

## BINAURAL INTERACTION IN THE COD

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### SUMMARY

1. Recordings were made from 93 single units in the acoustical lobes and the *torus semicircularis* of the cod during sound stimulation and anodal blocking of the posterior sacculus nerves.

2. Most medullary sound responses were phase locked to the stimulus to some degree. The phase locking was less pronounced in the *torus semicircularis*, and sound stimulation sometimes caused clear inhibition of activity in this area. A large fraction of the units in both recording loci was insensitive to our sound stimuli, which acted mainly via the swimbladder.

3. Peripheral blocking caused decreased activity and inhibition of sound responses in the acoustic lobes, indicating excitatory ascending input to this region. Binaural interaction was found in 8 of 29 medullary units tested during both ipsi- and contralateral block.

4. Single-sided blocking experiments revealed both inhibitory and excitatory input to the *torus semicircularis* region. Binaural interaction was found in 3 of the 5 units tested during both ipsi- and contralateral block in this area.

### INTRODUCTION

Directional hearing in terrestrial vertebrates is based on analysis of differences in sound intensity, phase and time of arrival at the two ears, and binaural processing in the central auditory system provides the neural basis for sound localization in mammals (see for instance Rosenzweig & Amon, 1955; Galambos, Schwartzkopff & Rupert, 1959; Hall, 1965; Masterton, Jane & Diamond, 1967; Goldberg & Brown, 1969). Feng & Capranica (1976, 1978) have recently demonstrated binaural interactions at different levels in the brain of anurans, and it is likely that central binaural analysis is a general feature among terrestrial vertebrates.

However, the mechanism for directional hearing is different in fish. The ratio between the speed of sound in water and in air is approximately five, making differences both in phase and arrival time at the two ears correspondingly less for a fish than for a comparable animal in air. Animal tissue in air is an effective barrier to sound, whereas a fish in water is nearly acoustically transparent. The sound intensity difference between the two ears of a fish is therefore negligible. Furthermore, the swimbladder in many teleosts has an auditory function, acting as a secondary sound source reradiating sound pressure as near-field particle motion. Sound pressure is

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a scalar quantity, and directional information cannot be mediated via a single pressure detector. These physical considerations led van Bergeijk (1964) to conclude that fish lacked directional hearing in the far field, but recent behavioural studies have demonstrated that even teleosts with auditorily active swimbladders possess this ability, both for the horizontal (Schuijf, Baretta & Wildschut, 1972; Schuijf, 1975; Chapman, 1973; Chapman & Johnstone, 1974) and the median vertical plane (Hawkins & Sand, 1977). The lateral line is not involved in the far field directional responses of teleosts (Schuijf & Siemelink, 1974).

The unaided fish ear is sensitive to the vectorial quantity particle motion (Chapman & Sand, 1973), and the angle of incident sound might thus be detected directly by comparison of the response from groups of hair cells with different directions of the axis of optimal sensitivity (Enger *et al.* 1973). The amplified vibrations emanating from the swimbladder will carry no relevant directional information, which implies that the intensity threshold for detection of the presence of a sound should be lower than the threshold for directional discrimination. Dual thresholds of this type have been reported in cod (Chapman & Johnstone, 1974).

By recording saccular microphonic potentials evoked by horizontal vibration of the perch (*Perca fluviatilis*), Sand (1974) found the optimal vibration direction to deviate about 20° from the long axis of the fish. The difference in optimal vibration direction between the two ears was thus about 40°, and he suggested that comparison of the response between the two sacculi might give the fish directional information about the horizontal position of the sound source. This idea was strengthened by Schuijf & Siemelink (1974), who observed that the ability to orientate towards a sound source in the horizontal plane was abolished after cutting the nerve roots innervating *pars inferior* of the left labyrinth in cod.

