J. exp. Biol. 133, 249–262 (1987)
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SELECTIVE AND REVERSIBLE BLOCKING OF THE LATERAL LINE IN FRESHWATER FISH

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Accepted 15 June 1987

SUMMARY

Fish possess two separate systems for detection of low-level sound and water motions in the low-frequency range: the inner ear and the lateral line. The relative roles of these systems in normal fish behaviour is still not clear. There is, for instance, a lack of experimental evidence showing the involvement of the lateral line and the inner ear in detection of infrasound, in directional hearing in the near field, and in detection and attack of swimming prey below the surface. To provide a useful tool for such studies, we have developed a pharmacological method for selective and reversible blocking of the lateral line in the roach (*Rutilus rutilus*). By recording multi-unit activity from the lateral line nerve and microphonic potentials from the inner ear, we have shown that cobalt ions in the external water may completely block the mechanosensitivity of the lateral line without affecting the utricular microphonic activity. This inhibiting effect of Co^{2+} is antagonized by Ca^{2+}, making the ratio between these ions the important blocking factor. For practical work, we recommend 12–24 h exposure to 0·1 mmol l^{-1} Co^{2+} at a Ca^{2+} concentration of less than 0·1 mmol l^{-1}. The fish showed no sign of general behavioural disorders even after 1 week in this solution, and the microphonic sensitivity of the inner ear was not reduced. The blocking effect of Co^{2+} was clearly reversible, and the recovery was dependent upon both the duration of the Co^{2+} exposure and the Ca^{2+} concentration of the recovery solution.

INTRODUCTION

Fish are able to detect low-level water motion with both the lateral line and the inner ear. However, the relative involvement of these sensory systems in normal fish behaviour is still not clear. The lateral line has traditionally been considered responsible for detecting disturbances of extremely low frequency, whereas perception of sound in the upper part of the detectable frequency range was thought to depend on the inner ear (see Sand, 1984). However, it has recently been suggested that the inner ear may show high sensitivity even for frequencies below 1 Hz, although involvement of the lateral line in the detection of infrasound has not been excluded (Sand & Karlsen, 1986). The major acceleration components of the local hydrodynamic flow fields generated by swimming goldfish are between 1 and 20 Hz

Key words: lateral line, inner ear, cobalt, calcium, mechanosensitivity, roach.

(Kalmijn, 1987; Kalmijn & Enger, 1987), making both the lateral line and the inner ear likely candidates for detection of swimming prey below the surface.

Directional hearing in fish is commonly thought to depend on vectorial weighing of the direct motional input to the otolith organs (see Schuijf, 1981). However, most natural sound sources are dipoles, and a single detection of the direction of particle motion will not, therefore, provide adequate information for source localization in the acoustic near field (Kalmijn, 1987). At extremely close range the lateral line may, nevertheless, give the information necessary for estimation of the position of a dipole source.

It is evident from the considerations above that more information is needed about the functional relationship between the lateral line and the inner ear. A method for specific and reversible blocking of one of these sensory systems would be helpful in gathering such information. Surgical methods for destruction of the lateral line have been frequently applied. However, complete elimination of the system, including the organs on the head, is not easy without causing severe side effects. Total surgical elimination of the lateral line system has therefore rarely been attempted (Dijkgraaf, 1973; Schuijf & Siemelink, 1974). This method has, furthermore, the disadvantage that it is not reversible. A pharmacological method thus seems more promising. Ototoxic antibiotics may be employed for the blocking of hair cell function (Wersäll & Flock, 1964; Harada, Musso & Mira, 1967; Matsuura, Ikeda & Furukawa, 1971; Konishi, 1979; Kroese & van den Bercken, 1982), although this effect is irreversible after prolonged exposure (Wersäll, Björkroth, Flock & Lundquist, 1973; Brown & Feldman, 1978; Hudspeth, 1983). Heavy metals, for instance Co^{2+}, have been shown to block the mechano-sensitivity of superficial neuromasts in both amphibians (Sand, 1975) and fish (Baumann & Roth, 1986). This effect of Co^{2+} is completely reversible, and is also antagonized by Ca^{2+}. It has, however, not been tested whether Co^{2+} in the external medium is able to block the hair cells of canal organs where the canal fluid contacts the exterior through pores only. The canal organs will, nevertheless, be more exposed than the hair cells of the inner ear, which may be influenced by agents added to the external medium only if these enter the circulatory system to a sufficient degree. It is therefore likely that selective blocking of the lateral line may be achieved by adding a suitable concentration of the inhibiting agent to the external medium.

In the present paper we have tested the effects of different Co^{2+}/Ca^{2+} ratios in the external water on the mechano-sensitivity of the lateral line and the inner ear in the roach, *Rutilus rutilus*. We show that it is possible to block the lateral line sensitivity completely at a Co^{2+} concentration which is tolerable to the fish, without affecting the sensitivity of the inner ear. This effect of Co^{2+} is reversible.

