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Infrasound initiates directional fast-start escape responses in juvenile roach *Rutilus rutilus*

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Summary

Acoustic stimuli within the sonic range are effective triggers of C-type escape behaviours in fish. We have previously shown that fish have an acute sensitivity to infrasound also, with acceleration thresholds in the range of 10^{-5} m s^{-2}. In addition, infrasound at high intensities around 10^{-2} m s^{-2} elicits strong and sustained avoidance responses in several fish species. In the present study, the possible triggering of C-escapes by infrasonic single-cycle vibrations was examined in juvenile roach *Rutilus rutilus*. The fish were accelerated in a controlled and quantifiable manner using a swing system. The applied stimuli simulated essential components of the accelerations that a small fish would encounter in the hydrodynamic flow field produced by a predatory fish. Typical C- and S-type escape responses were induced by accelerations within the infrasonic range with a threshold of 0.023 m s^{-2} for an initial acceleration at 6.7 Hz. Response trajectories were on average in the same direction as the initial acceleration. Unexpectedly, startle behaviours mainly occurred in the trailing half of the test chamber, in which the fish were subjected to linear acceleration in combination with compression, i.e. the expected stimuli produced by an approaching predator. Very few responses were observed in the leading half of the test chamber, where the fish were subjected to acceleration and rarefaction, i.e. the stimuli expected from a suction type of predator. We conclude that particle acceleration is essential for the directionality of the startle response to infrasound, and that the response is triggered by the synergistic effects of acceleration and compression.

Key words: fish, *Rutilus rutilus*, startle response, C-escape response, S-escape response, predator avoidance, infrasound, acceleration, compression, rarefaction, Mauthner neuron.

Introduction

Most fish and aquatic amphibians have the ability to perform conspicuous and rapid escapes in order to evade predatory attacks and thereby increase their probability of survival. The C-start is the most frequent of these startle responses and by far the best studied in terms of sensory motor control. Under natural conditions it is typically initiated by high intensity acousticolateral, somatic or visual stimuli activating either of the paired Mauthner cells (M-cells) within the brain stem (see reviews by Faber et al., 1989, 1991; Korn and Faber, 1996; Zottoli and Faber, 2000; Eaton et al., 2001). A key feature of the C-escape in fish is the orientation of the response away from the threatening stimulus source. Such a directionality mediated by input from the touch, lateral line and visual system has its natural basis in the well-established topographic organization of the central nervous representation of the visual field and mechanoreceptors in the skin. In addition, fish will consistently turn away from a sound source such as a ball dropped into the water above the fish (see review by Eaton et al., 1995). Eaton and Emberley (1991) demonstrated a simple inverse relationship between the angles of such stimuli and the angular components of the response movements, suggesting that the fish measures the sound source angle, which subsequently controls the magnitude or time span of the initial, rotational phase of the C-response.

The otolith organs of the inner ears in fish are inertial motion detectors directly stimulated by the particle accelerations of a sound wave, and fish may use these organs to determine the three-dimensional directionality of an incident sound wave (see review by Sand, 2002). Upon exposure to sound, the surface of a swim bladder may show amplified radial motions that are transmitted to the inner ear, providing an auditory gain to the fish (see review by Popper et al., 2003). Thus, fish with a swim bladder are sensitive to both the kinetic and pressure components of sound. By decoding the phase difference between these components, fish may be able to discriminate between opposing sound sources (180° apart) (Schuijf, 1975, 1981; Buwalda et al., 1983; Schellart and de Munck, 1987).

The phase comparison theory for sound source localization in fish has recently been extended to a neural model (called the XNOR-model) for how the different elements of the neural escape network may perform the left–right sound discrimination evident in acoustic startle behaviour (Eaton et

al., 1995; Guzik et al., 1999). In essence, the model predicts that directionality is determined by a time domain neural analysis of the initial polarities of the sound pressure and acceleration. Thus, an attack from the right will produce an initial right to left acceleration combined with a pressure increase, while a suction type of predator at this position will cause left to right acceleration and a rarefaction. Crucial assumptions of the model are that both these initial combinations of sound pressure and acceleration should inhibit the left and activate the right side Mauthner cell system, in order to elicit the appropriate escape to the left.

Numerous neurophysiological and behavioural studies have been conducted to clarify the sensory modalities involved in startle behaviours in fish. However, most of the behavioural studies performed to date have been hampered by insufficient control of the stimulus parameters. One of the few studies with a carefully controlled stimulus design is that of Blaxter et al. (1981), who found that herring *Clupea harengus* L. were able to perform C-starts away from an underwater sound source independently of whether the first sound cycle started with compression or rarefaction. However, the stimulus frequencies used were too high (26–160 Hz) to conclude whether the C-starts were elicited by sound compression, rarefaction, or both. Thus, behavioural data relevant for testing the validity of the XNOR-model is still lacking, as is also electrophysiological data. By performing intracellular recordings from M-cells in the goldfish, Canfield and Eaton (1990) showed that sound pressure was the salient stimulus for activation, but independent effects of sound compression *versus* rarefaction were not studied. In the later study by Casagrand et al. (1999), sound acceleration and pressure were found to be effective stimuli of both M-cells and other M-cell homologs in the brain stem. However, the frequencies used were again too high (100–2000 Hz) to reveal the relative effects of sound compression and rarefaction. In addition, the acceleration and pressure components were tested separately and not jointly in a manner comparable to natural conditions.

