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Dennis M. Higgs
University of Windsor

C. A. Radford

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RESEARCH ARTICLE

The contribution of the lateral line to 'hearing' in fish

D. M. Higgs[1,*] and C. A. Radford[2]

[1]Department of Biological Sciences, University of Windsor, Windsor, ON, Canada, N9B 3P4 and [2]Leigh Marine Laboratory, University of Auckland, PO Box 349, Warkworth, 0941, New Zealand

*Author for correspondence (dhiggs@uwindsor.ca)

SUMMARY

In the underwater environment, sound propagates both as a pressure wave and as particle displacement, with particle displacement dominating close to the source (the nearfield). At the receptor level, both the fish ear and the neuromast hair cells act as displacement detectors and both are potentially stimulated by the particle motion component of sound sources, especially in the nearfield. A now common way to test 'hearing' in fish involves auditory evoked potentials (AEPs), with recordings made from electrodes implanted near the auditory brainstem. These AEP recordings are typically conducted in enclosed acoustic environments with the fish well within the nearfield, especially for lower frequencies. We tested the contribution of neuromast hair cells to AEP by first testing intact goldfish (*Carassius auratus*), then ablating their neuromasts with streptomycin sulphate — disabling superficial and canal neuromasts — and retesting the same goldfish. We performed a similar experiment where only the superficial neuromasts were physically ablated. At 100 and 200 Hz, there was a 10–15 dB increase in threshold after streptomycin treatment but no significant difference at higher frequencies. There was no difference in threshold in control fish or in fish that only had superficial neuromasts removed, indicating that the differential responses were driven by canal neuromasts. Taken together, these results indicate that AEP results at lower frequencies should be interpreted as multimodal responses, rather than as 'hearing'. The results also suggest that in natural situations both the ear and lateral line likely play an integrative role in detecting and localising many types of 'acoustic' stimuli.

Key words: auditory evoked potential, sound detection, mechanosensory, sensory physiology, multimodal.

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INTRODUCTION

Sound propagates underwater as both a pressure and a displacement stimulus, with the relative contribution of each changing as the distance from the sound source increases (Rogers and Cox, 1988; Montgomery et al., 2006). In an unbounded medium (e.g. deep ocean waters) and with a monopole sound source, the relationship between pressure and displacement is fairly predictable near the source, with the displacement component attenuating as a square of distance from the source and the pressure component attenuating with the simple reciprocal of distance (Rogers and Cox, 1988). In a bounded medium (e.g. shallow water or experimental tanks), sound propagation is notoriously complex, however, and is not easily modelled (Rogers and Cox, 1988; Akamatsu et al., 2002), resulting in unpredictable relationships between displacement and pressure components. For a fish detecting acoustic stimuli, both the pressure and displacement components may be detectable, depending on the specific sensory structures involved (Popper and Fay, 1999; Montgomery et al., 2006; Higgs et al., 2006). At the receptor level, both the auditory and neuromast (lateral line) hair cells act as displacement detectors, albeit with presumed differences in frequency sensitivity (Coombs and Montgomery, 1999; Higgs et al., 2006). In the ear, movement of hair cells relative to the overlying otoliths, which lag behind when displaced with sound waves because of their greater density, results in the bending of stereocilia and opening of ion channels to transduce neural activity (Popper and Fay, 1999). Much the same mechanism works in neuromasts but here stereocilia stimulation is caused by differential shear on the overlying cupula (Webb et al., 2008). As such, both the ear and the neuromast can detect displacement components of sound stimuli and the contributions of each are difficult to truly differentiate (Braun and Coombs, 2000; Webb et al., 2008). Pressure components of sound waves are thought to be detected by vibration of internal gas-filled structures such as the fish's swim bladder, auditory bullae or various branchial bubbles (Popper and Fay, 1999). The gas-filled structures vibrate in response to sound waves, sending these vibrations to hair cells as displacement stimuli, although a few species have also been shown to have a connection between a gas bubble and the lateral line system (Webb and Smith, 2000; Webb et al., 2012) and thus may gain displacement information *via* this channel as well.

Despite the complexity of sound travel in experimental tanks (Akamatsu et al., 2002), the vast majority of acoustic testing of fish is done in small tanks because of the difficulty of performing experiments in open waters (but see Chapman, 1973; Chapman and Hawkins, 1973; Chapman and Sand, 1974). This is especially true of physiological measures of acoustic detection. Physiological measures of fish 'hearing' are often conducted by restraining a fish to a platform some distance from a speaker and measuring neural impulses at single-unit (e.g. Enger, 1967; Moeng and Popper, 1984; Sisneros and Bass, 2003; Maruska and Tricas, 2009), multi-unit (e.g. Enger, 1967; Sisneros, 2009) or whole-brain levels (e.g. Corwin et al., 1982; Kenyon et al., 1998; Wright et al., 2005, Vasconcelos and Ladich, 2008). The resulting responses are then taken to represent hearing thresholds and presented as audiograms for the species of interest. While there

