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Neuronal Encoding of Sound Direction in the Auditory Midbrain of the Rainbow Trout

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Wubbels, R. J. and N.A.M. Schellart. Neuronal encoding of sound direction in the auditory midbrain of the rainbow trout. *J. Neurophysiol.* 77: 3060–3074, 1997. Acoustical stimulation causes displacement of the sensory hair cells relative to the otoliths of the fish inner ear. The swimbladder, transforming the acoustical pressure component into displacement, also contributes to the displacement of the hair cells. Together, this (generally) yields elliptical displacement orbits. Alternative mechanisms of fish directional hearing are proposed by the phase model, which requires a temporal neuronal code, and by the orbit model, which requires a spike density code. We investigated whether the directional selective response of auditory neurons in the midbrain torus semicircularis (TS; homologous to the inferior colliculus) is based on spike density and/or temporal encoding. Rainbow trout were mounted on top of a vibrating table that was driven in the horizontal plane to simulate sound source direction. Rectilinear and elliptical (or circular) motion was applied at 172 Hz. Generally, responses to rectilinear and elliptical/circular stimuli (irrespective of direction of revolution) were the same. The response of auditory neurons was either directionally selective (DS units, $n = 85$) or not (non-DS units, $n = 106$). The average spontaneous discharge rate of DS units was less than that of non-DS units. Most DS units (70%) had spontaneous activities < 1 spike per second. Response latencies (mode at 18 ms) were similar for both types of units. The response of DS units is transient (19%), sustained (34%), or mixed (47%). The response of 75% of the DS units synchronized to stimulus frequency, whereas just 23% of the non-DS responses did. Synchronized responses were measured at stimulus amplitudes as low as 0.5 nm (at 172 Hz), which is much lower than for auditory neurons in the medulla of the trout, suggesting strong convergence of VIIIth nerve input. The instant of firing of 42% of the units was independent of stimulus direction (shift $< 15^\circ$), but for the other units, a direction dependent phase shift was observed. In the medial TS spatial tuning of DS units is in the rostrocaudal direction, whereas in the lateral TS all preferred directions are present. On average, medial DS units have a broader directional selectivity range, are less often synchronized, and show a smaller shift of the instant of firing as a function of stimulus direction than lateral DS units. DS response characteristics are discussed in relation to different hypotheses. We conclude that the results are more in favor of the phase model.

INTRODUCTION

Terrestrial vertebrates make use of interaural differences of the sound pressure signal to localize a sound source. Temporal, intensity, and spectral differences all depend on the position of the sound source with respect to the listener. However, the biological constraints for fish, living in an aquatic habitat, are quite different. The acoustic transparency of the fish body and the surrounding water are almost identi-

cal. For the fish auditory system best frequency ranges from 40 to 1,000 Hz, depending on species (Fay 1988; Schellart and Popper 1992). Therefore the wavelength exceeds the fish body length by far (velocity of sound in water is 1,450 m/s). For these reasons, the cues that terrestrial animals can use are thought to be subthreshold for these aquatic animals (Schuijf and Buwalda 1980). In general, however, sound appears most suitable to convey reliable environmental information (compared with light or olfactory agents) over long distances and within a short time. This environmental information may be of physical origin, such as the surf, waves, rapids, or human activities, or come from biological sources, e.g., prey, predators, and conspecific communication of sound producing fish (Schellart and Popper 1992). Behavioral experiments have demonstrated that fish are able to discriminate between different sound source directions (Buwalda et al. 1983; Schuijf and Buwalda 1980) and also between different source distances (Schuijf and Hawkins 1983).

The fish inner ear, comprising three otolith systems (i.e., saccule, utricle, and lagena) with specific hair cell orientation patterns (e.g., Popper and Fay 1993; Schellart and Popper 1992), may be considered as a three-dimensional accelerometer. A sound field causes displacement of the directionally sensitive hair cells relative to the otoliths, which lag the surrounding tissue because of their higher inertia (de Vries 1950). Diametrically opposed (monopole) sound sources yield identical particle motion, with the motion vector alternately directed toward and away from the fish. Therefore the fish has to deal with a directional ambiguity of 180° . This ambiguity, however, can be solved with an additional cue provided by the swimbladder. The gas-filled swimbladder functions as a sound pressure-to-displacement transducer, stimulating the inner ears indirectly from a body-fixed angle (Fig. 1). Theoretically, vectorial addition of direct displacement and swimbladder mediated displacement yields (for pure tones) elliptical displacement orbits (de Munck and Schellart 1987). Basically, two models have been proposed to solve the 180° ambiguity by making use of the availability of the indirect stimulus.