In earlier studies of acoustic escape responses in fish, the focus has mainly been on frequencies above 100 Hz. However, a typical head-on attack by a predatory fish produces complex hydrodynamic and acoustics stimuli with frequency components mainly below 100 Hz (Bleckmann et al., 1991). In fact, a swimming goldfish generates a hydrodynamic flow field with the main acceleration components below 10–20 Hz (Enger et al., 1989). The possible significance of such low frequency stimuli for escape behaviour is still unknown. We have previously shown that the otolith organs in fish are highly sensitive to the acceleration component of infrasound down to at least 0.1 Hz (Sand and Karlsen, 1986; Karlsen, 1992a,b). Typical behavioural threshold values are in the range of 10^{-5} m s^{-2}, or 4 orders of magnitude less than for detection of linear accelerations in humans. At higher intensities around 10^{-2} m s^{-2}, infrasound can initiate strong avoidance responses in fish (see review by Sand et al., 2001). For a prey fish, such sounds could indicate an approaching predator. We thus predicted that also infrasound might excite Mauthner neurons, and we have tested this assumption by using juveniles of the ostariophysan species roach *Rutilus rutilus* L. The fish were tested in a swing apparatus in which a suspended chamber was used to accelerate both the fish and surrounding water linearly in a quantifiable way (Karlsen, 1992b). Infrasonic accelerations did evoke C- and S-type startle responses. The responses were directional, and on average in the same direction as the initial phase of acceleration. In contrast to the postulations of the XNOR-model, only fish subjected to the combination of acceleration and compression readily responded, while those experiencing acceleration and rarefaction did not.

Materials and methods

Experimental Animals

Juvenile roach *Rutilus rutilus* L., 2–3 cm long, were caught by dip net from the shores of the freshwater lake Aarungen and transported gently 2 km to the test site at the Marine Biological Station in Drøbak, Norway. The fish were kept in aerated aquaria and fed either commercial fish food or freshly thawed chironomid larvae. The fish were held for at least 3 days in the storage tanks before experiments. A total of 54 fish were used in the experiments, which were conducted in accordance with the Norwegian Animal Welfare Act of 1974 and the Regulation on Animal Experimentation of 1996.

Experimental apparatus

The test apparatus was a swing system previously employed to study infrasound hearing in fish (see diagram in Karlsen, 1992b). In short, a Perspex chamber filled with water was suspended by four steel strings from a solid steel framework attached to a 150 kg concrete block. The block rested on a 20 cm layer of dry sand poured directly on the concrete basement floor. The horizontal background acceleration noise level measured at the chamber wall was less than 10^{-6} m s^{-2} in the frequency range 0.1–200 Hz. The dimensions of the chamber were 40 cm×20 cm×15 cm, corresponding to a volume of 12 l. The test chamber was accelerated by a Derritron VP3 vibrator (Riverside, CA, USA) secured to the concrete block and connected to the end wall of the suspended chamber by a horizontally aligned metal shaft. The displacement and acceleration of the chamber during testing were monitored by a linear variable differential transformer (LVDT) (Shaevitz 100 DC-D, Hampton, VA, USA) and an accelerometer (Entran EGCS-A2-2, Les Clayes-sous-Bois, France) attached to the chamber wall opposite to the vibrator. The pressures at different positions inside the chamber were measured using a hydrophone (Brüel & Kjær 8104, Nærum, Denmark). The sinusoidal driving voltage to the vibrator was produced by a function generator (Wavetek 186, Norwich, UK) and consisted of a single cycle. In order to avoid acceleration transients at the onset of the stimulus, the waveform was d.c.-shifted one peak value and phase shifted to start at –90° (Fig. 1). The waveform was pulse triggered and passed through