is a recent trend of presenting these results in terms of pressure and particle acceleration/displacement (e.g. Casper and Mann, 2006; Horodysky et al., 2008; Wysocki et al., 2009; Belanger et al., 2010; Radford et al., 2012), these studies invariably discuss the results as performance measures of the ear, largely ignoring lateral line inputs. While this approach is certainly justifiable when recording from single neurons or nerve bundles of the VIII nerve, it is less justified in whole-brain recordings such as those seen with auditory evoked potentials (AEPs; also called auditory brainstem responses, ABRs). As the nerve inputs for auditory and lateral line nerves lie in close contact along the dorsoventral axis (reviewed in Higgs et al., 2006), whole-brain potentials are likely to detect both nervous inputs. As the behaviour of sound waves along tank walls of various constructs are difficult to accurately measure, it is likely that a sound stimulus presented in such a situation will have the capability to contain significant amounts of particle motion even some distance from the source that would be difficult to predict based on free-field equations. The unpredictability of the particle motion component therefore makes it difficult to state with confidence whether whole-brain responses truly represent 'auditory' inputs *sensu strictu* as opposed to some mixture of auditory and lateral line responses. Until this dichotomy is better resolved, it will remain impossible to state whether these responses are tests of fish hearing or rather represent a more integrated response to mechanosensory stimulation.

The purpose of the current study was to quantitatively assess the contribution of both auditory and lateral line impulses in response to 'acoustic' stimulation in a typical AEP setup. We used the common auditory model the goldfish, *Carassius auratus* (Linnaeus), to test whole-brain responses to sound stimuli and then tested the same fish again following treatment with streptomycin – a known ablation agent of superficial and canal neuromasts (Brown et al., 2011; Buck et al., 2012) – or physical removal of just superficial neuromasts (Baker and Montgomery, 1999). In this manner we were able to quantify differential responses with only auditory *versus* auditory and lateral line inputs. We show that what had been thought to represent 'hearing' in AEP experiments is in actuality due to both auditory and lateral line inputs, at least at frequencies of 100 and 200 Hz. These results demonstrate the need for caution in the interpretation of AEP results and further show that detection of acoustic inputs in typical shallow water environments is probably accomplished by integrative auditory and lateral line inputs to form a more inclusive gestalt of the sensory information.

MATERIALS AND METHODS

Fish handling

Goldfish were purchased from a local supplier and immediately (within 15 min) transported to the laboratory. Fish were kept in a large, recirculating, aquarium filled with conditioned tap water until used in experiments. Before experiments, fish were lightly anaesthesised with 0.004 mol l^{-1} 2-phenoxy-ethanol (Sigma-Aldrich, St Louis, MO, USA) to allow placement into the testing apparatus and were kept under light anaesthesia with a constant flow of 0.002 mol l^{-1} 2-phenoxy-ethanol over the gills. Previous experiments showed that there was no effect of anaesthetic on fish hearing thresholds (Radford et al., 2012). All fish were tested twice, first as a normal AEP and second after removal to one of the three experimental treatments (see below) for 3 h. In all cases the fish was still alive at the end of the re-test and fully recovered from the manipulations. The AEP recording electrodes were placed in the same location across trials on the same fish.

AEP testing

All testing of evoked potential responses was done on fish in a polyvinyl chloride (PVC, 0.5 mm thick) tank 1.11 m long with a diameter of 0.25 m (Wright et al., 2005). An underwater speaker (UW-30, Lubell Labs Inc., www.lubell.com) was placed at one end of the tank and a Plexiglas fish holder was placed at the opposite end, 0.75 m from the speaker. Sound stimuli were generated with SigGen software (Tucker-Davis Technologies, TDT, www.tdt.com) and delivered to the underwater speaker through a TDT electrophysiology workstation. Tone bursts were delivered at 100, 200, 400, 600, 800, 1000 and 2000 Hz with a 10 ms duration, gated through a Hanning window with a 2 ms rise/fall time. Evoked potentials in response to the tone bursts were collected through two stainless steel subdermal electrodes (Rochester ElectroMedical; www.rochestermed.com); one electrode, the recording electrode, was placed under the skin in the dorsal midline in line with the opercular edge to standardise locations, while the other electrode, the reference, was placed under the skin in the snout.