Even if the mechanisms for directional hearing are qualitatively different in aquatic and terrestrial vertebrates, the central processing responsible for sound localization might thus depend upon binaural interaction in both groups. However, no neurophysiological data on binaural interaction in fish have been reported, probably due to the technical difficulties involved in presenting stimuli selectively to one ear in fish. Sound pressure stimulation via the swimbladder will affect both ears equally and stimulation by local vibration will cause severe mechanical cross-talk between the two ears. We have solved this problem by stimulating both ears simultaneously and then applying reversible anodal block to the right or left posterior saccular nerve. Our data demonstrate that binaural interaction occurs in the cod brain.

#### MATERIALS AND METHODS

##### *Preparation*

Single-unit activity in the brain was recorded from 12 cod (*Gadus morhua*) of 30–40 cm in length. This fish was chosen since it is the one in which directional hearing has been most thoroughly studied. The fish was immobilized by intramuscular injection of tubocurarine chloride (3 mg/kg) and clamped in a stereotaxic holder. The dorsal part of the skull was then opened and the brain exposed. The experiments were conducted in air, but the fish was kept continuously moist and was artificially respired by running sea water over the gills.

### Sound stimulation

Pure tone signals were generated by an oscillator and passed through a gating circuit to provide transient-free pulses with variable rise-time and duration. The gated signal passed via a variable attenuator and a 20 W power amplifier to a loud-speaker mounted 40 cm above the fish. The sound pressure was measured using a calibrated microphone, and at the selected frequencies the sound pressure variation was less than 4 dB within the area occupied by the fish. The presented sound levels are given as decibels relative to 1 microbar.

Due to the large difference in density between air and fish tissues, air-borne sound will be ineffective in vibrating the fish directly. Our sound stimuli therefore acted mainly via the swimbladder. In fish it is the particle motion component of the incident sound which carries directional information in the natural habitat (Schuijff, 1976), and we therefore tested possible binaural interaction even for units which seemed not to respond to the presented sound stimuli.

### Recording of nervous activity

Micropipettes filled with 3 M-KCl were used for the extracellular recordings of single-unit activity. The electrode resistances were in the range of 10–20 M $\Omega$ . The electrodes were connected to a high input impedance amplifier and the recorded responses were stored on magnetic tape. The stereotaxic holder was provided with a micromanipulator where electrode advancement was achieved by means of a remote-controlled step-motor. During a specific track the electrode was usually advanced in 2–10  $\mu$ m increments. Fig. 1A indicates the gross areas in the brain from which recordings were obtained. In the *medulla oblongata* the area was in the acoustical lobes at the level of the eighth nerve root, and in the *mesencephalon* recordings were made from the *torus semicircularis* region. We did not mark the exact electrode positions by dye or current injections, and the tentative map in Fig. 1 is drawn from estimates based on electrode angles and tracking depths.

### Blocking of the posterior saccular nerve

The blocking electrodes consisted of a pair of glass insulated silver wires 100  $\mu$ m in diameter. The distance between the wires at the tip was 0.5–0.8 mm. This gap was parallel with the nerve when the electrode was positioned on the surface of the posterior saccular nerve. Positioning of the electrodes was performed using two manually operated micromanipulators attached to the stereotaxic holder. The saccular nerves in cod are short and not easily accessible. They are shielded from any dorsal view, and during electrode insertion the *cerebellum* and acoustical lobes therefore had to be pushed gently medially. In Fig. 1D, which gives a lateral view of the left electrode in proper position, the sacculus is hence considered transparent, with only its outline marked.

The blocking electrodes were connected to a stimulator via an isolator unit. Block of transmission through the nerve was achieved during d.c. pulses of 2–6 V and 0.5–1.5 s duration, judged from central effects. The presented recordings of blocking effects were obtained with the anode proximal to the brain. For the medullary units which were affected, this polarity decreased the spontaneous firing rate in addition to