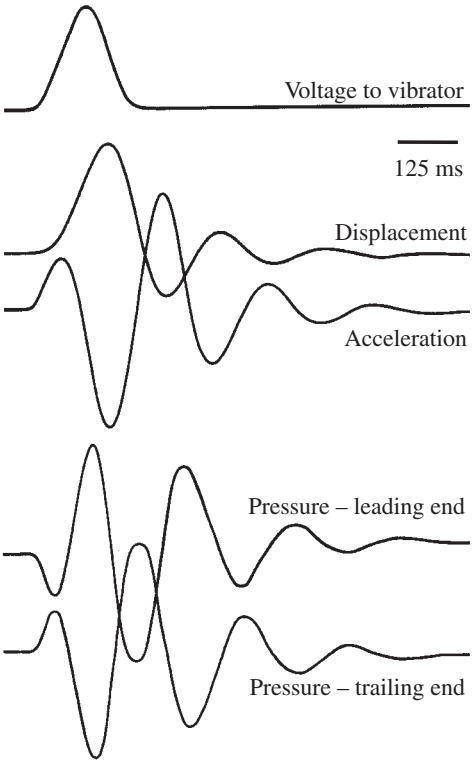

Fig. 1. Comparison of the waveform of a 4 Hz driving voltage to the vibrator and the resulting acceleration and displacement of the chamber, as well as the pressure changes inside the chamber. The pressure was measured close to the leading and the trailing chamber wall, respectively. The figure reflects relative waveforms only, and all parameters are presented in arbitrary units. The driving waveform comprised a single cycle of a sinusoid that was d.c.-shifted one peak value and phase-shifted to start at –90°. The waveform of the initial acceleration approached a sinusoid of about 1.7× the driving frequency, whereas the frequency of the initial compression or rarefaction inside the chamber was about 2.1× the driving frequency.

an attenuator and a power amplifier before reaching the vibrator. The pulse triggering the function generator was initiated manually using a stimulator (Grass S4, West Warwick, RI, USA).

The behaviour of the fish was monitored by video recording at 25 frames s^{-1} using a Sony DCR-VX 1000E video camera looking down at the fish through the transparent roof of the test chamber. The bottom of the tank was light grey, in order to make the darker fish stand out when seen from above, and marked with thin centre lines. The behaviour of the fish was observed in real time on a TV screen, and the video recordings were simultaneously stored on a Hitachi Super VHS video recorder. A small red LED display driven by the triggering pulse to the function generator was placed at the corner of the camera view. A digital oscilloscope was used to simultaneously record the driving voltage to the vibrator and the outputs from the LVDT, accelerometer and hydrophone. Hard copies of the respective waveforms were obtained using an *x,y* plotter connected to the oscilloscope.

Stimulus waveform

The waveforms of the driving voltage to the vibrator and the outputs from the different transducers are compared in Fig. 1. The shape of these waveforms were inverted, but otherwise unaffected, by changing the initial acceleration from push to pull mode. Evidently, the simple one cycle, phase- and d.c.-shifted driving voltage induced chamber accelerations and displacements of rather complex waveforms. However, the escape responses were evoked during the period between stimulus onset and the initial acceleration peak. Within this period, the acceleration approached the initial half-cycle of a sinusoidal waveform of 1.7× the driving frequency, starting at –90°. Hence, assuming that acceleration was the relevant stimulus parameter, the effective stimulus frequency was about 1.7× the frequency of the driving voltage to the vibrator. The frequency of the corresponding initial compression or rarefaction inside the chamber was approximately 2.1× the driving frequency. In the result section, the stimulation frequency is presented as the frequency of the initial acceleration. In most of the tests, the driving frequency was 4 Hz, and the frequency of the initial acceleration was then approximately 6.7 Hz.

Testing procedure

Before testing began, about eight fish at a time were transferred gently to the test chamber and allowed to rest for at least 8 h, usually overnight, before the first stimulus. Care was taken to ensure that no air bubbles remained in the chamber after transfer of the fish. The test chamber was normally filled with water from the lake. However, in some tests the lateral line was blocked by adding 0.1 mmol l^{-1} Co^{2+} to virtually Ca^{2+}-free artificial freshwater. This procedure completely abolishes the mechanosensitivity of the lateral line system in roach without affecting the inner ear (Karlsen and Sand, 1987). A total of 54 fish were tested in the main series of experiments, employing the 6.7 Hz stimulus at 15 dB above threshold, and each group of 7–8 fish in the chamber was stimulated four times. The test fish were not tagged, and we were thus unable to examine the responses and responsiveness of each individual fish. All experiments were recorded on videotape, and a resting period of minimum 30 min was allowed between tests. The swing apparatus was kept in a separate room isolated from the control room, in which the investigators were conducting the experiments. All the electronic instruments, apart from the camera, the vibrator and the transducers attached to the chamber, were also kept in the control room.

Data analysis

During playback analysis, the frame-by-frame movement of each fish in a given trial was traced by hand onto a clear plastic film taped to the front of the TV monitor. The frame corresponding to stimulus onset was identified by the flashing LED. Tracing was done for 5 frames, or 200 ms, starting at the frame before stimulus onset. A distance calibrator within the chamber made it possible to calculate the swimming distances

and velocities between frames. For plots of startle trajectories, the position of the head of the fish in each frame was converted into *x,y* coordinates, starting at (0,0) for the frame before stimulus onset. Coordinates were entered into a computer and the trajectories were plotted using the program Sigmaplot 2000. The final escape angle relative to the direction of acceleration was calculated using the head coordinates of last two frames of the 5-frame sequence.

