditioning stimulus presented during testing. Negative relative response values occurred infrequently when the response elicited by a test stimulus resulted in an increase in respiration. Raw data (mean absolute SRs) for each experiment were tested for parametric distribution using Shapiro-Wilk tests. Differences in response strength between stimuli, within experimental conditions, were analyzed using repeated measures ANOVAs with post hoc corrected 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.

Results

Experiment 1. Generalization as a function of stimulus frequency

Animals showed a complete failure to generalize to any of the novel test frequencies (ranging between 10 and 160 Hz). This complete difference in response strength was significant when comparing the CS and all novel dipole test stimuli (10, 20, 80, 160 Hz) (*F* (5, 20) = 23.45, *p* < .05). Post hoc analysis using adjusted (α level = .01) paired samples *t*-tests confirmed this difference in response strength between the CS and the novel dipole stimuli (*p* < .01 for each dipole stimulus). In contrast, animals did generalize to the 40 Hz aerial loudspeaker stimulus, showing about 40% of the response strength shown to the CS (see Figure 3.3). This large decrease in response strength to the loudspeaker was not statistically significant with respect to responses elicited by the CS. That is, although they were reduced by more than half, responses to the loudspeaker were statistically indistinguishable from responses to the dipole.

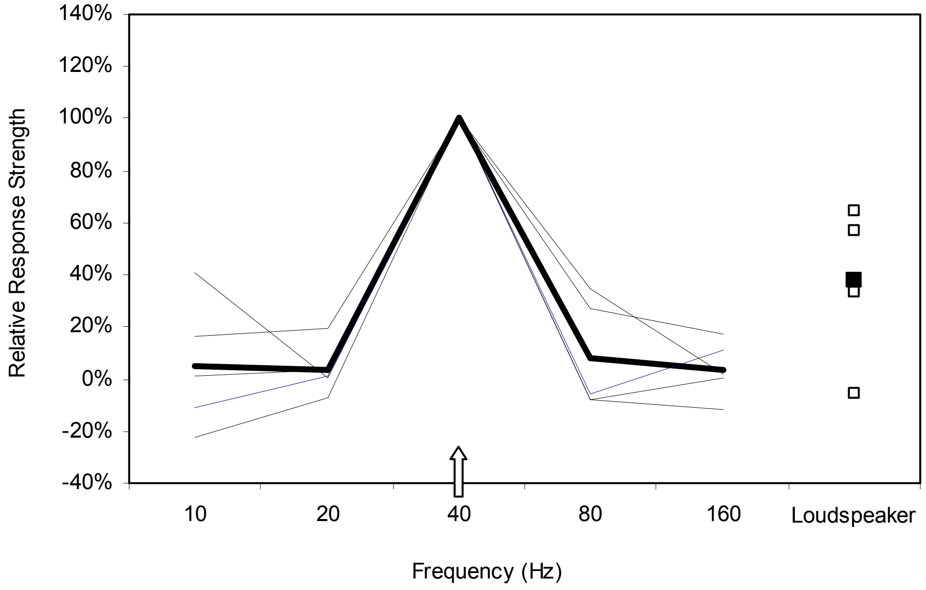

Figure 3.3. Normalized responses to frequency for dipole and loudspeaker stimuli. Training stimulus (CS) is indicated by the upward facing arrow. The mean for dipole stimuli is indicated by the heavy black line and individual median responses by the thinner lines. The group mean response for the loudspeaker stimulus is indicated by the solid black square and individual median responses by white squares. The dipole CS is indicated by the upward facing arrow.

Experiment 2. Generalization as a function of stimulus amplitude

Relative response strength decreased as a function of increasing difference in amplitude between the CS and test stimuli (see Figure 3.4). 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, $p < .05$), but an adjusted paired samples *t*-test (α level = .0125) post hoc analysis failed to detect significant differences (p = *ns*) between mean absolute SR's for individual test stimuli and the training condition.