MATERIALS AND METHODS

Experimental animals

Roach (*Rutilus rutilus*) is an ostariophysine species, and thus has acute hearing sensitivity. Furthermore, it has a well-developed canal lateral line system. Along the

sides of the body the canals possess one pore and one neuromast per lateral line scale. A number of superficial neuromasts are in addition situated on each of these scales. The lateral line nerve in roach has one main branch along each side of the trunk, and this nerve innervates both canal organs and superficial neuromasts. Afferent fibres from superficial neuromasts show a high degree of convergence. At the level of the pectoral fin, where recordings were made, fibres innervating canal organs therefore dominate. The detailed anatomy of the lateral line system in the roach will be described elsewhere (H. E. Karlsen, in preparation).

The roaches used in this study ranged in length from 12 to 18 cm. The fish were stored in large tanks with a continuous flow of dechlorinated tap water at 8–10°C. The electrophysiological recordings were performed under amytal anaesthesia (Keys & Wells, 1930). The barbiturate was given intraperitoneally ($50\,mg\,kg^{-1}$), and produced a deep anaesthesia for several hours without blocking the respiratory movements.

Recording from the lateral line nerve

The methods for recording from the lateral line nerve and mechanical stimulation of the lateral line have previously been described in more detail (Sand, 1981). A sketch of the experimental set-up is shown in Fig. 1. The anaesthetized fish was fixed side-down on the angled bottom of a chamber recirculated with the test solutions. The water inlet was also used for artificial respiration of the fish. A superficial incision was made above the lateral line nerve at the level of the pectoral fin, and the nerve was dissected free for a length of 0·5–1 cm. Only the part of the fish posterior to the operated area was submerged during these experiments.

The nerve was then cut, and multi-unit activity was recorded from the posterior end by a platinum electrode centred in a sliding polyethylene tube. During the recordings, the tube was slid forward to enclose the cut end of the nerve, and the signal-to-noise ratio was improved by filling the tube with silicone oil (Wilkens & Wolfe, 1974). The electrode was connected to standard recording equipment, including a window discriminator which produced a 2 ms rectangular pulse for each selected spike. The firing frequency was measured by feeding the rectangular pulses to a voltage stepper reset at 0·5 s intervals by a time mark generator. To measure the degree of synchronization between vibrational stimuli and nerve activity, the rectangular pulses were averaged (64 sweeps) by a signal analyser triggered by the oscillator generating the sine wave stimuli. The averaged value for a certain stimulus phase thus gives the probability of occurrence of a spike within ±2 ms of this phase, the amplitude of the original rectangular pulse representing a probability of 1. At 50 Hz, which was the frequency used in this study, 2 ms corresponds to 36°. The term 'synchronization index' is defined here as the difference between the highest and lowest probability for occurrence of a spike during a stimulus cycle (Sand, Ozawa & Hagiwara, 1975).

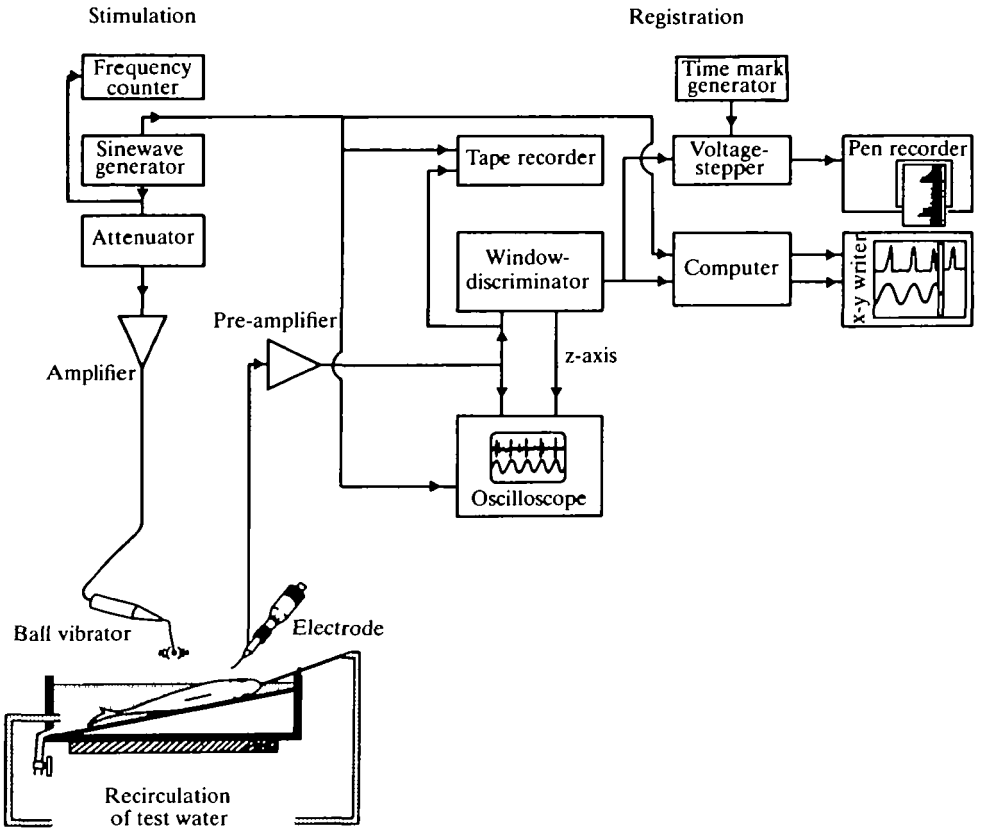

Fig. 1. The experimental arrangement for testing the mechano-sensitivity of the lateral line in the roach. See text for details.