Results

The response

Before stimulation, the fish moved calmly through the camera field of view at a swimming speed of about 1 cm s^{-1}. After onset of a stimulus above the threshold level for a behavioural response, some of the fish would immediately shoot off, usually in a direction deviating from the pre-stimulus cruising course. The maximum swimming speeds during the response were in the order of 25 *BL* s^{-1} (*BL*, body lengths), or 63 cm s^{-1} for the 2.5 cm long test fish. By about 5–7 frames after initiation of the response, the fish had resumed their original gliding behaviour. The acceleration threshold of the response, which was taken as the minimum acceleration needed to trigger at least one fish out of several, was 0.023±0.004 m s^{-2} (mean ± S.D., *N*=6) at 6.7 Hz acceleration (r.m.s. of the initial half-cycle). Even close to threshold, the fish in the trailing half of the chamber (see later) responded vigorously in an all-or-none fashion, while the remaining fish seemed unaffected. There was typically no gradation of the responses, which clearly displayed the characteristic short latency and initial body bending patterns of Mauthner startle behaviours. A total of 84 responses to an initial 6.7 Hz acceleration stimulus about 15 dB above the response threshold were studied in detail, which revealed the following body shapes in the first video frames after stimulus onset: 'C' (57), 'S' (10), 'J' (10) and 'I' (7). In fish of the 'J' category, only a tail bend was seen before the forward spurt, while an 'I' shape, or no obvious body bend, was typical of fish about to shoot straight forward with no change in trajectory.

The fish did show some variability in the apparent latency of the response. This was mainly due to lack of synchronization between the video frame timing and the manually initiated stimulus. The frame containing the LED monitor flash was termed frame 0. The starts of the 84 escapes were distributed as follows: frame 0, 1; frame 1, 30; frame 2, 43; frame 3, 7; frame 4, 1; frame 5, 2. To be sure that the fish were responding to the initial phase of acceleration at 6.7 Hz, and to minimize the chance of secondary responses to other responding fish, the 10 startle responses occurring in frames 3–5 were excluded when response latencies and final escape angles were estimated and when escape trajectories were traced. The average latency of the remaining 74 responses was approximately 63 ms. These responses were initiated within the duration (about 80 ms) of the initial compression or rarefaction in the chamber (Fig. 1).

We also made a series of preliminary experiments on the threshold of the acceleration response as a function of frequency. Each frequency was calculated from the rise time of the accelerometer waveform. Our tentative conclusion was that the threshold acceleration needed to trigger the response became higher as frequency was decreased within the tested range between 3.4 and 32 Hz.

Polarity of the response

During the early stages of the project, we noticed that the fish showed a tendency to jump or shoot in the same direction as the initial acceleration of the swing. To study this further, we changed the polarity of the initial swing movement between push and pull, in a quasi-random fashion. The frequency of the initial acceleration was 6.7 Hz and the stimulus level was approximately 15 dB above the response threshold. Fig. 2 shows the resulting flight trajectories of the response to initial movements of the test chamber from right to left, push-mode (A), and from left to right, pull-mode (B). The direction of the flight trajectories showed a wide scatter, but the average direction coincided with the direction of the initial acceleration. The final escape angle relative to the stimulus direction, as defined by Domenici and Blake (1993),

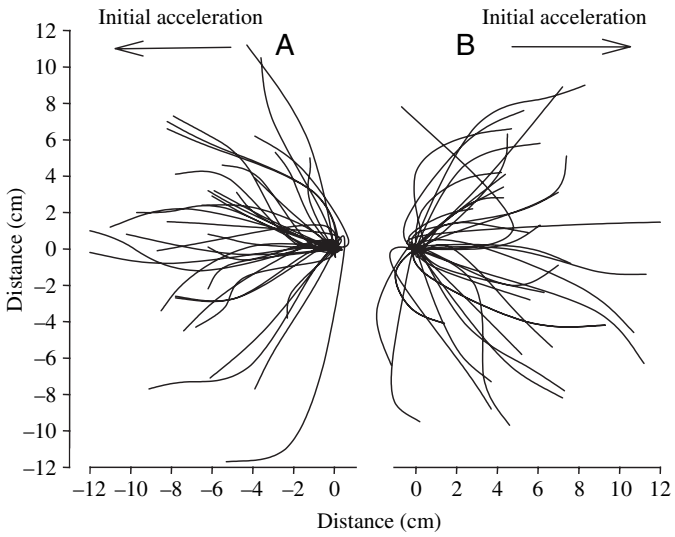

Fig. 2. Smoothed startle trajectories displayed by juvenile roach in response to the initial half-cycle of an acceleration of about 6.7 Hz, at a stimulus level approximately 15 dB above the response threshold. Movements of the fish where measured in the horizontal plane from video frames recorded by a camera looking down on the fish through the transparent roof of the test chamber. The trajectories show movements of the head of the responding fish during a 160 ms period, i.e. from the video frame before stimulus onset (0,0) and through the subsequent four frames. (A) Trials in push mode with the initial acceleration to the left resulted in 36 startle responses from fish in the trailing (right) half of the chamber, which experienced compression, and no responses from fish exposed to rarefaction in the leading (left) half. (B) Tests in pull mode with the initial acceleration to the right resulted in 35 startle responses from fish in the trailing (left) half of the chamber and 3 startle responses (not illustrated) in the leading (right) half of the chamber. Startle responses in both stimulus situations were on average in the same direction as the initial acceleration.

approximated a symmetric and unimodal distribution as shown in Fig. 3. There was no obvious difference between the average response direction of the first fish responding in a group and the more delayed startle responses, strengthening the assumption that all the analysed responses were to the initial stimulus and not secondary to other responding fish.