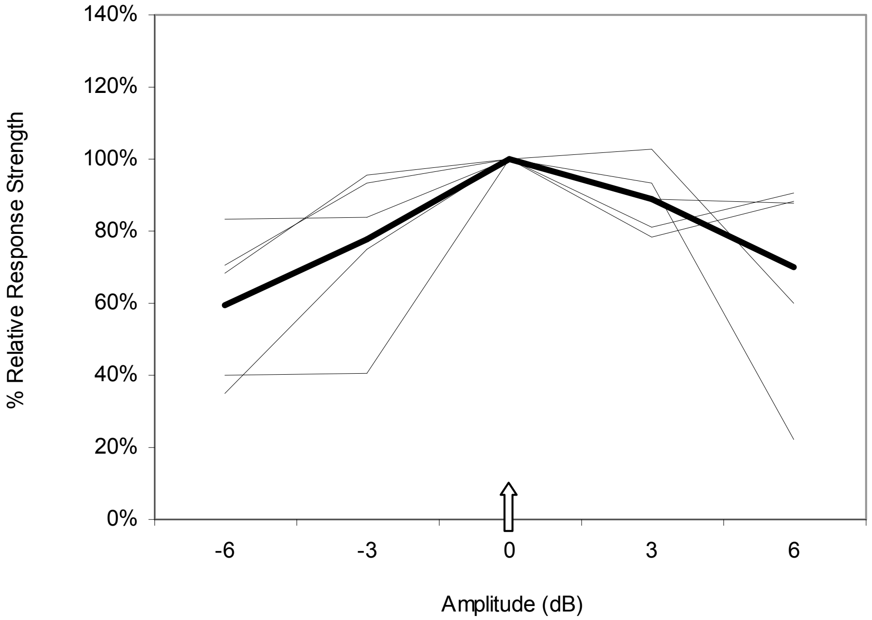

Figure 3.4. Relative generalization gradient plotted as a function of mean normalized responses for animals tested over a range of amplitudes (experiment 2). The CS is indicated by the upward facing arrow.

Experiment 3. Generalization as a function of source position

In these experiments, subjects were split into four groups that differed in the distance of the training stimulus and the range of amplitudes presented. Animals in groups A, B and C (conditioned to a randomized intensity source, trained and tested along the 3, 6 and 12 cm transects respectively) generalized fully to all stimuli regardless of position (see Figure 3.5). No clear gradients in responding were noted for these three groups (see Figure 3.1). 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, $p > .05$)].

In group D, when animals were conditioned to a 3 cm source of fixed amplitude, responses to test stimuli differed significantly from the conditioned stimulus (group D) ($\chi 2$ (4, n=5) = 11.20, $p < .05$). However, none of the pairwise comparisons were significant (adjusted Wilcoxon Signed Ranks α level = .0125; p = *ns* for all test stimuli) and visual inspection of these responses (see Figure 3.5D) is consistent with the results of the post hoc analysis, in that no particular gradient or pattern may be discerned.