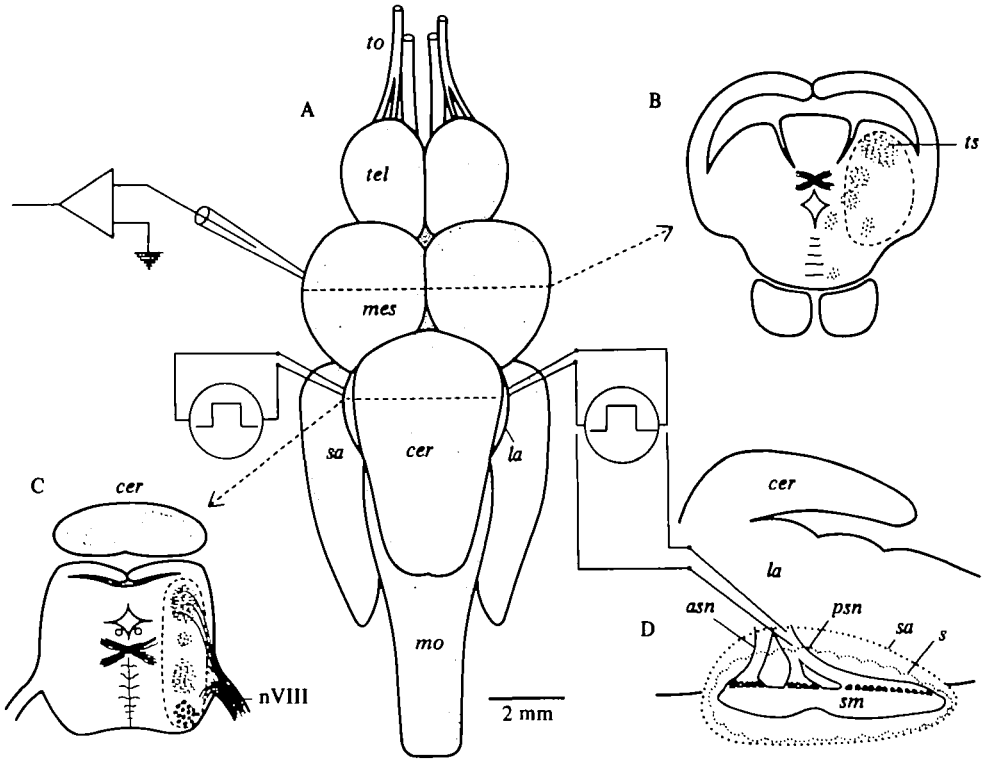


Fig. 1. Sketch of the preparation. (A) Dorsal view of the brain with the recording and blocking electrodes positioned in the *torus semicircularis* and on the posterior saccular nerves, respectively. (B, C) Cross-section of the *mesencephalon* and *medulla oblongata* at the levels indicated by broken lines in A. The encircled parts indicate areas from which recordings were obtained. (D) Lateral view showing the position of the left blocking electrode. The sacculus and sagitta are drawn transparent, and their outlines are indicated by dotted lines. *asn* anterior saccular nerve, *cer* cerebellum, *la* lobus acusticus, *mes* mesencephalon, *mo* medulla oblongata, *nVIII* nerve VIII, *psn* posterior saccular nerve, *s* sagitta, *sa* sacculus, *sm* saccular macula, *tel* telencephalon, *to* tractus olfactorius, *ts* torus semicircularis. The dorsal view A is redrawn after Dale (1976).

blocking the sound response. The opposite polarity often induced increased spontaneous firing, even if the sound response was blocked. The standard pulses were alternated with pulses of reversed polarity in order to avoid electrode polarization. This procedure only blocked a fraction of the peripheral acoustic fibres, since the lagenar and the anterior saccular nerve were unaffected. This limitation was acceptable in the present project, but it was essential that central units were not influenced directly by the blocking pulses due to current spread through the tissues. This possibility was ruled out by applying pulses of ten times normal magnitude with the electrodes in intracranial fluid but lifted off the nerve. In these cases no effect was observed on central units.

