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Anechoic aquarium for ultrasonic neural telemetry

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An acoustic neural telemetry tag has been developed for recording from free-swimming aquatic animals. Microwire electrodes were implanted into the VIIIth nerve of the toadfish, *Opsanus tau*, and interfaced to the subdermally implanted tag. The telemetry tag frequency modulates the neural signal, converting it into a varying frequency, which is amplified and transmitted acoustically (centre frequency of 90 kHz and a 20 kHz bandwidth). This acoustic signal is detected by a receiver hydrophone, and the receiver reconstructs the full neural waveform from the acoustic signal. However, due to the multipath environment in the experimental aquarium, the acoustic signal is quickly degraded as the hydrophone is moved away from the source. In order to receive the signal independent of fish position, an anechoic aquarium was designed. Streams of microbubbles (*ca.* 70 μm diameter) were generated to produce a curtain of sound-absorptive material along the walls and water surface of the aquarium. Microbubble generation significantly reduced the multipath artefacts, and allowed signal discrimination independent of fish and hydrophone position. The anechoic aquarium will allow the recording of neural activity from free-swimming fishes in quasi-natural habitats, thus allowing better understanding of the neural mechanisms of behaviour.

Keywords: telemetry; anechoic; acoustical

1. INTRODUCTION

It has long been a goal of neuroethologists to continuously record neural activity from unrestrained, naturally behaving animals. Recent studies have produced stable neural recordings in free-swimming toadfish (*Opsanus tau*) from sieve microelectrodes chronically implanted into the regenerated VIIIth nerve (Mensinger & Highstein 1999; Mensinger *et al.* 2000). An acoustic transmitter tag has been developed to interface with the sieve electrode to allow acoustic telemetry of neural signals from a free-swimming animal in a quasi-natural environment (Mensinger & Deffenbaugh 1998).

In contrast to the situation with terrestrial animals, recording from mobile aquatic animals presents a different array of problems. The increased drag of the aqueous medium dictates that telemetry devices must be small and streamlined or implanted subcutaneously. Implantation greatly reduces the risk of infection or damage to the device, but is offset either by limited battery life or by the need for invasive battery changes. Additionally, the conductivity of seawater precludes the use of radio-telemetry for practical bio-monitoring frequencies except at ranges less than 1 m (Simon *et al.* 1994). In contrast, acoustic telemetry offers sufficient range, but can be limited by echoes (multipath) in enclosed environments such as tanks.

We have previously reported the development of an implantable, inductively powered telemetry tag that addresses the problems of size and power (Mensinger & Deffenbaugh 1998). In this paper, we report the development of an anechoic aquarium that eliminates multipath artefacts, and allows acoustic telemetry of full neural waveforms from free-swimming fishes.

2. MATERIAL AND METHODS

(a) *Telemetry tag*

The transmitter tag is a flat cylinder (2 cm diameter × 1 cm high). Contained within the tag are miniature 0.2 F ultra-high-capacity energy storage capacitors, which provide power to the tag electronics, a circular inductive coil used to recharge the tag, a piezoelectric transducer for transmitting data acoustically, and electronic circuitry. The circuitry includes two input electrodes, which are connected to a differential amplifier. A voltage-controlled oscillator converts the electrode voltage into a varying frequency, which is amplified and transmitted acoustically by the piezoelectric transducer. This acoustic frequency modulated signal has a centre frequency of 90 kHz and a 20 kHz bandwidth. The tag is powered by magnetic induction either by a recharging 'wand' before submersion or underwater by a magnetic field generated by a specially designed recharging habitat.

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(b) *Receiver system*

The acoustic signal from the tag is detected by a receiver hydrophone. The received signal is filtered and demodulated to reconstruct the full neural waveform. The demodulator emits a constant duration pulse at each zero-crossing of the received signal, to convert the frequency modulated signal into a pulse position modulated (PPM) waveform. The PPM waveform is then filtered by an eight-pole elliptic low-pass filter, reconstructing the neural waveform.