Lateral line stimulation

The lateral line was stimulated by a sphere (radius, r, 1·5 mm) vibrated parallel to and directly above the canal. In a previous study it was shown that the canal organs in the roach are optimally stimulated by water vibrations at 50 Hz parallel to the canal (Sand, 1981), and such a stimulus was therefore chosen in the present experiments. The distance between the centre of the ball and the skin was 4·5 mm (3r), and the stimulus intensity was altered by varying the vibration amplitude of the ball. The vibrator was calibrated using a microscope with stroboscopic illumination, and the water displacements caused by the vibrating sphere were calculated according to the equations given by Harris & van Bergeijk (1962). The presented stimulus intensities are the estimated water displacements (root mean square, rms, values) at the fish surface directly beneath the ball, disregarding possible distortions due to the presence of the fish. In the case of poor sensitivity of the lateral line, due to partial inhibition, massive, unquantified pipette stimuli were given in addition by ejecting a water jet along the canal.

Recording of utricular microphonic potentials

The method employed in these experiments is illustrated in Fig. 2. The anaesthetized fish was clamped with five pairs of steel bars in a fish holder attached to a vibrating table, as previously described (Sand & Michelsen, 1978). The foremost pair clamped the fish head securely just above the eyes. The table was suspended by four steel strings, and horizontal vibration of the table parallel to the long axis of the fish was achieved using an electromagnetic vibrator. Vibration of the fish in air effectively stimulates the otolith organs, and constitutes a good simulation of the kinetic part of sound stimulation in water (Enger, Hawkins, Sand & Chapman, 1973; Sand, 1973). The movements of the table were measured with three orthogonally positioned velocity transducers. Below 100 Hz the vibrations were linear with negligible vertical wobbling, and the recordings were restricted to this frequency range.

The skull was opened dorsally, and the brain carefully removed by suction. The utriculus and parts of the semicircular canals were then exposed, and microphonic potentials were recorded with carbon fibre microelectrodes (Armstrong & Millar, 1979; Fox, Armstrong & Millar, 1980) from the anterior part of the utriculus. The tips of the electrodes were sharpened by etching (Armstrong, Fox & Millar, 1980) to facilitate penetration of the utricular membrane. The electrode was connected to standard recording equipment. We recorded from the utriculus since it is directly accessible once the brain has been removed, while the pars inferior is situated in a groove in the skull floor covered by a thin bony shield. Moreover, the utriculus in the

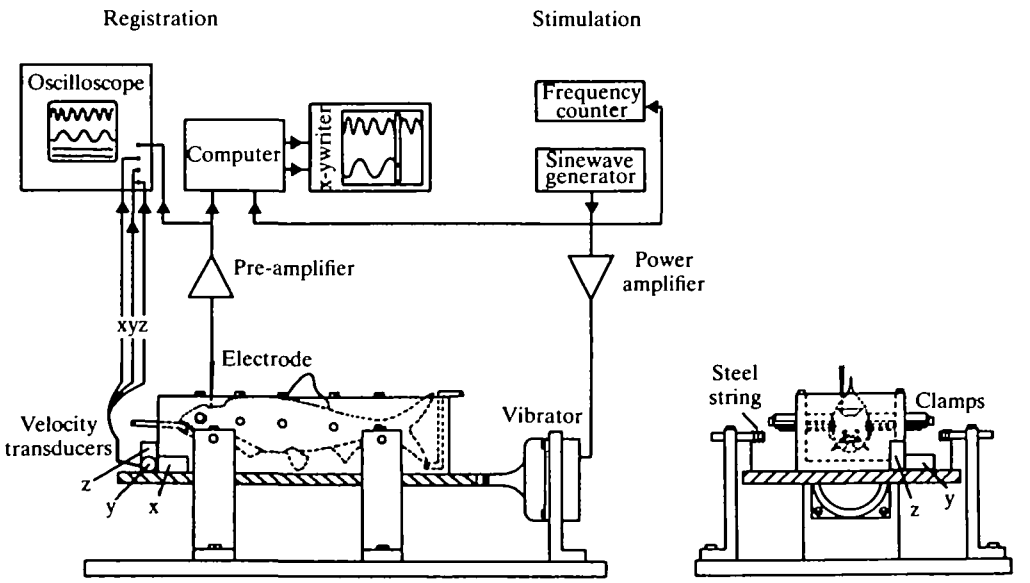

Fig. 2. Sketch of the experimental set-up for recording of microphonic potentials from the inner ear in the roach. The fish was clamped in a holder fixed on a vibrating table. Utricular microphonic potentials were recorded in response to vibration of the whole fish in air. See text for further details.