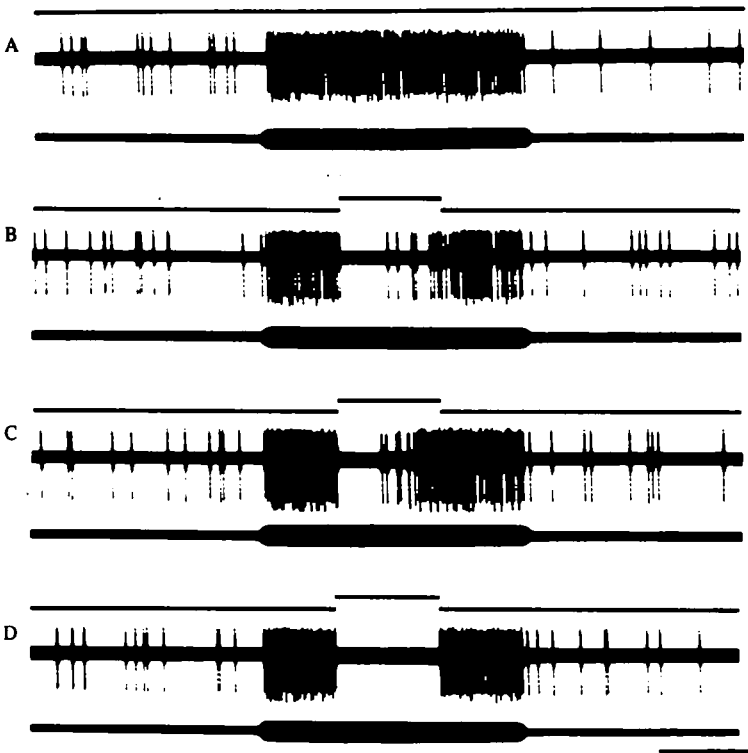


Fig. 2. Responses of a unit in the acoustical lobe. (A) Response to a 160 Hz tone at 21 dB. (B, C) Anodal blocking of the ipsi- and contralateral posterior saccular nerve, respectively, in the middle of the tone stimulus. The decreased firing rate during blocking indicates excitatory input to the unit. (D) Simultaneous peripheral blocking of both sides during sound stimulation. The blocking period in this and later figures is indicated on the upper trace of each recording, whereas the sound stimulus is monitored on the lower trace. Vertical and horizontal calibration bars: 2 mV and 1 s, respectively.

## RESULTS

### *Recordings from the acoustical lobes*

Single-units in the acoustical lobes usually displayed irregular spontaneous activity. The firing rate varied from  $< 5$  to  $> 100$  impulses/s, but was approximately constant for a specific unit. In some units spontaneous activity occurred in regular bursts. A clear response to sound stimuli was seen in 39 of the total number of 53 recorded medullary units. The different types of auditory neurones in cod and the frequency dependency of the sound responses will be described in detail elsewhere (K. Horner, in preparation). The sound responses of medullary units were phase locked to the stimuli. Sound stimulation caused an increase and/or a decrease of the firing rate, depending on stimulus frequency and the spontaneous activity. The degree of phase locking varied, and was for many units only revealed after computer analysis of the response. The response to variation in sound pressure amplitude was often a varying degree of phase locking between stimulus and the unit activity, rather than an altered firing rate. Sound never induced a complete inhibition of the spontaneous activity of

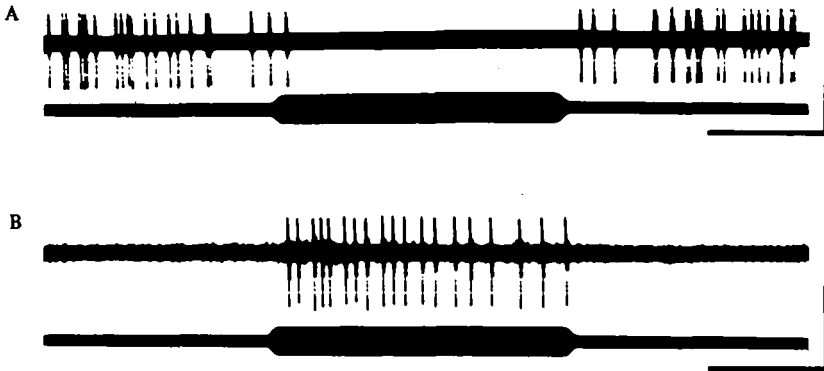


Fig. 3. Sound responses of two different units in the *torus semicircularis*. (A) Inhibition of the spontaneous activity caused by a 190 Hz tone at 20 dB. (B) Excitation of a non-spontaneously active unit induced by 170 Hz sound stimulation at 21 dB. Vertical and horizontal calibration bars: 0.3 mV and 1 s, respectively.