(c) *Anechoic aquarium*

A 1 m diameter fibreglass tank (Rowland Fiberglass, Inc., Ingleside, TX, USA) served as the experimental aquarium (figure 1). The aquarium was filled with 320 l of filtered seawater to a depth of 45 cm. Water was recirculated through the aquarium via an external water pump (Flotec, Delavan, WI, USA). Water was drawn through a submerged intake in the centre of the tank, 5 cm beneath the surface. Air was mixed into the return flow via an aerator positioned above the water surface. The macrobubbles in the water return were then cleaved into microbubbles by the impeller of the water pump. This microbubble-laden water was equally distributed around the inside walls of the tank by a 2.5 cm diameter outflow ring (polyethylene tube), which was positioned on the bottom of the tank in contact with the wall. The water containing the microbubbles was forced out of 1.5 mm pores in the outflow ring equally spaced at 2.5 cm intervals. The microbubbles rose to the surface along the sides of the tank, and were prevented from mixing with the central water column by a circular plastic partition (2 mm thick), positioned 5 cm from the tank wall. The partition terminated *ca.* 8 cm below the water surface, allowing microbubbles to be drawn just below the water surface of the tank by the centrally positioned water intake. This system generated a continuous flow of bubbles along the aquarium walls and water surface.

(d) *Electrode implantation*

Toadfish were anaesthetized with 0.001% MS-222 (Sigma Chemical Co., St Louis, MO, USA) and injected with 0.1 ml 1% pancuronium bromide. A small craniotomy was made directly over the anterior ramus of the VIIIth nerve. Twin microwire electrodes of insulated 20 μm diameter platinum–iridium wire (Sigmund Cohn Corporation, Mt Vernon, NY, USA) were connected with conductive epoxy to 50 gauge multistranded gold wires, 6 cm in length, which terminated into a multipin connector. The post-microwire assembly was encased in silastic (PI Medical, Portland, OR, USA) to ensure water tightness. The electrodes were inserted proximal to the anterior ramus of the VIIIth nerve. Neural activity was confirmed by recording differentially from the two microwires with an A-M Systems 1800 differential amplifier (A-M Systems, Inc., Carlsborg, WA, USA). The electrode was affixed with cyanoacrylate to the edge of the craniotomy and the remaining opening sealed with cyanoacrylate gel, the wound sutured and the fish allowed to recover overnight. The microwire connector was then inserted into the telemetry tag mounted on the fish's head. All animal care procedures conformed to institutional animal care guidelines.

(e) *Experimental design*

To demonstrate the anechoic qualities of the aquarium, the telemetry tag was initially hardwired to a function generator that served as a model fish that produced waveforms approximating the frequency and amplitude of normal VIIIth nerve activity (Mensinger *et al.* 1997). The telemetry tag was charged via magnetic induction and submerged in the test aquarium. Acoustic telemetry was conducted in the presence and absence of microbubbles. The trials were then repeated with the telemetry tag attached to a microwire-implanted fish.

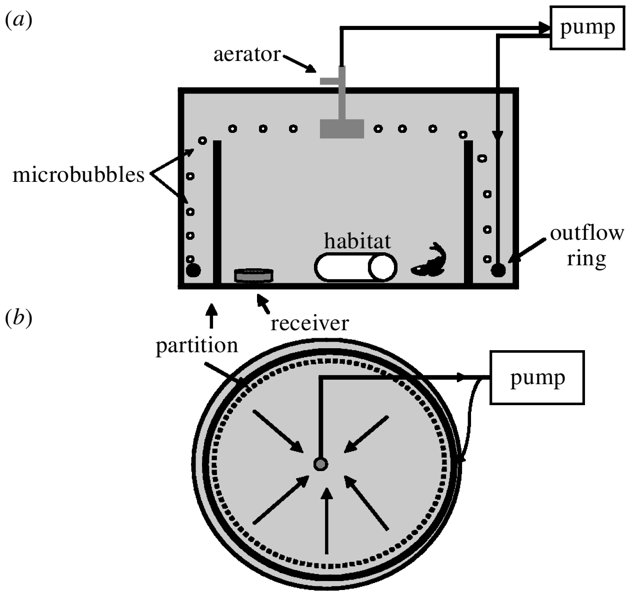

Figure 1. Diagrammatic representation of the anechoic aquarium, (*a*) cross-section and (*b*) top view. An external water pump recirculates the water through the tank. Water is drawn through a centrally positioned, subsurface intake. The water flow draws macrobubbles into the return flow through the aerator. The impeller of the water pump turns the macrobubbles into microbubbles, some of which have the desired 70 μm diameter. The microbubbles are returned to the tank via an outflow ring. A plastic partition restricts the microbubble output to the walls of the aquarium, until the current draws them back into the intake. Thus the aquarium walls and water surface are insulated with a stream of microbubbles. Note that the figure is not drawn to scale.