After initial AEP testing, fish were subjected to one of three treatment conditions; control (N=5), streptomycin treatment (N=5) or a superficial scrape treatment (N=5). For the controls, fish were removed from the AEP apparatus and placed into a recovery tank of conditioned tap water for 3 h. After the 3 h time period, fish were placed back into the AEP apparatus and retested. For the streptomycin treatment, fish were removed from the AEP apparatus and placed into a 0.05% (w/v) solution of streptomycin sulphate in conditioned tap water for 3 h. At the dosage used in the current study, streptomycin sulphate ablates both superficial and canal neuromasts (Montgomery et al., 1997; Brown et al., 2011; Buck et al., 2012), leaving auditory epithelia unaffected (Matsuura et al., 1971; Buck et al., 2012). After streptomycin treatment, fish were placed back into the AEP apparatus and retested. For the superficial scrape treatment, the superficial neuromasts were removed while leaving the canals intact to investigate which part of the lateral line system was mediating the response. Fish were anaesthetised to the point where movements ceased but they were still ventilating their gills, and were then wiped with paper towels to remove any mucus and excess water. The entire surface area of the fish was then scraped several times – first in the rostrocaudal direction then in the caudorostral direction – with a sterilised scalpel blade. The fish was subsequently placed in fresh water and left for 3 h to recover before being placed back into the AEP apparatus and retested. After physiological testing, six fish were anaesthetised and then killed to assess the effects of the experimental manipulations on the auditory epithelia (N=5 each for control and streptomycin treatments). Additional fish were also stained (see below) to assess the effect of streptomycin treatment on canal and superficial neuromasts.

For all AEP recordings, tone bursts were played at subthreshold levels and increased in 5 dB increments until stereotypical AEP traces were seen (Fig. 1). For each frequency and level combination, 500 responses were collected at 90 deg stimulus presentation and 500 responses were collected at 270 deg, with the traces averaged to reduce stimulus artifacts (thus, 1000 response traces were averaged for each frequency–level combination). Sound level was calibrated in terms of pressure at the start of each day's experiments by placing a hydrophone (Reson model LC-10, www.reson.com) at the location of the fish holder and adjusting output so that each frequency played sounds at equivalent levels. Particle acceleration of each sound played was measured directly by placing a waterproofed triaxial accelerometer (Bruel and Kjear model 4524, 100 mV g^{-1} sensitivity, www.bksv.com) directly on the fish holder.

Fluorescence microscopy

To determine whether the streptomycin treatment had unintended consequences on auditory epithelia, three control and three streptomycin-treated fish were killed and placed in 4% paraformaldehyde for fixation. After fixation, the auditory epithelia were dissected from the head, rinsed, and stained in Oregon Green phalloidin (Invitrogen Corporation, www.invitrogen.com) to stain hair cell bundles (Higgs et al., 2002). Stained epithelia were imaged on an inverted fluorescence microscope (Leica DMI6000B, Leica Microsystems, www.leica-microsystems.com) and qualitatively examined for areas of hair cell damage. An additional two goldfish (one control and one streptomycin treated) were also processed to verify that the streptomycin treatment had the intended effects. Fish were placed in a 1 mol l^{-1} solution of DASPEI {2-[4-(dimethylamino)styryl]-*N*-ethylpyridinium iodide; Invitrogen} in conditioned tap water for 1 h (Buck et al., 2012), anaesthetised in 0.004 mol l^{-1} 2-phenoxy-ethanol and imaged with a fluorescence dissecting microscope (Leica MZFLIII) under a GFP-2 filter set.

Statistics

Data were tested for normality with the Kolmogorov–Smirnov statistic and none showed significant deviations from a normal distribution. Comparisons of threshold responses were done separately for each treatment by examination of thresholds between pre- and post-treatment fish. For control, streptomycin-treated and scraped fish, two-way repeated measures ANOVA were conducted, with frequency and condition (pre-treatment *versus* post-treatment) as the main effects. Where differences were found within condition, sequential Bonferroni comparisons were made within each frequency class by comparing frequencies of the largest difference first, stopping when significance was no longer found and adjusting the alpha level by the comparison number.

RESULTS

There was no difference in the shape of the evoked potential traces in goldfish before or after any of the treatments (Fig. 1). In all cases, at low frequencies (100 and 200 Hz) the response traces showed repeated peaks and troughs in the response waveform that persisted throughout the recording duration (Fig. 1A,C). At higher frequencies the response traces showed a predominant trough in the waveform approximately 9–10 ms from the beginning, coincident with the offset of the stimulus (Fig. 1B,D).