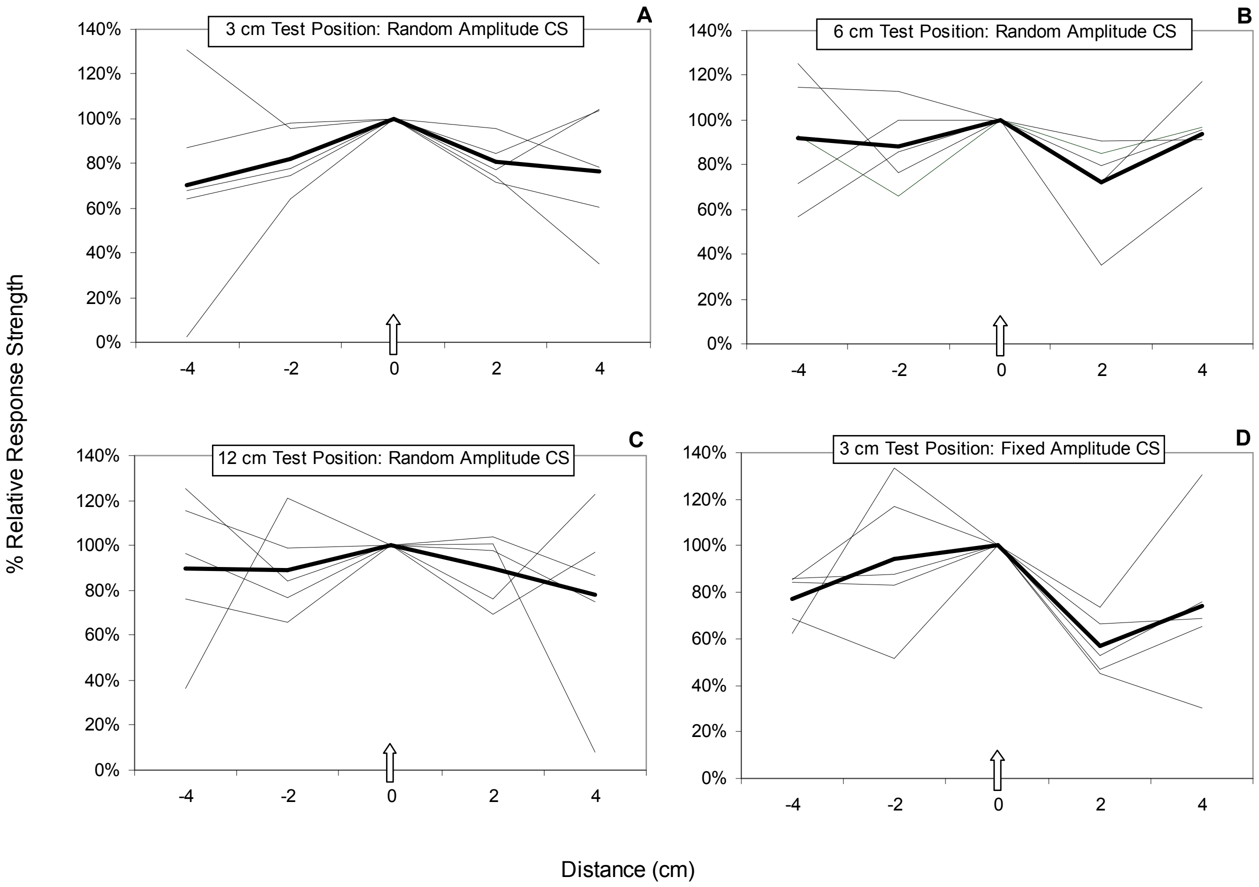

Figure 3.5. Relative generalization gradients plotted as a function of mean normalized responses for location tests (experiment 3). Training stimuli are indicated by upward facing arrows. Figures A, B, C and D illustrate responses for location tests. Figures A (3 cm transect), B (6 cm transect) and C (12 cm transect) contain data for animals trained using a range of amplitudes and figure D (3 cm transect) responses for animals trained using a fixed amplitude.

Discussion

Response similarity and stimulus perception

Similarity in responses to a test stimulus and a conditioned stimulus (CS) reflects a perceived similarity between the test stimulus and the CS (Kimble, 1961; Guttman, 1963). If the similarity of responses declines as the test stimulus varies with respect to the CS along a particular dimension, it is likely that this physical dimension gives rise to a perceptual dimension for the subject (Guttmann & Kalish, 1956; Kimble, 1961; Shepard, 1987). Complete generalization indicates an overlap of features characteristic to both the test stimulus and the CS and incomplete generalization reflects a less than identical match of these features (Mackintosh, 1974).

Fay (1970, 1992) conditioned and tested animals using classical respiratory conditioning, a stimulus generalization paradigm, and single tones delivered using a loudspeaker source to explore the perceptual dimension of pitch in goldfish. The resulting response curves in both experiments clearly show gradients in responding generally characterized by uniform decrements in response strength with increasing differences in frequency between the CS and novel stimuli. Animals in these experiments demonstrated a pitch-like perceptual dimension similar to that possessed by humans.

Fay (1995a) has also established evidence supporting a timbre-like perceptual dimension in goldfish. In this series of experiments he employed a similar training and testing paradigm. Stimuli consisted of pure tones, harmonic complexes and pulsed signals, delivered by a loudspeaker. Results from this study indicate that both stimulus pulse repetition rate and spectral envelope influence behavior. As differences in these physical dimensions between the CS and the test stimuli increased, response strength decreased. In addition to demonstrating that goldfish have perceptual dimensions of pitch and timbre (Fay, 1972, 1994, & 1995a), Fay has also shown that these animals perceive asymmetries in amplitude modulation in much the same way as humans (Fay et al., 1996).

Goldfish generalize to stimulus amplitude and position

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 3.4). 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 positions, 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. In general, increasing change in source position elicited slightly weaker responses, both for sources that were rostral (headward) and caudal (tailward) to the training position. It should be noted however that there was a marginally more obvious gradient in responses when tested along the 3 cm transect (see Figure 3.5A) as compared to the 12 cm transect (see Figure 3.5C). 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 position 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 position. 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 positions.