medullary units, as might have been expected if the units received inhibitory peripheral input.

To demonstrate input to medullary units from the contralateral ear, initial experiments were performed with single-sided peripheral block. Blocking of the contralateral posterior saccular nerve had effect on 3 of the 8 sound sensitive units tested, while ipsilateral block affected 9 of a total number of 12 sound-sensitive units. Both the sound response and the spontaneous firing rate diminished during the block when the anode was proximal to the brain. Four sound-insensitive units were also tested, and in 2 of these reduced spontaneous activity was induced by ipsilateral block. It is clear then that some of the medullary units receive peripheral input from the contralateral side, but the results are not conclusive evidence for binaural input to these units. A total number of 19 sound-sensitive units were therefore tested during both ipsi- and contralateral peripheral block. Six of these units were not affected at all, 7 units were only influenced by ipsilateral block and the remaining 6 units were affected by blocking of the posterior saccular nerve on either side.

The response pattern displayed by the last group clearly indicates that these units receive binaural input (Fig. 2). The response to sound only is seen in Fig. 2A, whereas blocking of the ipsi- and contralateral posterior saccular nerve is performed during sound stimulation in Fig. 2B and C, respectively. It is clear that peripheral blocking on either side causes a nearly complete inhibition of sound response. The block is relieved towards the end of the blocking pulse, but the unit activity is completely inhibited when peripheral block is applied to both sides simultaneously (Fig. 2D). Ten sound insensitive units were also tested for binaural input. Four of these were unaffected by peripheral block and 4 were only influenced by ipsilateral block. In the remaining 2 units peripheral block on either side caused a pronounced decrease of the spontaneous firing rate, indicating binaural input to these units via the posterior saccular nerves.

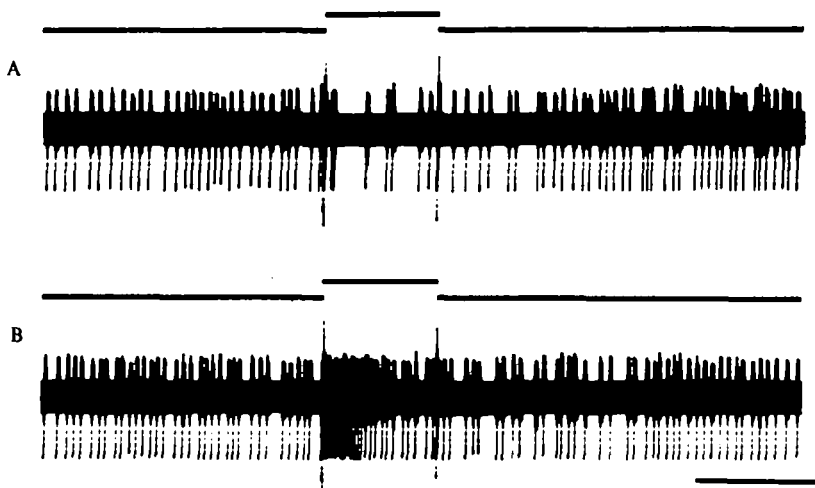


Fig. 4. Anodal blocking of a sound insensitive unit in the *torus semicircularis*. (A) Blocking of the contralateral posterior saccular nerve reduced the firing frequency, indicating excitatory input to the neurone from this side. (B) Blocking of the ipsilateral nerve increased the firing rate, indicating inhibitory input to the neurone via this nerve. Vertical and horizontal calibration bars: 0.3 mV and 1 s, respectively.