There was a significant interaction between frequency and streptomycin treatment in the two-way repeated measures ANOVA for both pressure ($F_{6,24}$=20.27, P<0.001) and acceleration ($F_{6,24}$=29.45, P<0.001). Visual analysis of the data showed clear differences in threshold with streptomycin treatment at lower frequencies but not at higher frequencies (Fig. 2A, Fig. 3A). Sequential Bonferroni comparisons showed a significant elevation in pressure threshold at 100 Hz (t=–4.47, P=0.011) and 200 Hz (t=–6.67, P=0.003) following streptomycin treatment, with mean threshold differences of 9.7±2.2 and 16.1±2.4 dB between pre- and post-treatment conditions, respectively (Fig. 2D). The next largest frequency difference was at 400 Hz (5.3±2.1 dB) (Fig. 2A) but this difference was not statistically significant (t=–2.5, P=0.07); therefore, further comparisons between pre- and post-treatment threshold were not made. Similar trends were seen with respect to acceleration data, with elevation of acceleration thresholds evident at 100 Hz (t=–4.47, P=0.01) and 200 Hz (t=–6.67, P=0.003) but at no other frequencies (Fig. 3A,D).

For control fish there was not a significant interaction between frequency and treatment ($F_{6,24}$=0.39, P=0.87) for pressure threshold, nor was there a significant treatment effect on threshold ($F_{1,4}$=5.4, P=0.08) but there were significant effects of frequency ($F_{6,24}$=26.9, P<0.001) for pressure data (Fig. 2B). Across all frequencies, differences within a fish pre- and post-treatment were less than 5 dB, less than one step size in intensity level in the stimulus presentation protocol used. In terms of acceleration data, there was no interaction between frequency and treatment ($F_{6,24}$=0.75, P=0.49), a significant effect of frequency ($F_{6,24}$=30.31, P<0.001), but also a treatment effect ($F_{1,4}$=27.25, P=0.006). For acceleration, thresholds were higher after treatment but only by approximately 4.5 m s^{-2}, well within the minimum step size of 4.7 m s^{-2} used in the stimulus presentation protocol (Fig. 3B).

For the scrape treatment, there was a significant effect of frequency on pressure threshold ($F_{6,24}$=35.6, P<0.001) but not of treatment ($F_{1,4}$=0.63, P=0.47), nor was there a significant interaction between frequency and treatment ($F_{6,24}$=0.51, P=0.79) (Fig. 2C). Pairwise comparison likewise showed little difference within a frequency, with mean differences being between 0–2 dB at any given frequency (Fig. 2C). Likewise, for acceleration thresholds there was no interaction between frequency and treatment ($F_{6,24}$=0.54, P=0.77) and no treatment effect ($F_{1,4}$=18.46, P=0.47) but there was a

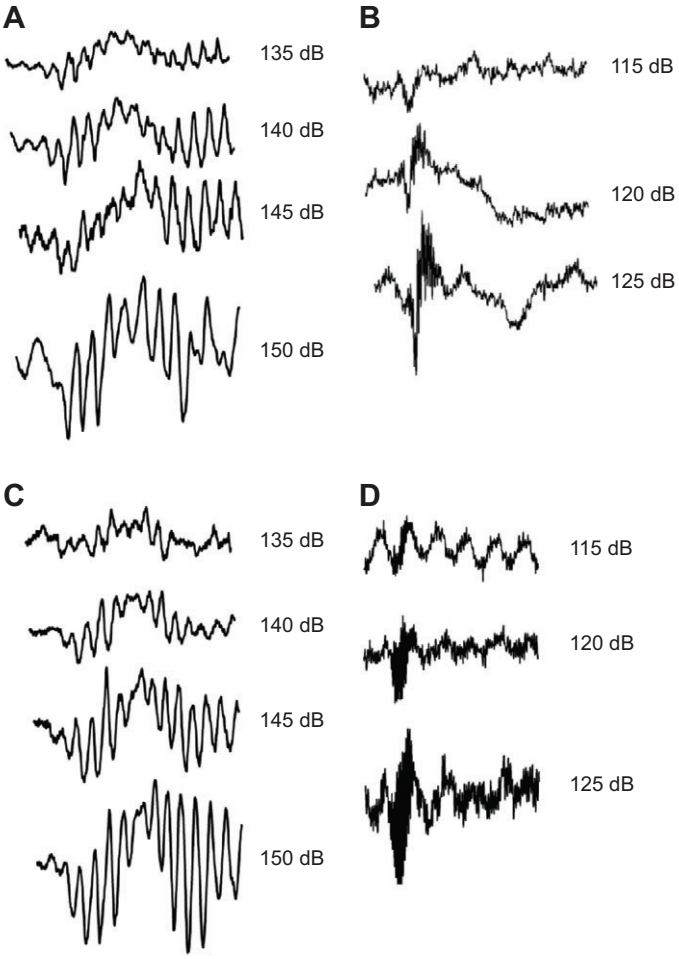

Fig. 1. Evoked potential traces from (A) control goldfish in response to a 100 Hz tone pip, (B) control goldfish in response to a 1000 Hz tone pip, (C) streptomycin sulphate (0.05%)-treated goldfish in response to a 100 Hz tone pip and (D) streptomycin-treated goldfish in response to a 1000 Hz tone pip.