For initial accelerations in both push and pull modes, it was noticed that nearly all escape responses occurred in the trailing half of the chamber, where the initial acceleration was associated with a pressure increase. Only 3 of the 74 startle responses studied in detail occurred in the leading half of the chamber, where the initial acceleration coincided with a pressure decrease. A summary of these data is shown in Fig. 4. In preliminary tests, the ambient pressure within the tank in the absence of acceleration was abruptly increased by rapidly lifting the outlet tube. Such treatment had no behavioural effects at all, even for sudden pressure elevations much larger than those encountered in the acceleration studies, suggesting that the observed responses were not induced by increased pressure alone. In the future, experiments allowing independent control of acceleration and pressure changes of variable waveforms will be performed.

Control for involvement of the lateral line or visual cues

The swing system used in the experiments was designed to move the fish and the surrounding water together as a unit, in order to minimize the possibility of lateral line activation (Karlsen, 1992b). To completely eliminate the possible involvement of this sensory modality in the response, we also tested a group of fish after 24 h in virtually Ca^{2+}-free water containing 0.1 mmol l^{-1} Co^{2+} (Karlsen and Sand, 1987). Cobalt treatment had relatively little effect on the resting behaviour of the fish. The most noticeable deviation from normal behaviour, indicating effective blocking of the lateral line, was that individual fish would occasionally bump into each other. This behaviour was never observed in fish in normal freshwater. Under cobalt blocking, the fish still responded selectively to the polarity of the stimulus, but for a few occasional exceptions. There was still a strong tendency for responses to occur only in the compression side of the chamber, and the trajectories were still on average in the same direction as the initial acceleration (Figs 4, 5). However, the shooting distances were reduced slightly, and this correlated with a slower between-stimulus cruising speed in Co^{2+}-containing water.

All lighting in the test room was arranged to avoid any cues to the fish in the form of moving edges or shadows. In addition, we did a number of tests with no acceleration, but with either a moving light or a series of opaque objects and edges moving against a lit background. In no case did these stimuli evoke flight behaviour, and we conclude that the observed escape responses were not to visual cues.

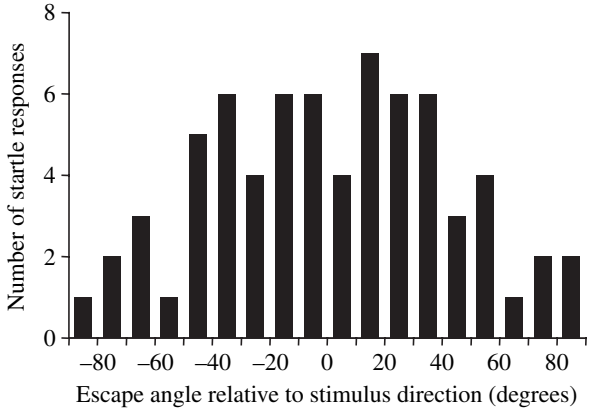

Fig. 3. Histograms showing the angle between the direction of initial acceleration (0°) and the final escape direction. The presented data were calculated using the last two coordinates of the 71 escape trajectories shown in Fig. 2. A total of 69 final escape angles fell within ±90° of the stimulus direction, and approximated a symmetric and unimodal distribution.

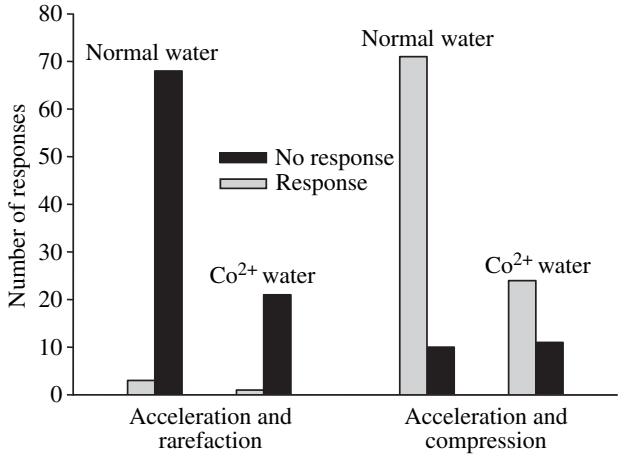

Fig. 4. Histograms presenting the numbers of responsive and non-responsive juvenile roach (2.5 cm) in the rarefaction and compression half of the test chamber, respectively. The frequency of the initial half-cycle of the acceleration was about 6.7 Hz, and the stimulus level was approximately 15 dB above response threshold in all trials. The fish mainly responded to the combination of linear acceleration and a pressure increase. Blocking of the lateral line system by adding 0.1 mmol l^{-1} Co^{2+} to the water (Co^{2+} water) did not significantly change the observed response patterns. The escape responses (see Fig. 2) were therefore triggered by stimulation of the inner ear.