#### Recordings from *torus semicircularis* region

Recordings were made from 40 single units in the *torus semicircularis* region. Twenty-two of these units responded to our sound stimuli, usually in a similar way as described for medullary units. However, the phase locking between stimulus and response was often less pronounced. Most of the units showed irregular spontaneous activity, and units of the bursting type were also observed. Some of the units in this region were not spontaneously active. An example is given in Fig. 3 B, which shows how a continuous tone evoked a slowly adapting spike train. Fig. 3 A presents another type of unit where the spontaneous activity was completely inhibited during sound stimulation. This inhibition clearly differs from the reduced firing rate seen in some phase locked responses of medullary units.

The majority of blocking experiments involving units from the *torus semicircularis* was performed with single-sided peripheral block. Blocking of the contralateral posterior saccular nerve had effect on 5 of the 12 sound sensitive units tested, while ipsilateral block affected 7 of 9 units. Ipsilateral block affected 5 of the 9 sound insensitive units tested, whereas contralateral peripheral block influenced 3 of 5 units. An increase in firing rate during the blocking period was seen in 2 and 4 of the total number of units affected by contra- and ipsilateral blocking, respectively, but only 1 of these 6 units was clearly sound responsive. A decreased firing frequency was observed during the single-sided block in the remaining 14 units. These data indicate the existence of both excitatory and inhibitory ascending inputs to auditory units in the *torus semicircularis* region.

Five units, of which only one was sound responsive, were tested during both ipsi- and contralateral peripheral block. Two of these units were not affected, and two displayed an inhibited firing rate during peripheral blocking on either side. The

remaining unit (Fig. 4) was not sound responsive, but peripheral block on the contralateral side reduced the firing rate. Ipsilateral peripheral block clearly enhanced the firing rate. This unit possibly received inhibitory and excitatory ascending input from the ipsilateral and contralateral labyrinth, respectively.

#### DISCUSSION

At the turn of the century Herrick (1899) described fibres crossing between the two acoustic lobes, and between the acoustic lobe and the contralateral *torus semicircularis* in teleosts. These findings have been supported by later anatomical studies (Pearson, 1936; Aronson, 1963; Sarnat & Netsky, 1974), while our results, from the single-sided blocking experiments, provide physiological confirmation. One prerequisite for binaural interaction is thus present in teleosts: auditory fibres which cross from one side of the brain to the other. Our main purpose was to discover whether binaural interaction occurs in cod. The double-sided blocking experiments clearly show that the acoustical lobes and the *torus semicircularis* contain neurones which receive ascending input from both ears.

We have limited our study to input carried centrally via the posterior saccular nerves. It is therefore not surprising that about 40% of the sound-responsive units were not affected by peripheral block. Our blocking experiments revealed no evidence for inhibitory ascending input to medullary units, but this negative result must be interpreted with care. The involvement of different parts of the *pars inferior maculae* in directional hearing is likely to differ, and blocking of other auditory nerve branches might reveal a different pattern. In the bullfrog (*Rana catesbeiana*) the majority of binaural neurones in the first central auditory nucleus were excited by the contralateral ear and inhibited by the ipsilateral ear, and only 20% of the cells received excitatory input from both ears (Feng & Capranica, 1976).

Seven of the 23 units in the *torus semicircularis* region which were affected by peripheral block received inhibitory ascending input, which came from the ipsilateral ear in 5 of these cells. Six of the cells receiving inhibitory input was insensitive to our air-borne sound stimuli, which acted via the swimbladder. However, these cells might be particularly interesting regarding directional hearing, since fish are able to extract directional information from the particle motion components of the incident sound.

The present paper thus demonstrates the existence of binaural interaction in the acoustical lobes and the *torus semicircularis* of cod. Future studies should employ more refined techniques to explore possible monaural and binaural central interactions of inputs from different parts of the fish ear. The central neurones should also be tested for sensitivity to both sound pressure and particle motion.

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