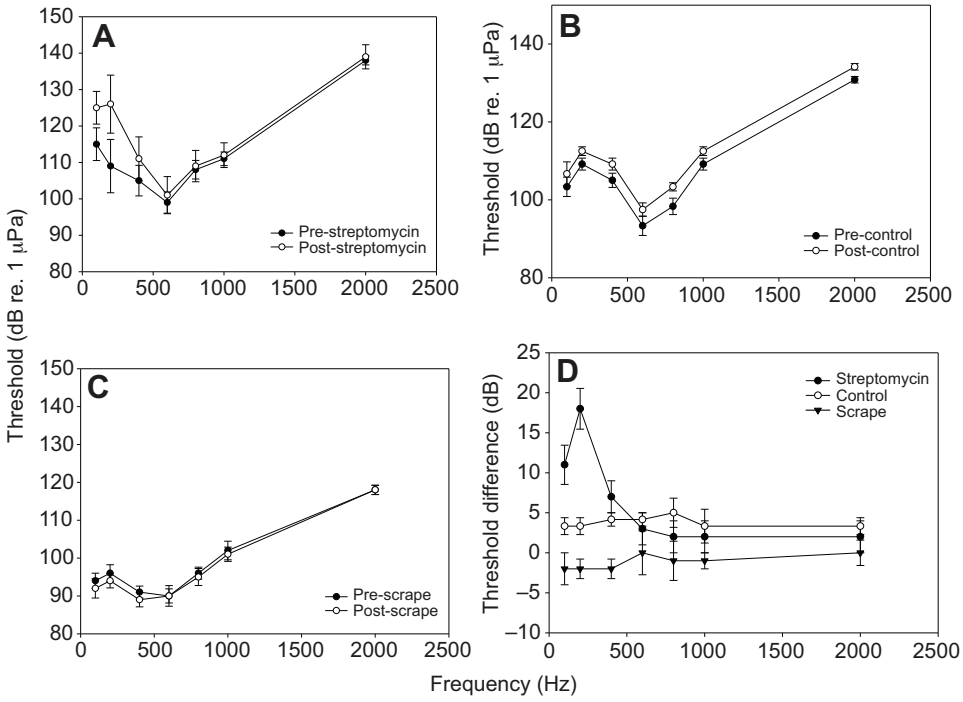

Fig. 2. Auditory pressure thresholds of (A) fish before and after treatment with 0.05% streptomycin sulphate, (B) control fish before and after sham treatment, and (C) fish before and after scraping to remove superficial neuromasts. (D) Threshold difference (post-treatment minus pre-treatment) of goldfish under each treatment condition. All plots show means ± s.e.m.

significant difference between acceleration thresholds at different frequencies ($F_{6,24}$=41.42, P≤0.001).

Treatment with streptomycin had no discernible effect on auditory epithelia (Fig. 4). Control fish (Fig. 4A,B) hair cells were equally stained across all epithelial areas examined. All saccules (Fig. 4C,D) and lagenae (Fig. 4E,F) of streptomycin-treated fish also had fully intact auditory epithelia after treatment, with even phalloidin staining of hair cell microvilli across the epithelial surface and no evidence of damage.

As expected, treatment with streptomycin did affect both canal and superficial neuromasts (Fig. 5). Control fish (Fig. 5A,C) had strong DASPEI staining of both canal and superficial neuromasts all along the body. Streptomycin-treated fish had no positive staining of either canal or superficial neuromasts (Fig. 5B,D).

DISCUSSION

The objective of the current study was to test the potential role of the mechanosensory lateral line system in AEP responses previously thought to be a measure of 'hearing' in fish. We have demonstrated here that pharmacological ablation of both the superficial and canal neuromasts results in an increase in acoustic thresholds at low frequencies but that physical ablation of just the superficial