Goldfish do not generalize to frequencies separated by octave intervals

Novel test frequencies located at full octave intervals from the CS failed to elicit any notable response by the animals. The lack of response to the dipole test stimuli suggests that animals perceived categorical 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. 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).

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 characterized by monotonic decrements as a function of increasing spectral distance from the CS 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. Fay (1969) using a 40 Hz dipole CS found a very steep gradient in responding over a test stimulus range of 50 Hz. 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, 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). Fay (1969, 1970) conditioned goldfish using single tones between 40 and 1600 Hz and tested animals using single tones within ~ ± 1 octave of each CS. He found that generalized responses fell off monotonically as a function of increasing difference between test stimuli and the CS. However, for CS frequencies ≤ 400 Hz, responses to test frequencies outside of 1 octave from the CS approached chance levels. This is corroborated by the present results. Fay (1992) conditioned goldfish using a stimulus tone consisting of duel frequency components (166 Hz and 724 Hz) and tested over a range (95-1514 Hz) of single frequency tones. He found that animals generalized most to the single frequency tones closest to those used to produce the duel tone CS (Fay, 1992). Further support for the existence of a pitch-like dimension in goldfish is evident in two additional studies (Fay, 1995a, 2005). Fay (1995a) found that training with a 100 Hz pure tone and testing with pure tones, ranging between 200-500 Hz, resulted in a monotonic generalization gradient. Moreover, training animals using a harmonic complex with a 100 Hz fundamental and testing with a series of harmonic complexes (fundamental frequencies ranging between 60 and 200 Hz) also resulted in a steep monotonic gradient in responding (Fay, 2005). In both of these experiments decrements in responding increased as the difference between the CS and test stimuli increased.

When considering the results of the present experiment and those of Fay (1969, 1970, 1994, 1995a, & 2005) it appears that goldfish definitely have a perceptual dimension that is pitch-like, but only for frequencies within close spectral proximity to a reference stimulus (e.g. the CS). They do not generalize across octaves.

It has been suggested that since a perceptual dimension corresponding to stimulus frequency is present in so many widely-disparate groups [fishes (Fay, 1969, 1970, 1994, 1995a, 2005), birds (MacDougall-Shackleton & Hulse, 1996; Njegovan, Ito, Mewhort, & Weisman, 1995; Cynx, 1993 and Page, Hulse, & Cynx, 1989) and non-human primates (Brosch, Selezneva, Bucks, & Scheich, 2004)], pitch perception may have been a primitive vertebrate sensory ability ,Fay, 1998; Popper & Fay, 1999). Although frequency selectivity is common to all vertebrates studied to date, only one common contributing 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.

Another potential difference in the pitch perception of vertebrate groups is the phenomenon of relative pitch perception. The internal representations of frequency for human listeners are often described as being either absolute or relative (relational). Absolute pitch perception refers to the ability to identify or reproduce a previously experienced tone without an immediately available reference (Bachem, 1937; Ward, 1999; Parncutt & Levitin, 2001; Zatorre, 2003) and relative pitch perception refers to the use of interval relationships between tone frequencies (Burns & Ward, 1982; Burns & Campbell, 1994) in other words the frequency ratio between temporally adjacent tones. These descriptions of pitch perception have been extended to more loosely describe the use of absolute and relative pitch cues by animals. In the present study goldfish also failed to recognize the harmonic relationship (octave intervals) between test stimuli. Instead they demonstrated a more absolute pitch-like percept in the sense that they were able to recognize a stimulus of specific frequency presented in the absence of a reference stimulus.

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 potent octavolateralis stimulus. Additionally, we found that these animals seemed to perceive stimuli of the same frequency that vary along the dimensions of intensity and position to be qualitatively similar. Further investigation using a broader range of test stimuli, separated by larger intervals, and under similar conditions where animals generalized to the stimuli used in the present study (e.g. position and intensity stimuli) may be warranted. Data collected in the present study suggest that position may be perceived more saliently at close range, but is generally not of paramount salience in octavolateralis detection of dipole sources. Testing this hypothesis by presenting stimuli at distances closer to the animal may contribute to a more complete conception of the role of the octavolateralis system in providing a functional sensory image of the immediate environment. Experiments such as these may provide useful information concerning how perception of particular stimuli (e.g. vibratory sources) change with increasing physical distance and which dimensions of a stimulus (e.g. frequency and amplitude) field animals are attending to as location in space changes relative to the animal.