3. RESULTS

Figure 2 shows the effects of microbubble generation on signal discrimination. Figure 2*a–c* was generated during acoustic telemetry by the model fish. The lower trace in each set is the direct recording of the signal from the model fish and the upper trace is the reconstruction of this signal following telemetry. In figure 2*a*, the pump is off, and the multipath dominates the signal at a distance beyond a few centimetres. Figure 2*b* illustrates the initial effects of microbubble production following 10 s of water flow. Note that the reconstituted signal is marginally clearer as the initial microbubble generation begins to absorb the extraneous sound energy. Figure 2*c* shows the effect of maximal microbubble production. The water pump has been active for over 30 s. This type of signal discrimination was independent of fish or hydrophone position anywhere within the confines of the plastic partition. Figure 2*d* shows the reconstruction of spontaneous neural signals from an unrestrained experimental fish placed in the test aquarium during microbubble generation.

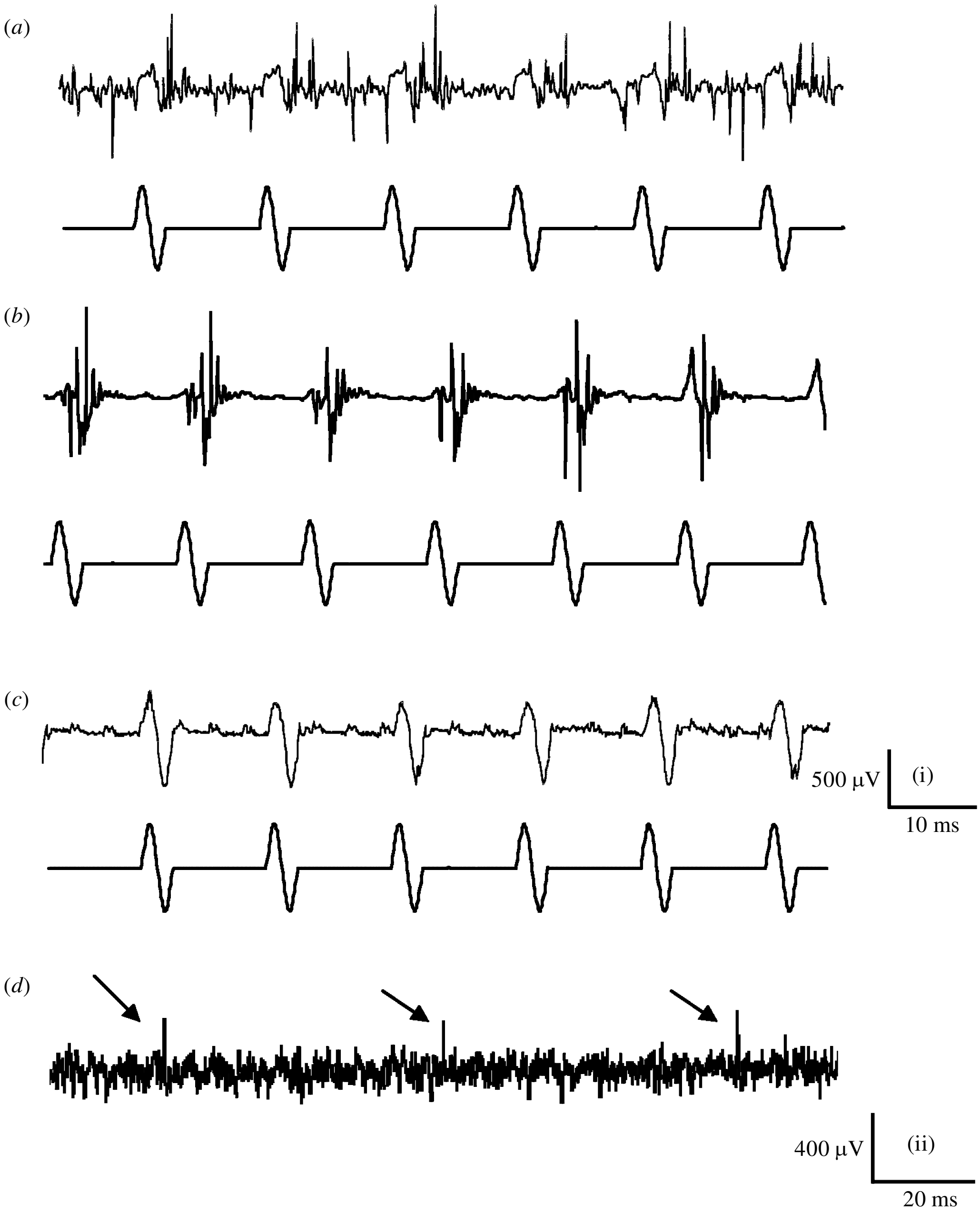

Figure 2. The effects of microbubble generation on signal discrimination. (*a–c*) Generated during acoustic telemetry by the model fish. The lower trace in each set is the direct recording of the signal from the model fish, and the upper trace is the reconstruction of this signal following acoustic telemetry. In (*a*), the pump is off, and the multipath environment degrades the signal at a distance of 10 cm. (*b*) The initial effects of microbubble production following 10 s of water flow. Note that the reconstituted signal is marginally clearer as the initial microbubble generation begins to absorb the extraneous sound energy. (*c*) The effect of maximal microbubble production. This type of signal discrimination was independent of fish or hydrophone position anywhere within the confines of the plastic partition. (*d*) Discrimination of acoustically transmitted VIIIth nerve signals from an unrestrained experimental animal placed in the test aquarium during microbubble generation. The arrows indicate individual action potentials.

4. DISCUSSION

The results demonstrate that microbubbles act as effective sound absorbers, and can transform even small aquaria into anechoic chambers at acoustic telemetry frequencies. The microbubble generation allowed acoustic neural telemetry independent of fish and hydrophone position.