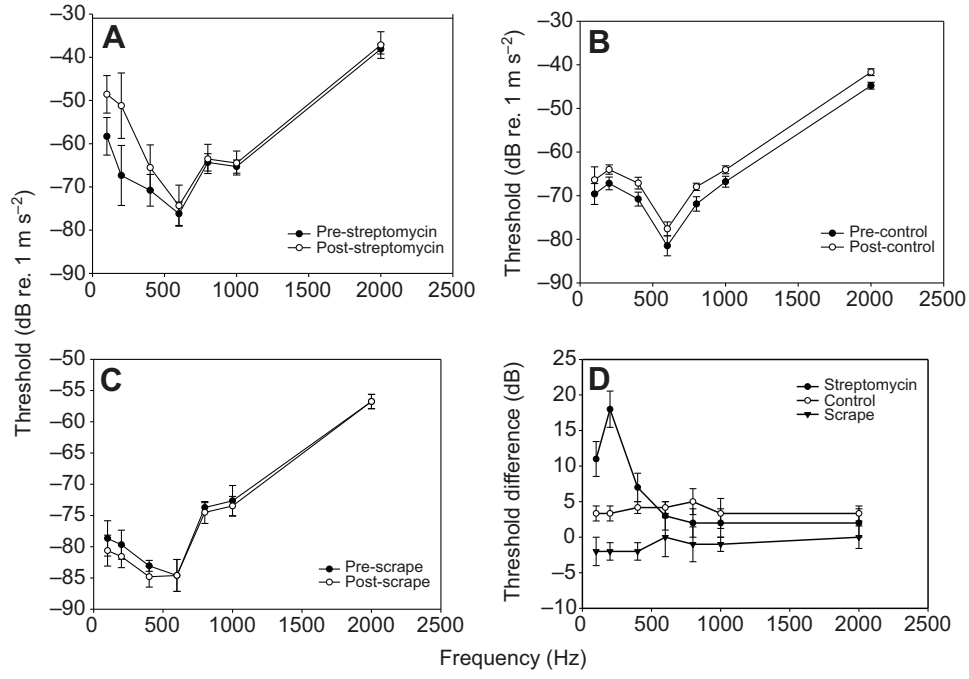

Fig. 3. Auditory acceleration thresholds of (A) fish before and after treatment with 0.05% streptomycin sulphate, (B) control fish before and after sham treatment, and (C) fish before and after scraping to remove superficial neuromasts. (D) Threshold difference (post-treatment minus pre-treatment) of goldfish under each treatment condition. All plots show means ± s.e.m.

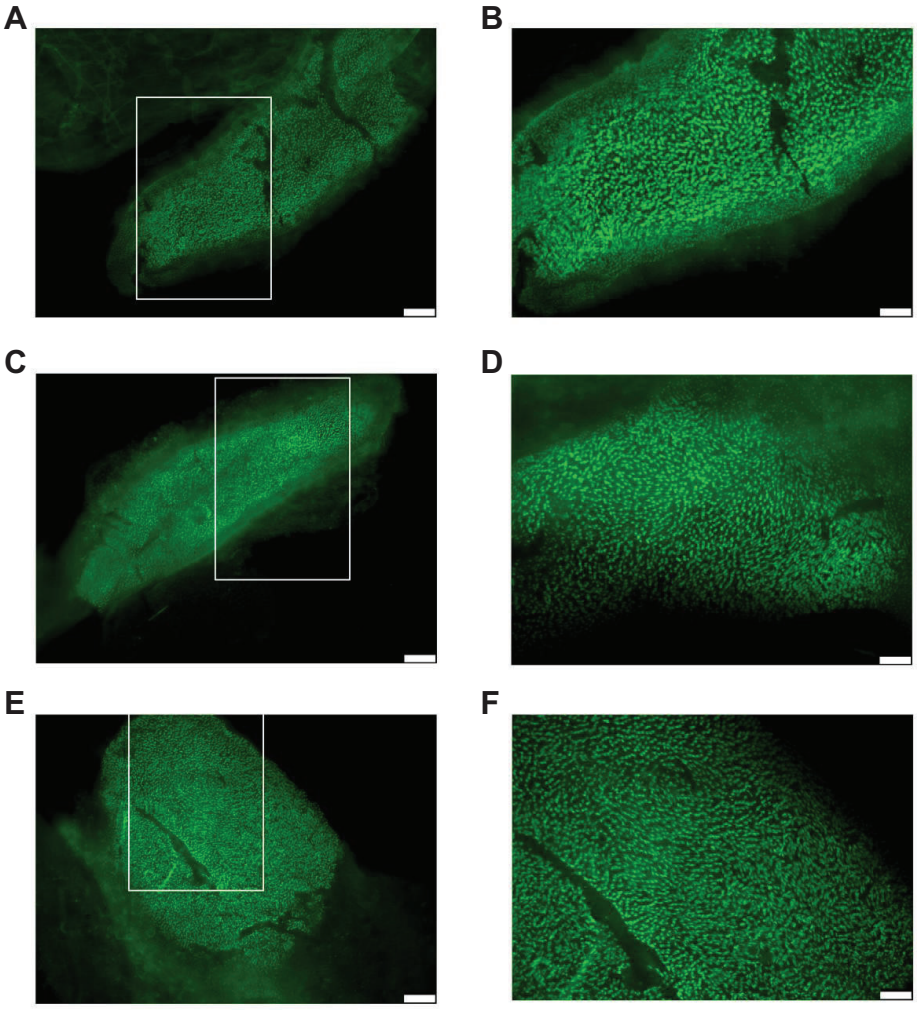

Fig. 4. Fluorescently stained hair cells of auditory epithelia in control (A,B) and streptomycin-treated (C–F) goldfish. (A) Overview and (B) higher magnification image of the sensory epithelium of a saccule from a control fish, showing fully intact sensory hair cell microvilli stained with Oregon Green phalloidin. (C) Overview and (D) higher magnification images of the sensory epithelium of a saccule, and (E) overview and (F) higher magnification images from a lagena of a streptomycin-treated fish, also showing fully intact sensory hair cell microvili. In all cases of streptomycin treatment, there was no sign of hair cell loss, indicating that streptomycin did not impact auditory hair cells. Boxes in A, C and E delineate the epithelia area focused on in B, D and F, respectively. Scale bars: A, C and E, 100 μm; B, D and F, 50 μm.