Ostariophysi is at least as sensitive to horizontal vibrations as the sacculus, whereas the lagena is less sensitive (Fay, 1984). The electrode was fixed to a light-weight manipulator firmly clamped to the fish holder, causing negligible relative movements between the fish and electrode at the frequencies employed. The microphonic potentials are presented as dB re 1 μV rms.

Test solutions

The fish were exposed to standard fresh water with varying added amounts of $CoCl_2$ and $CaCl_2$. The standard fresh water had the following composition (in mmol l^{-1}): KCl, 0·025; KNO_3, 0·05; NaH_2PO_4, 0·05; NaCl, 0·2; $MgSO_4$, 0·1 (Kishimoto, 1966; Sand, 1975). The pH was adjusted to 7·2 with NaOH. Water with no added $CaCl_2$ is called Ca^{2+}-free in the present study, although atomic absorption measurements showed that such water nevertheless had a Ca^{2+} level of 1–10 μmol l^{-1}.

RESULTS

Effects of Co^{2+} on the lateral line mechano-sensitivity

Initially the effects of Co^{2+} were studied during continuous recordings from the lateral line nerve. Before switching to the test solution, the mechano-sensitivity of the lateral line was measured in water from the storage tank. The vibrating ball was then slowly moved directly above the canal, and the recorded spike activity usually became synchronized to the stimulus for a few separate and rather restricted ball positions directly above individual pores. At these positions, ball vibration also increased the firing frequency. The location of these sensitive spots indicated that recordings were mainly obtained from canal organs rather than from superficial neuromasts. The effects of Co^{2+} were then tested at the optimal ball position, and the stimulus intensity was kept at a sub-maximal level at 4×10^{-4} cm.

Fig. 3 demonstrates the effect of switching to Ca^{2+}-free fresh water with 1 mmol l^{-1} Co^{2+}. The response to vibrational stimuli showed a gradual decline. After 60 min no responses were evoked by this type of stimulation, whether measured as increased firing rate or as the degree of synchronization. However, pipette stimulation still produced weak responses for a further period of 10–15 min before the mechano-sensitivity was completely blocked. Co^{2+} treatment also reduced the spontaneous firing rate during the initial 10–20 min. However, to avoid losing sensitive units owing to possible decline of spike amplitudes, the window discriminator was adjusted during the experiment to keep the recorded spontaneous activity constant.

Control experiments in test solution without Co^{2+} showed no decline in sensitivity. It is evident from the figure that the time course of the inhibiting effect of Co^{2+} was the same whether the response was measured as degree of synchronization or increased firing rate. A similar comparison was performed on four additional fish, and the results are pooled in Fig. 4. In all cases complete blocking of the responses to vibrational stimuli was obtained after an exposure period of 50–80 min.

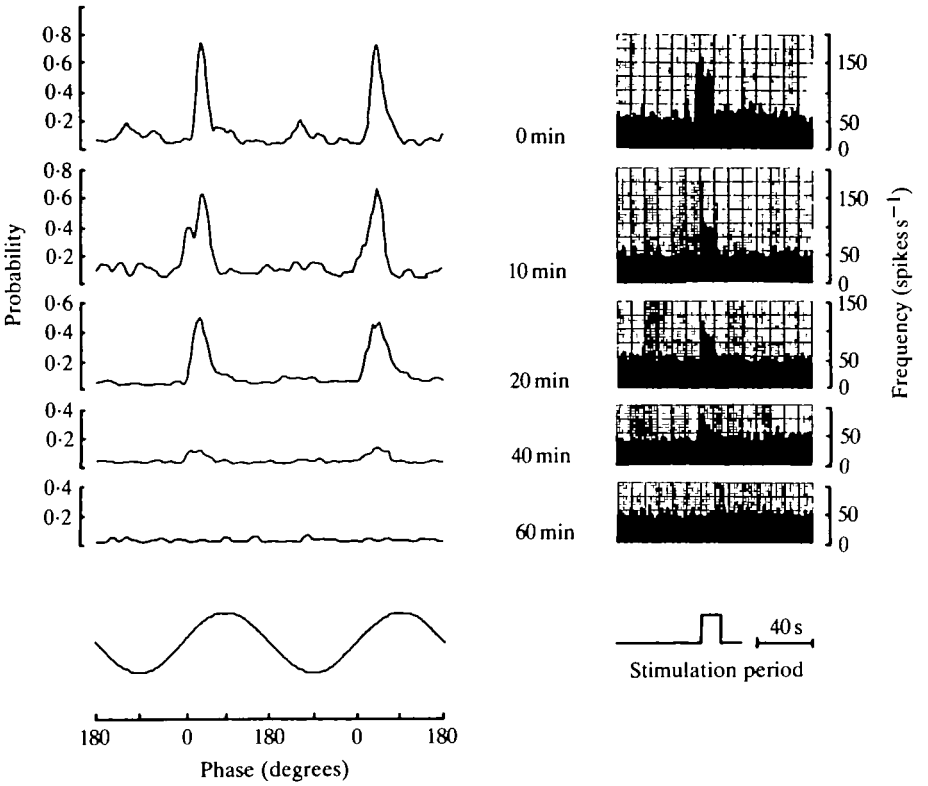

Fig. 3. The inhibiting effect of 1 mmol l^{-1} Co^{2+} in Ca^{2+}-free solution on the mechano-sensitivity of the lateral line. The left part presents probability plots, showing the degree of synchronization between ball vibration and spike activity, and the increase in spike frequency induced by the 50 Hz vibrational stimulus parallel to the canal is shown to the right. The estimated amplitude of the water displacements at the lateral line just beneath the ball was kept constant at 4×10^{-4} cm. Recordings are presented at different times after switching from normal fresh water to the test solution. The preparation showed no sensitivity to this stimulus after 60 min exposure.