Chapter 4

Summary and General Discussion

Objectives of the present study of fish bioacoustics

The first objective of the present body of work was to examine the role of the octavolateralis system sub-channels (i.e. inner ear and lateral line) of the goldfish with regard to detection of a low frequency vibratory stimulus source. Specifically addressed were questions concerning the sensitivity of each sensory sub-channel to a dipole source and which octavolateralis channel(s) were controlling behavior. The second major objective in this undertaking was to discern what, if any, perceptual dimensions and salience are associated with the stimulus properties of frequency, amplitude and position, by the goldfish. How are these physical properties perceived? These avenues of inquiry were pursued using classical conditioning and examining both detection of and generalization to a submerged vibratory (dipole) stimulus source and aerial stimuli.

Detection and perception of vibratory stimulus sources

Vibratory stimulus sources produce both particle movement (oscillations and hydrodynamic flow) and sonic energy (pressure fluctuations). Detection of either or both particle movement and pressure fluctuation components of a stimulus field are an important means of gathering information involved in an aquatic animal’s perception of and interaction with the surrounding environment. The octavolateralis system of the goldfish is sensitive to both hydrodynamic and sonic stimuli. Existing research has established that the lateral line and inner ear of the goldfish octavolateralis system have overlapping ranges of sensitivity (Coombs, 1994; Plachta et al., 1999; Weiss & Martini, 1970; Enger, 1966; Jacobs & Tavolga, 1967; Offutt, 1968; Weiss, 1966; Yan, 2001). If both of these octavolateralis components are activated by some environmental stimulus either one or both sub-channels has the potential to influence behavior.

Objects that oscillate or vibrate (multi-polar stimuli) are common stimulus sources in aquatic environments (e.g. moving appendages of a swimming animal). Even the lowest order multi-polar sources (i.e. dipoles) produce complicated stimulus fields having both hydrodynamic and sonic components (Kalmijn, 1988). Therefore, the vibrating stimulus source used in this research (e.g. dipole) potentially stimulated multiple octavolateralis sensory channels simultaneously.

Summary of Findings

Although goldfish are not known to produce sound, for the purpose of communication, they do use sound (i.e. sonic energy) in some behaviors as demonstrated in the present study. The goldfish is a fish specialized for sound pressure detection (sonic audition) and sonic audition was found in the present study to be the prevailing mode of detection for vibratory stimuli. When behavior was classically conditioned to a 40 Hz vibratory stimulus sonic audition was the primary means of detection (see study 1). Individual sensory channels of the octavolateralis system were inactivated and sensitivity of the remaining intact systems was assessed as it related to the behavior of interest. Under the conditions of the present study, inactivating the lateral line system had virtually no consequence with respect to sensitivity to a low frequency vibratory stimulus. The inner ear was sufficient for detecting the vibratory source. Inactivating the gas bladder resulted in a significant decrement in detection threshold. This indicates that the animals were responding to the acoustic sound field (sonic energy) emanating from the dipole. It was sound pressure as opposed to hydrodynamic flow or inner ear stimulation by particle oscillations that most strongly influencing behavior.

Under the conditions of the present study, the lateral line and inertial inner ear were sufficient to detect a vibratory low frequency sound source. However, when acting in concert with the gas bladder (Weberian apparatus) hearing sensitivity was greatly improved. Study 1 of the present research confirms that, unaided by the gas bladder, the inertial inner ear and the lateral line are capable of influencing behavior. Either one is sensitive to a low frequency dipole source located within the nearfield, but inner ear sensitivity is more acute with the gas bladder intact. The combined results of the detection experiments in study 1 of the present research suggest that, as is the case with human hearing, sonic audition is an important mode of hearing in the goldfish as evidenced by its influence on the animal’s behavior.