neuromasts has no effect on response thresholds. We also demonstrated that ablation of the lateral line has no effect on higher frequency responses, consistent with what is known about frequencies of best sensitivity of the lateral line system (Montgomery et al., 1995; Braun et al., 2002; Webb et al., 2008). The present data strongly suggest that measures of acoustic responses in fish using the AEP technique represent responses of both the ear and the lateral line and that, at least for frequencies below 400 Hz, it is incorrect to judge AEP data as representing fish hearing alone.

The use of AEP recording technology has now become a common way to estimate 'hearing' thresholds in fishes, with well over 100 different studies in the 30 years since the seminal paper by Corwin et al. introducing this technique for non-human vertebrates (Corwin et al., 1982). The majority of these studies, including previous studies by the current authors, conduct AEP tests in small laboratory tanks with complex and largely undefined acoustics. Despite the complex nature of sound propagation in bounded media such as experimental tanks (Rogers and Cox, 1988; Akamatsu et al., 2002), previous AEP work discusses the results in terms of the auditory abilities of the fish species of interest. Trunk lateral line canals have previously been shown to interact with the swim bladder to enhance hearing (Kratochvil and Ladich, 2000) but the effect was due to vibrations coming directly from the swim bladder activating the neuromasts, rather than the neuromasts reacting to direct vibrations from the particle motion of the sound itself. It is clear from the current study that, at least for low frequencies (100–200 Hz), AEP measurements in laboratory tanks do not solely measure hearing in fish but rather measure sound detection across sensory modalities. This argument is not meant to deride past studies or suggest they are invalid but it does suggest caution in the interpretation of mechanistic explanations for changes in response thresholds.

That there was no change in the shape of the response waveform with pharmacological ablation is interesting. Previous studies that have shown AEP response waveforms across frequencies consistently found that responses to low frequency (100–200 Hz) tone pips have multiple response peaks while those to higher frequencies (400 Hz and above) typically have one peak or trough (depending on electrode positioning) followed by a quick response to baseline levels (e.g. Ladich and Yan, 1998; Wysocki and Ladich, 2001; Higgs et al., 2002; Higgs et al., 2003). It has previously been suggested (Higgs et al., 2003) that perhaps the differences in response at 100 and 200 Hz are due to lateral line contributions but the results of the present study clearly show this not to be the case, as neuromast ablation had no effect on waveform shape. Analysis of AEP responses in fish lags behind work in other vertebrates as it remains unclear what different peaks in the AEP traces correspond to, unlike the situation in birds and mammals in which each waveform peak corresponds to defined centres of neural activity (e.g. Katayama, 1985; Hall, 1992; Brittan-Powell et al., 2002). A better understanding of the neural architecture driving AEP