To test if this effect of Co^{2+} was reversible, the test solution was changed to Co^{2+}-free standard fresh water with 1 mmol l^{-1} Ca^{2+} immediately after complete blocking had been achieved. All the fish then displayed weak responses to massive pipette stimulation within 10–30 min, whereas the responses to ball vibration started to reappear after 1–2 h.

It was not practical to test the blocking effect of Co^{2+} concentrations lower than 1 mmol l^{-1} by continuous recordings. Exposure to a Ca^{2+}-free test solution with 0·1 mmol l^{-1} Co^{2+} decreased the responses to ball vibration by only 20–50 % within 2 h. During prolonged recordings it was sometimes difficult to distinguish between genuine blocking of the mechano-sensitivity and deterioration of the recording conditions. The fish were therefore exposed to the different test solutions for 24 h prior to the recordings, and the mechano-sensitivity was then measured as the increased firing rate evoked by 4×10^{-4} cm vibrations at the optimal position along

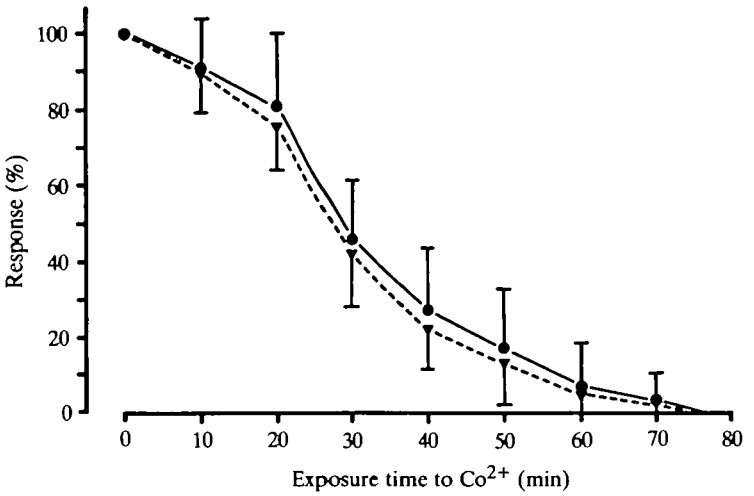

Fig. 4. Time course of the inhibiting effect of 1 mmol l^{-1} Co^{2+} in Ca^{2+}-free solution on the mechano-sensitivity of the roach. The response to the same stimulus as described in Fig. 3 was measured either as synchronization index (▼) or increased firing frequency (●). The mean values ± S.D. for five fish are included, and both types of response were evidently blocked by Co^{2+} to a similar degree.

the lateral line canal. In most cases both sides of the fish were tested. During the exposure period, the fish were kept separate in tanks containing 25 l of test solution. To minimize the influence of the presence of the fish on the ionic composition of the water, the test solution was changed after each exposure.

Fig. 5 presents the results from the experiments testing the blocking effect of 24 h of exposure to standard fresh water with different Co^{2+}/Ca^{2+} ratios. A Co^{2+} concentration of 0·03 mmol l^{-1} was sufficient to block completely the mechano-sensitivity in test solutions containing 0·03 mmol l^{-1} Ca^{2+} or less. Even 0·01 mmol l^{-1} Co^{2+} completely blocked the response to ball vibration at this Ca^{2+} concentration, whereas massive pipette stimulation evoked a small increase in the firing frequency. Fig. 5 clearly shows that elevation of the Ca^{2+} concentration effectively antagonized the inhibiting effect of Co^{2+}, and Table 1 summarizes the main results from this series of experiments. At effective Co^{2+}/Ca^{2+} ratios complete blocking may, of course, be achieved after shorter exposure than 24 h. The mechano-sensitivity was, for instance, completely abolished after 12 h in fresh water containing 0·1 mmol l^{-1} Co^{2+} and 0·03 mmol l^{-1} Ca^{2+}. However, the relationship between the necessary exposure time and the different Co^{2+}/Ca^{2+} ratios was not studied further.

In fish in which the normally increased firing rate in response to mechanical stimulation had been abolished by Co^{2+} treatment, a sudden drop of the spontaneous firing frequency upon termination of the stimulus was often observed. This curious effect was seen after both ball vibration and pipette stimulation, although it was most pronounced after the latter stimulus (Fig. 6). A more modest post-stimulus inhibition of this type was also occasionally seen following the normal excitation by pipette stimulation in Co^{2+}-free solution.