The difficulty in transmitting data acoustically through an enclosed area like a tank is multipath. The signal from the fish is reflected off the water surface and the bottom and sides of the aquarium, so that the receiver detects not only the direct signal from the fish, but also multiple echoes of the signal from all directions. In fact, at ranges beyond a few centimetres, the vast majority of the energy that the receiver detects is reflected and not direct energy. Because of the dominance of the scattered field, it is insufficient simply to scatter reflections by roughing up the tank surfaces (e.g. lining the bottom with gravel).

Acoustic energy must actually be absorbed from the system by conversion into heat.

At their resonant frequency, air bubbles in water have an extinction cross-section, which is about 100 times larger than their geometrical cross-section. That is to say, they absorb acoustic energy in a region very much larger than their own size. The resonance frequency of an air bubble near the water surface is *ca.* $f = 6.5/D$, where f is the frequency in Hertz, and D is the diameter of the bubble in metres (Clay & Medwin 1977). Thus, for the 90 kHz frequency of the telemetry signal, the diameter of a resonant bubble is 72 μm. The sound absorption by the microbubbles proved sufficient to reduce the reverberation within the tank to levels where acoustic telemetry of high bandwidth neural signals is feasible.

Although a variety of approaches to underwater sound absorption have been proposed in the acoustics literature (Hinders *et al.* 1995; Hladky-Hennion & Decarpigny 1992; Lastinger 1972), the use of resonant microbubbles has several distinct advantages for biotelemetry applications. The microbubbles are essentially invisible to the eye, making observation possible from any angle if a transparent tank is used. The system is easy to clean and maintain, and the introduction of microbubbles raises little biocompatibility concern. Finally, the system is very inexpensive to implement, even for large tanks.

The use of sound-absorbing microbubbles increases the feasibility of using acoustic telemetry in any size tank. It is especially useful for the marine environment where the conductivity of seawater renders radio-telemetry ineffective at biologically relevant ranges. Although we have confirmed that the tank is anechoic for ultrasonic frequencies, future studies will determine effects of microbubble production on the sound generation within the hearing range of the fish.

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