Discussion

Startle thresholds

The primary purpose of the present study was to examine whether the characteristic startle responses of fish may be initiated by infrasonic water vibrations. The rationale was that such low frequency water motions are produced by a potential predator swimming calmly about (Enger et al., 1989), and they will form essential parts of the bow waves produced during striking attacks. Importantly, it has been shown that fish are highly sensitive to the acceleration component of infrasound by using their inner ears (see review by Karlsen and Sand, 1991), and behavioural studies have also documented that

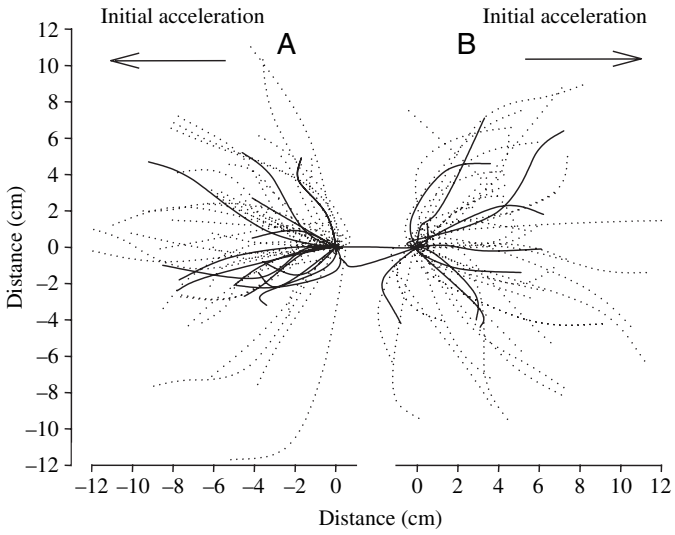

Fig. 5. Smoothed escape trajectories obtained as described in Fig. 2, but from juvenile roach having the lateral line system blocked by 0.1 mmol l^{-1} Co^{2+}. Eliminating the sensory function of the lateral line organs did not significantly change the response patterns. Response trajectories were still mainly in the direction of the initial acceleration, and 25 of the 26 observed startle responses occurred in the compression (trailing) half of the test chamber. For comparison, the trajectories of fish in normal freshwater, as shown in Fig. 2, are included as dotted lines.

infrasound readily elicits escape and other evasive actions in different species (see review by Sand et al., 2001). We have found that high intensity infrasonic vibrations could indeed elicit typical startle behaviours in juvenile roach. The acceleration threshold was about 0.023 m s^{-2} (r.m.s.) at 6.7 Hz, estimated from the initial half-cycle of the stimulus. This threshold is a conservative value since most startle movements started before the initial acceleration peak was reached, i.e. 75 ms after stimulus onset. Still, the threshold is approximately 60 dB above the absolute thresholds for detection of infrasound in the roach (H. E. Karlsen, unpublished observation). Our results are thus in agreement with earlier work showing that the fast-start startle is a high threshold behaviour. The observed threshold is still far below the close range infrasonic hydrodynamic noise produced by swimming fish (*BL* = 5–20 cm; Bleckmann et al., 1991).

The initial acceleration created rarefaction in the leading half of the test chamber and compression in the trailing half. Maximum positive and negative pressure amplitudes were created at the end walls while negligible pressures appeared at the midline of the chamber. Since the test fish were moving freely about and responses occurred at different positions in the chamber, we were unable to relate precise pressure thresholds to the startle responses. However, the hydrophone measurements that were performed indicated that they were in the order of a few tens of pascals. Even though numerous studies have characterized the kinematics and the neural basis of startle behaviour in fish, few studies have been concerned with response thresholds. Still, the limited data that exist are in agreement with the thresholds of the present study. Casagrand et al. (1999) found the acceleration thresholds for detection of excitatory postsynaptic potentials (EPSPs) from M-cells in goldfish to be around 0.01 m s^{-2} at 125 Hz. Casagrand et al. (1999) also recalculated the sound pressure threshold given by Lewis and Rogers (1998), for eliciting directional startle behaviours in goldfish, to an acceleration threshold of 0.03 m s^{-2}. Blaxter et al. (1981) and Blaxter and Hoss (1981) found the threshold for acoustic startle in the herring to be approximately 70 dB above the absolute threshold, corresponding to pressures in the range 2–18 Pa (70–200 Hz) and to accelerations within the range 0.01–0.04 m s^{-2}.