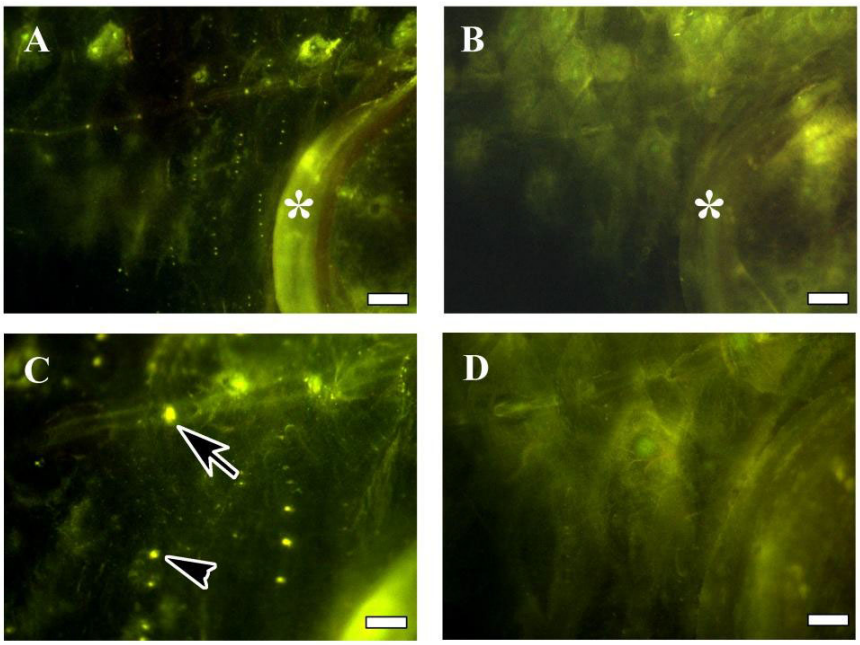

Fig. 5. DASPEI staining of goldfish in control (A,C) and streptomycin-treated (B,D) groups. All images were taken along the trunk of the goldfish, just posterior to the opercular margin (asterisks in A,B). In control fish, positively stained neuromasts are clearly evident at low magnification (A), with one canal neuromast (arrow in C) between each canal pore and superficial neuromasts (arrowhead in C) in lines below the canal. No positive staining was ever seen in streptomycin-treated fish at either low magnification (B) or higher magnification (D). Scale bars: A and B, 1 mm; C and D, 500 μm.

responses in fish would allow a finer resolution of changes in auditory responses with different developmental or environmental conditions and would represent a powerful step forward in the field of fish hearing. Understanding the neural architecture driving AEP response will also provide valuable insight into the potential mechanisms underlying sound source localisation in fish, which remains a 'black box' (Fay, 2005; Zeddies et al., 2012).

While we did not quantify the morphological effects of our streptomycin treatment on neuromasts, it is clear that streptomycin did disrupt neuromast function and physically ablated the majority of canal and superficial neuromasts. Recent work has conclusively demonstrated that the streptomycin dosage used here does in fact ablate both superficial and canal neuromasts in goldfish (Brown et al., 2011) and zebrafish [*Danio rerio* (Buck et al., 2012)], although it might be less effective on other species (Brown et al., 2011). That we saw clear effects on response thresholds at low frequencies, where the neuromasts are known to be sensitive – but not at higher frequencies outside the neuromast response range (Montgomery et al., 1995; Coombs and Montgomery, 1999) – also demonstrates that the chemical treatment used ablated neuromasts. While it is possible that some neuromasts survived streptomycin treatment, it is clear from the physiological results that a reduction in neuromast inputs significantly affects AEP responses, showing that AEP response at low frequencies is a combination of auditory and mechanoreceptive inputs. The effects of streptomycin were not due to changes in auditory hair cells as we found no indication of hair cell damage in the stained preparation, which showed saccular and lagenar epithelia clearly intact with an entire field of auditory hair cell microvilli. Also, previous research (Matsuura et al., 1968; Matsuura et al., 1971) has indicated that streptomycin only impacts auditory responses when it is injected directly into the auditory lumen, not when it is applied extraluminally. We also found no evidence that the results were due to a generalised effect of streptomycin on the health of the fish as there is no expectation that such an effect would only manifest itself at low frequency.

To a fish it should not essentially matter whether the sound is detected by the ear, the lateral line or both. Detection of environmental stimuli is an integrative process whereby animals take in information across multiple sensory modalities and synthesise inputs in the brain to drive behavioural responses (Hebets and Papaj, 2005; Brø-Jørgensen, 2010). In terms of sound stimuli, the auditory nerve and the lateral line nerves innervate a similar area of the brainstem in fish and send inputs from there to the torus semicircularis, a primary integrative centre (reviewed in Higgs et al., 2006). Ablation of the lateral line increases the response latency of the 'acoustic' escape response in goldfish and alters the directionality of this response when tested with an underwater sound source (Mirjany et al., 2011), presumably due to electrical synapses with the Mauthner cells forming the motor aspects of the escape response (Mirjany and Faber, 2011). Interestingly, when goldfish startle responses are triggered with an airborne sound source, ablation of lateral line inputs improves escape responses (Canfield and Rose, 1996), suggesting that the relationship between particle motion and pressure waves in the stimulus can affect the relative role of the ear and lateral line. It is likely that in nature fish use inputs from both auditory and lateral line systems either simultaneously or in series (Braun and Coombs, 2000; Webb et al., 2008) to ascertain the true nature of 'sound' stimuli and make appropriate behavioural decisions.

In summary, our results show that 'auditory' evoked responses to low frequency sound stimulation should not be considered measures of hearing ability in fish but rather a multimodal mechanosensory response driven by both the ear and the lateral line system. As it remains difficult, if not impossible, to separate the roles of these two systems when obtaining whole-brain recordings it is imperative that researchers recognise the contributions of both systems when interpreting acoustic experiments. If the goal of such experiments is simply to measure response capabilities to sound stimuli, it may not matter which system is being used but if the goal of these studies is to examine how sound detection has evolved or the relevant distances for sound detection, then it is imperative to accurately identify the sensory systems involved.

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AUTHOR CONTRIBUTIONS

Both authors contributed equally to all aspects of this work.

COMPETING INTERESTS

No competing interests declared.

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