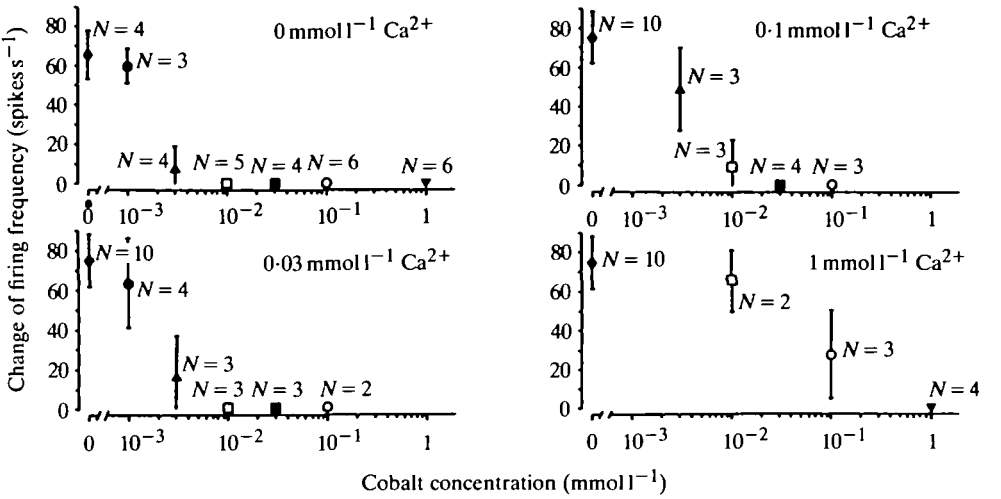

Fig. 5. Blocking of the lateral line mechano-sensitivity by 24 h exposure to water with different Co^{2+}/Ca^{2+} ratios. The response to the same stimulus as described in Fig. 4 is presented as mean values ± S.D. of the increased firing rate. The number of fish tested at each ratio is indicated on the figure. The inhibiting effect of Co^{2+} was clearly antagonized by Ca^{2+} in a competitive manner.

When using Co^{2+} to block the mechano-sensitivity of the lateral line in behavioural experiments, it is desirable to employ a concentration sufficiently high to provide a reasonable safety factor. However, the concentration must, of course, be below the level giving disordered behaviour. Long-term exposure to 1 mmol l^{-1} Co^{2+} proved lethal to the fish, which died after 1–2 weeks. However, no general behavioural change was observed in roaches after 1 week in 0·1 mmol l^{-1} Co^{2+}. It is reasonable to suggest that Co^{2+} might disturb the ionic transport across the gills, thus inducing hypotonicity of the body fluids in freshwater fish. In agreement with this notion, the dying fish exposed to 1 mmol l^{-1} Co^{2+} for 1 week showed a decline of the plasma

Table 1. *Summary of the blocking effect of Co^{2+} on the lateral line mechano-sensitivity at different Ca^{2+} concentrations*

Cobalt concentration (mmol l^{-1})	Calcium concentration (mmol l^{-1})			
	0	0·03	0·1	1
1	−	−	−	−P
0·1	−	−	−P	+
0·03	−	−	−P	+
0·01	−P	−P	+	+
0·003	+	+	+	+

The symbol − indicates that none of the tested fish showed responses to vibrating ball stimulation.

The symbol + indicates that some or all of the tested fish displayed sensitivity to this type of stimulus.

P indicates that some of the fish showed weak responses to strong pipette stimulation.

The exposure time to all the test solutions was 24 h.

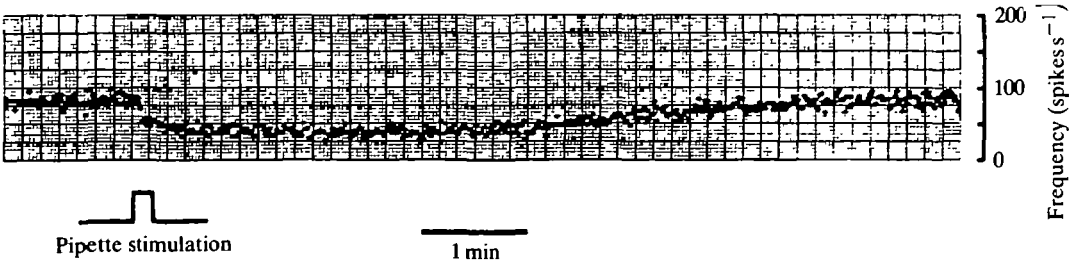

Fig. 6. Post-stimulus depression of the spontaneous firing rate after pipette stimulation in a fish exposed to water containing 0·1 mmol l^{-1} Co^{2+} and 0·03 mmol l^{-1} Ca^{2+} for 24 h. No excitation was seen during the stimulation, which instead was followed by a reduced firing rate for about 8 min.

chloride concentration to about 50 % of the control values. However, the plasma osmolality and chloride concentration showed no change after 1 week of exposure to 0·1 mmol l^{-1} Co^{2+} in Ca^{2+}-free fresh water at 8°C.