The latency of acoustic or vibratory startle responses in fish are typically in the range 5–40 ms depending on the stimulus intensity and frequency (Eaton et al., 1977; Blaxter et al., 1981). This is significantly shorter than the average response latency of 68 ms we observed at approximately 15 dB above threshold. Although the time resolution of the employed video system is inadequate for estimating the short latencies of startle responses evoked by intense acoustic stimuli at sonic frequencies, the low time resolution is sufficient for a rough estimate of the relatively long average response latencies to infrasonic stimuli observed in the present study. Many factors may influence latency, i.e. temperature, fish species, size etc. However, the relatively long latency in our study may mainly reflect the low stimulus frequency, and thus an increased time to reach threshold levels. In any event, the observed latencies were far shorter than the latency of visually evoked startles, which are typically in the range 100–120 ms. The startle responses to infrasonic vibration were not triggered by the lateral line organs, because selectively blocking this system by using the cobalt technique (Karlsen and Sand, 1987) did not eliminate the responses. Thus we feel confident that all the observed startle responses were triggered by the inner ear. Lateral line stimulation may still participate in evoking startle responses under natural conditions. As noted, the swing system was designed to avoid possible lateral line stimulation, by moving the fish and surrounding water as a unit. However, a striking predator in the field may be approximated by an acoustic dipole source, and will generate potent lateral line stimuli at close range.

Types of startle responses observed

Fish are known to display different types of fast-start escape behaviours as defined by the pattern of the initial body bending (Domenici and Blake, 1997; Hale, 2002; Hale et al., 2002). In the present study we found that all variants of initial body shapes (C, S and J) described for fast-start responses in fish could be triggered by infrasound and thus by output from the inner ear otolith organs alone. The classical C-response comprised 68% of the infrasound-induced startles we studied in detail. In freely moving ostariophysan fish, acoustically triggered C-starts are generally accepted to always involve the Mauthner cell proper, being the first spinal-projecting neuron

activated in the brain stem (Zottoli, 1977; Eaton and Bombardieri, 1978; Eaton et al., 1981; see review by Zottoli and Faber, 2000). In addition to the M-cell, activation of morphologically homologous commissural neurons in adjacent hindbrain segments are currently also believed to be important for the full initial C-bend of the body (Eaton et al., 1982; Kimmel et al., 1982; Metcalfe et al., 1986; Eaton and Lee, 1991; Lee et al., 1993; Forman and Eaton, 1993). The propulsive phase of the C-response, which essentially involves a counter bend of the body, is postulated to be controlled by a functional group of more caudal medullar neurons having ipsilateral spinal projections (Forman and Eaton, 1993; Eaton et al., 2001; Hale, 2002). Direct evidence for the existence of an extensive and hierarchic brain stem escape network has recently been obtained using calcium imaging to monitor the activity in reticulospinal neurons in the transparent larvae of zebrafish *Danio rerio* H. (see review by Fetcho and O'Malley, 1997; Liu and Fetcho, 1999; Ritter et al., 2001; Gathan et al., 2002).

A typical S-escape response, characterized by significant but opposite initial anterior and posterior body curvatures, was observed in 12% of the infrasound startles. The initial body shape strongly suggests that this response most likely does not involve activation of the Mauthner neuron proper. Hale (2002) instead proposed that it may be initiated by a parallel activity of M-cell serial homologs and ipsilateral reticulospinal neurons, activating nearly simultaneously the frontal contralateral trunk musculature and the caudal ipsilateral trunk muscles respectively. In addition to being employed to avoid predators, the S-start behaviour is also used offensively during prey strikes (Webb and Skadsen, 1980; Harper and Blake, 1991; Frith and Blake, 1995; Johnston et al., 1995).

The J-response also comprised 12% of the startle responses. It was characterized by the largest curvature appearing posterior in the animal, with the tail moving almost perpendicular to the anterior axis. Like the S-response, the J-response is also typically used both for escape and attack. Following the model of Hale (2002), the J-response may reflect activation of the same pool of ipsilateral reticulospinal neurons that participate in the typical S-response, but differs from this by showing no activation of commissural neurons.

The I-response (8%) was characterized by an apparent lack of initial body bending apart from a very small tail bend, and it was typically observed in fish about to shoot straight forward with no change in trajectory. It was unclear to us if the I-response was a true startle response, or if it involved a direct activation of neural circuits for fast undulatory swimming rather than the escape network.

It has been suggested that different startle responses may be viewed as a degrading series of escape behaviours initiated by different subsets of the startle neural circuit. Our data seem to fit this idea. In the present study we did not correlate response type to initial movement velocity or orientation of the responding fish. Modulation of the escape response by afferent inputs is, however, known from other studies (Eaton and Emberley, 1991) and away-from-stimulus responses are known to occur significantly more often than toward-stimulus responses (Domenici and Blake, 1993).

Eaton et al. (1977) suggested that fish startles should be unpredictable in order to prevent predators from learning any fixed patterns of response and compensating for it. Escape trajectories have also been found to vary considerably after the initial turn away from the stimulus (Eaton et al., 1981; Eaton and Emberley, 1991). In the present study we found the escape responses of juvenile roach to have both a strong deterministic component (following the direction of initial acceleration) and a significant stochastic secondary component (wide scatter of the trajectories). The final escape angles relative to the stimulus direction, as shown in Fig. 3, indicate a unimodal distribution and thus no definite preferred escape trajectories as was found in angelfish and other animals (Domenici and Blake, 1993).