Animals generalized to changes in stimulus intensity and position for a single frequency (40Hz) vibratory source (see study 2). Changes in stimulus intensity at the source and location were accompanied by changes in pressure at the animal and yet they generalized to variations of both stimulus dimensions. The constant variable in both the intensity and location experiments was the 40 Hz energy of the stimulus source. Furthermore, animals did not generalize to changes in stimulus frequency, not accompanied by changes in pressure at the animal, strongly suggesting that animals were responding to frequency. For this particular vibratory stimulus and under the specific conditions of study 2 stimulus frequency was a more salient cue than cues associated with position & intensity (e.g. changes in pressure at the animal).

The fact that fish generalized to changes in position is most parsimoniously explained by recognizing that changes in pressure via the gas bladder provide non-vector information. Although both the directionally sensitive inertial inner ear and spatially sensitive lateral line array are capable of detecting vibratory stimuli, this information might have been discounted or superseded in a perceptual hierarchy of importance by simultaneous stimulation of the pressure sensitive inner ear.

In comparing goldfish auditory abilities to that of other vertebrates, including humans, a pitch-like perceptual sense is cited as one fundamental similarity among others in hearing by goldfish, non-human vertebrates and humans (Fay, 2005). In the present study frequency appears to have been a particularly relevant physical stimulus feature for goldfish. Previous research has established that goldfish and humans appear to have so much in common with regard to the functional aspects of hearing. This makes the finding that goldfish do not generalize to frequencies separated by octave intervals note worthy, with respect to the evolution of vertebrate hearing, because octave generalization is an ability shared by some other vertebrates including humans.

Significance of Findings and Suggestions for Future Inquiry

The present expands upon our knowledge of the multichannel octavolateralis system with regard to sensory sensitivity and perception of low frequency vibratory stimulus sources in aquatic environments. Physiological studies help us to better understand what a system is capable of, its limitations. However, behaviorally derived data, such as that in the present work, give us an idea of what may occur naturally while still recognizing that the carefully controlled and manipulated experimental environment is probably much less complex than the animal's natural environment. Nonetheless, the data produced in behavioral studies have the potential to provide essential clues when investigating the knotty enigma of the functional evolution of auditory processing.

Continuing this line of research to include testing the sensitivity of the inner ear, by simultaneously inactivating both the lateral line and the gas bladder, may reveal yet additional information concerning the interaction of the octavolateralis sub-channels. It may also be instructive to conduct a series of generalization experiments similar to those in the present study but testing animals with the gas bladder both intact and inactivated, over a smaller range of frequencies and using smaller increments (e.g. 3-5 Hz) for greater resolution. Similar location and amplitude experiments using a greater range of stimuli and larger increments between test stimuli may reveal gradients in responding not detectable under the conditions of the present study. Replicating these experiments using fish species with other types of hearing specializations (physiological structures other than the Weberian apparatus) may also be revealing especially with regard to multisensory interaction and functional hearing similarities between species.

The present study raises some thought provoking questions. Could the experimental protocol used in the present experiments have influenced the outcome? Braun and Coombs (2009) provide recent evidence that in fact the experimental protocol used to test octavolateralis subsystems may indeed influence the outcome of a behavioral experiment. Did motivational factors (e.g. fear and anxiety) influence responding? The low voltage electric shock used as the US could have been perceived as a noxious stimulus. Is a specific stimulus dimension, such as frequency, a more or less salient cue when associated with a noxious or aversive stimulus as compared to a non-aversive stimulus? Would conducting experiments, such as those used in the present study, using an operant conditioning paradigm and a rewarding outcome associated with responses to the CS yield results similar to those in the present study? These questions are yet to be answered.

Behavioral experiments using non-human animals, such as those in the present research, are generally labor intensive and relatively time consuming making their execution costly. So why bother? Knowledge gained using comparative models of other biological sensory systems may be used to develop artificial systems for the detection of sound and development of guidance systems for autonomous robots or vehicles. Furthermore, developing a more complete understanding of vertebrate hearing may be of assistance in better understanding hearing damage, loss and abnormalities in humans, and in turn may result in improved and new therapies, corrective measures and technological aids. Another benefit of better understanding the sensory / perceptual world (the *umwelt*) of other species is that we can better grasp how our past, present, and future actions influence the way that other animals experience their environment.

The exact nature of the functional relationship between the inner ear and the lateral line system is yet to be completely defined. However, each new study that illuminates some previously unanswered question, that confirms or fails to provide support for some previous hypothesis or that incites new questions, advances progress in better understanding the octavolateralis system of fishes and vertebrate hearing in general.

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