To study the recovery of mechano-sensitivity after complete blocking of the lateral line, the fish were initially exposed to Ca^{2+}-free solution with 0·1 mmol l^{-1} Co^{2+} for 24 h. Thereafter the fish were transferred to standard fresh water with different Ca^{2+} concentrations, and throughout the recovery period different fish were taken out for measurement of the mechano-sensitivity of the lateral line. No recovery was seen after 24 h in Ca^{2+}-free solution, whereas weak responses to massive pipette stimulation were evident after only 3 h in water containing 1 mmol l^{-1} Ca^{2+}. However, an amazingly long period of 2–3 weeks was needed to reach normal sensitivity to vibrating ball stimulation.

Effects of Co^{2+} on the inner ear microphonic potentials

Fig. 7 presents stimulus–response curves for utricular microphonic potentials recorded from a roach taken directly from the storage tank. The potentials were evoked by vibrating table stimuli of different frequencies. To test if Co^{2+} added to the external medium affected the microphonic sensitivity, a group of fish was exposed to Ca^{2+}-free water containing 1 mmol l^{-1} Co^{2+} for 24 h. A comparable control group was kept in standard fresh water for the same period. This Co^{2+} treatment is far in excess of that needed for complete inhibition of the mechano-sensitivity of the lateral line. Fig. 8A shows the stimulus–response curves at 75 Hz for both the exposed group and the control fish, and it is evident that the Co^{2+} treatment had no effect on the microphonic sensitivity.

The long-term effect of Co^{2+} exposure was tested in standard fresh water containing 0·1 mmol l^{-1} Co^{2+}. This concentration is sufficiently low to avoid obvious behavioural disorders in roach, but still causes complete blocking of the lateral line mechano-sensitivity after just 12 h if the Ca^{2+} concentration is kept low. Fig. 8B displays the stimulus–response curves at 75 Hz for three fish after 5 days exposure to this solution, and the microphonic sensitivity is clearly not reduced compared to the controls presented in Fig. 8A.

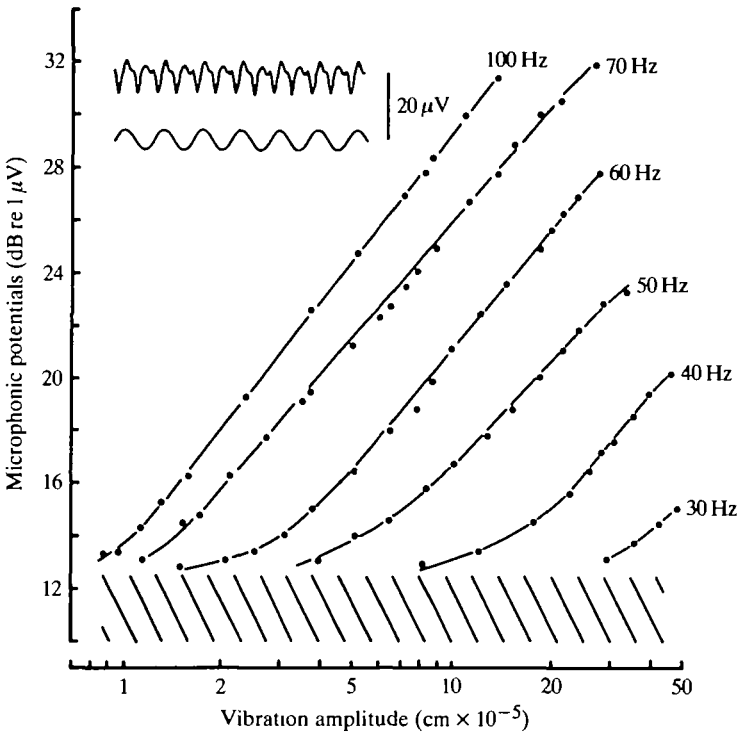

Fig. 7. Stimulus–response curves for utricular microphonic potentials evoked by vibrating a roach in air at different frequencies. Hatching indicates the electric background noise. Inset: sample recording of microphonic potentials (upper trace) during 70 Hz vibrations of 5×10^{-5} cm (lower trace).

DISCUSSION

It is clear from the present experiments that it is possible to block completely the mechano-sensitivity of the canal lateral line organs in the roach by Co^{2+} treatment. Only the trunk organs were tested, although the roach also has separate lateral line canals and a large number of superficial neuromasts on the head. However, the head canals communicate with the exterior through pores in a similar way to the canals on the trunk, and it is reasonable to assume that Co^{2+} added to the external water also inhibits the head organs.

The inhibiting effect of Co^{2+} was antagonized by Ca^{2+} in a competitive manner, which has previously been shown for superficial neuromasts in amphibians (Sand, 1975) and fish (Baumann & Roth, 1986). The critical blocking factor is therefore the Co^{2+}/Ca^{2+} ratio. It is difficult to keep the Ca^{2+} concentration of the test solution extremely low. Traces of Ca^{2+} will always be present, and the fish will also continuously excrete Ca^{2+}. Co^{2+} may, in addition, to some extent be chelated or adsorbed, and a relatively high initial Co^{2+}/Ca^{2+} ratio should therefore be employed to block the lateral line in behavioural experiments. In experiments involving inhibition of the lateral line for several days, it is recommended that the concentrations of Ca^{2+} and Co^{2+} are measured regularly to avoid critical reduction of the