Directionality of the startle responses

All the different types of infrasound-induced startle responses that we recorded were directional, and thus in concurrence with earlier studies of acoustic startles in fish (Moulton and Dixon, 1967; Zottoli, 1977; Blaxter et al., 1981; Blaxter and Fuiman, 1990; Eaton and Emberley, 1991; Foreman and Eaton, 1993). Even though the flight trajectories showed a wide scatter, with an envelope occupying approximately a hemifield (see Figs 2, 3), the average response direction coincided with the direction of the initial acceleration experienced by the fish. Fish are assumed to be able to determine the direction to simple monopole sound sources by comparing the phase of sound pressure to the phase of the incident particle acceleration. According to this phase model (Schuijf, 1981), the direction of acceleration during sound compression should be interpreted by the fish as movement away from the source. Movements experienced during rarefaction, on the other hand, will be towards the source. In our experiments the startle movements were generally in the direction of the initial acceleration and during compression, which we interpret as an adaptive movement away from a threatening sound source such as an advancing predator.

A prerequisite for the phase model is that the fish is able to separately encode and compare the phases of sound pressure and incident particle motion. In the ostariophysan hearing specialists, including the roach, such a task is clearly feasible. The sacculus in these fish detects extremely low pressure levels by being connected *via* the Weberian ossicles to the swim bladder. The sensory hair cells in the sacculus are arranged in two oppositely oriented populations; one set that depolarises on the compression phase and the other on the rarefaction phase (Furukawa and Ishii, 1967; Hama, 1969; Sento and Furukawa, 1987). Goldfish is known to discriminate behaviourally between compressions and rarefactions (Piddington, 1972). While the sacculus in the ostariophysi appears to be dedicated to encoding phases of sound pressure, the direction of initial acceleration may be encoded by the utricle and the lagena (see review by Popper and Edds-Walton, 1995). In both these otolith organs the hair cells are distributed

with sensory axes at a variety of angles across the sensory epithelium (Popper and Platt, 1983; Platt, 1993).

Role of compression in initiation of startle responses

Casagrand et al. (1999) have shown that both sound pressure and acceleration cause excitatory postsynaptic potentials in Mauthner neurons and homologous cells, but sound pressure was a much more efficient stimulator than acceleration. The authors therefore concluded that acceleration alone would not be able to initiate a startle response. In the present study we were not able to elicit startle responses by pressure increases alone. In natural conditions, neither pressure nor acceleration will appear separately, and it is thus not surprising that both components are necessary to elicit startle in fish. Even though acceleration conveys the crucial directional information to the fish, several studies have shown that the startle escape appears to be triggered only when the pressure reaches a critical value (Blaxter and Hoss, 1981; Blaxter et al., 1981; Blaxter and Fuiman, 1990; Canfield and Eaton, 1990).

Eaton et al. (1995) and Guzik et al. (1999) have adopted the original phase model for directional hearing to explain the directional startle responses. Their model is based on the extensive afferent input from the ear converging on the Mauthner system, and both phases of pressure are assumed to be able excite and drive the Mauthner neurons. Contrary to this assumption, we observed that only fish experiencing acceleration and compression responded, while those that experienced acceleration in combination with rarefaction did not. Rather unexpectedly, this suggests that only a pressure increase may elicit acoustic startles, while rarefactions somehow cancel or inhibit this response – at least at low frequencies. In their behavioural study, Blaxter et al. (1981) found the directionality of acoustic startle responses in herring to be independent of the initial stimulus polarity being compression or rarefaction. This has been interpreted as if the herring were responding equally well to both pressure polarities (Eaton et al., 1995). However, this may not be true since Blaxter et al. (1981) found the threshold to initial rarefaction to be about 3 dB lower than for initial compression. The stimuli used comprised a single cycle of a 80 Hz sine wave, and due to the mass load (resonance properties) of their setup the amplitude of the first half cycle was 3–6 dB lower than for the second half cycle. The increased sensitivity to initial rarefaction may therefore reflect the fact that their fish were responding only to compression irrespective of the stimulus starting with compression or rarefaction. Further studies are clearly needed to clarify these questions.

Conclusions

Fish have an acute sensitivity to infrasound, or linear accelerations, and have been proposed to exploit this ability in a number of behaviours including predator–prey interactions. In the present study we have demonstrated for the first time that intense infrasound is efficient in eliciting classical startle responses in fish. The behaviour is triggered by the synergistic effects of initial acceleration and compression, corresponding to the stimulus generated by (for example) an approaching predator. This finding supports the idea that predator detection may have played a significant role in the evolution of fish hearing and the Mauthner neural system (see review by Eaton and Popper, 1995). Unexpectedly, responses were inhibited by rarefaction, suggesting a need for more behavioural studies designed to further test the validity of current models for neuronal computation of startle directionality.

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