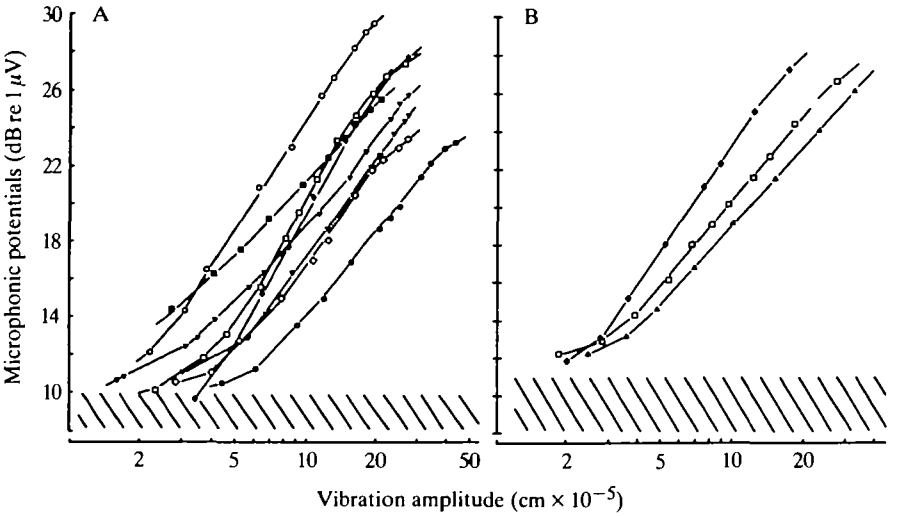

Fig. 8. Utricular microphonic responses of different roaches to 75 Hz vibrations. Hatching indicates mean electric background noise. (A) Open symbols represent fish exposed to Ca^{2+}-free fresh water containing 1 mmol l^{-1} Co^{2+} for 24 h; filled symbols represent data from control fish. (B) Comparable recordings from three roaches exposed to Ca^{2+}-free test solution containing 0·1 mmol l^{-1} Co^{2+} for 5 days. The microphonic sensitivity was clearly not reduced by the Co^{2+} treatment.

Co^{2+}/Ca^{2+} ratio. High Co^{2+} concentrations have toxic effects on the fish: the roach died after 1–2 weeks exposure to 1 mmol l^{-1} Co^{2+}. However, no behavioural disorders were observed after 1 week in water containing 0·1 mmol l^{-1} Co^{2+}, whereas complete blocking of the lateral line was observed after 12 h if the Ca^{2+} concentration was kept below 0·1 mmol l^{-1}. A similar blocking solution and exposure time may therefore be recommended for behavioural experiments.

Among possible side effects of this treatment are influence on other superficial sensory systems, such as olfaction and taste, and reduced ionic transport capacity of the gill epithelium. However, olfaction in fish is not inhibited by Co^{2+} (Yoshii & Kurihara, 1983), and no change in plasma osmolality and chloride concentration was seen after a 1-week exposure to 0·1 mmol l^{-1} Co^{2+} at 8 °C. The utricular microphonic sensitivity was not reduced even after 5 days in this solution. The microphonic potentials are directly connected to the hair cell receptor potentials (see Sand, 1976). We therefore conclude that the recommended treatment selectively inhibits the lateral line, leaving the inner ear sensitivity intact. This method for selective blocking of the lateral line mechano-sensitivity is probably transferable with minor modifications to most other freshwater fish possessing lateral line canals with regular pores. The method has, for instance, been successfully employed to elucidate the role of the lateral line in the predatory behaviour of the bluegill (*Lepomis macrochirus*) (A. J. Kalmijn, O. Sand & P. S. Enger, in preparation). However, the method cannot be directly applied to marine species, owing to the high Ca^{2+} concentration of sea water.

The recovery of the mechano-sensitivity of the lateral line after Co^{2+} exposure was clearly a slower process than the blocking. The recovery was dependent upon both

the duration of Co^{2+} exposure and the Ca^{2+} concentration of the recovery solution. In the present experiments, recovery periods between a few hours and 2–3 weeks were required to reach full restitution after a complete block of the lateral line. However, the minimum recovery time to reach normal sensitivity was not studied in detail for the different blocking protocols.

The physiological mechanism for the blocking effect of Co^{2+} on the hair cell sensitivity and the antagonizing effect of Ca^{2+} is still not clear (Sand, 1979). Co^{2+} is a well-known competitive inhibitor of Ca^{2+} fluxes through membrane channels (Hagiwara & Byerly, 1981), and Ca^{2+} is probably involved at several levels in the hair cell transduction process. This ion may be an important carrier of the receptor current in lateral line hair cells (Sand, 1975; Baumann & Roth, 1986), or it may modify the permeability of the transducer membrane to other ion species (Russell & Sellick, 1976). Ca^{2+} may also be important for maintaining the stiffness of the stereocilia (Orman & Flock, 1983) or the mechanical properties of the cytoskeleton (Hudspeth, 1983).

This work was supported by grants from The Norwegian Council for Science and the Humanities and The Nansen Foundation.

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