The first model (the phase model) hypothesizes two detector systems, one for the direct motion component of the sound field and the other for the swimbladder-mediated, or pressure, component (Schuijf 1976a,b; Schuijf and Buwalda 1980). These detector systems are supposed to be separated spatially, because somehow the motion component and the pressure component have to be segregated

FIG. 1. Stimulation of inner ear of swimbladder-bearing fish. Motion component of sound field causes displacement of directionally sensitive hair cells relative to otolith, which lags surrounding tissue because of its higher inertia. Gas-filled swimbladder functions as a sound pressure to displacement transducer, stimulating inner ear indirectly from a body-fixed angle. Vectorial addition of direct displacement and swimbladder mediated displacement yields an elliptical displacement orbit.

by the auditory system for further analysis. A differential neuronal mechanism could accomplish this. For instance, because of the relatively fast attenuation of the pressure component (in the tissue between swimbladder wall and inner ear), comparison of the input of two, equally oriented, populations of hair cells (at different distances from the swimbladder wall) would provide a measure for the pressure component (elimination of identical motion component). Comparison of bilateral input from hair cells with a symmetrical orientation relative to the median plane provides a measure for the direct motion component (elimination of identical pressure component).

Analysis of the phase difference between the pressure and motion components solves the 180° ambiguity. Others have elaborated on this model, e.g., with respect to the physiological realization and the processing in the auditory system (Rogers et al. 1988) or with respect to the performance of this model with various sound sources or under noisy conditions (van den Berg and Buwalda 1994). Recently, a neural model, incorporating the phase model, has been put forward as a possible mechanism for left-right discrimination preceding the Mauthner-initiated escape response (e.g., Eaton et al. 1995). It has been demonstrated psychophysically that the phase relation between the pressure and motion component is a cue for discrimination between otherwise identical sound fields (Wubbels and van den Berg 1996).

The second model (the orbit model) recognizes that analysis of the displacement orbits may provide the location of the sound source. The orbit, which is characterized by the orbit parameters orientation of the long axis, ratio of the long and short axes, and direction of revolution within the orbit, is specific for each location of a (monopole) sound source (de Munck and Schellart 1987; Schellart and de Munck 1987). Theoretically, however, the performance of a monaural mechanism would be very poor for some directions. A binaural mechanism, making use of the interaural differences between the displacement orbits caused by the symmetrically opposed angle of incidence of the indirect stimulus vector, would improve the

performance of this model (Schellart and de Munck 1987; Schellart and Buwalda 1990). Evidence for binaural interaction has been obtained in the cod (Horner et al. 1980). Also, a fish in which the VIIIth nerve was cut unilaterally appeared unable to discriminate between different sound directions (Schuijff 1976a). Therefore we expect fish to use binaural input for localization.

The midbrain torus semicircularis (TS; homologous to the inferior colliculus) is the first area along the fish auditory pathway with considerable binaural input (e.g., de Wolf et al. 1983). It is known from other studies that auditory processing, such as frequency and temporal analysis, occurs here (Crawford 1993; Lu and Fay 1993; Nederstigt and Schellart 1986; Schellart et al. 1987) and that input from the lateral line system (Schellart and Kroese 1989) and visual system (Coughlin and Hawryshyn 1994; Schellart and Rikkert 1989; Wubbels et al. 1995) are processed in this nucleus as well.

A directional response code for auditory stimulation has been observed in the TS of the frog (Feng 1981). Mapping of the auditory space has been demonstrated in the avian homologue of the inferior colliculus (Knudsen and Konishi 1978; see also Irvine 1992) and in the superior colliculus of mammals (Middlebrooks and Knudsen 1984; see also Irvine 1992). In a previous report, we have shown that sound direction is encoded by neurons in the TS of fish, and that these directionally selective (DS) units are topographically mapped (Wubbels et al. 1995).

The acoustic properties of water confront investigators of fish hearing with a technical problem, i.e., stimulus control. First, for audible frequencies, wavelengths are several meters long, whereas the experimental tank is much smaller. Therefore, reflections from the walls and the water surface may cause large local amplitude variations. Second, the amplitude and phase of direct and indirect stimulation of the inner ear (Fig. 1) are known only from model studies (de Munck and Schellart 1987; Schuijff 1976a,b). To overcome this problem of stimulus control, we have chosen another approach.

In the experiments presented here, a pure motion stimulus was applied by means of a vibrating platform, a method used before by investigators of the fish auditory system (Enger et al. 1973; Fay and Olsho 1978), and the contribution of the swimbladder was eliminated (see METHODS). The motion stimulus was either rectilinear or elliptical, mimicking the direct motion stimulus, or the combined direct motion stimulus plus the indirect pressure stimulus, respectively.

We investigated the neuronal encoding of sound direction in the TS of the rainbow trout. This species has moderate hearing abilities and lacks the specializations of the peripheral auditory system observed in some other species (Schellart and Popper 1992). In the phase model, temporal information from the two independent detector systems (for the motion component and the pressure component) is essential, requiring strong synchronization of the spike response. Somewhere in the CNS, the temporal information contained in the pressure and motion systems has to be analyzed. In the orbit model, the auditory system has to decompose the orbit parameters (i.e., orientation of the long axis, axes ratio, and direction of revolution) into their vectorial counterparts. Orbit parameters have to be encoded by the relative amount of stimulation of populations of hair cells with a different directional orientation. This would imply a spike density code. Thus according to the two models treated above, the cues that are used for directional hearing, and their neuronal encoding in the fish auditory system, are quite different. Therefore, knowledge of the encoding properties of sound source direction in the brain may provide indications about the validity of these theoretical models.

We encountered auditory neurons with a directional-selective response and auditory neurons that responded independent of the stimulus direction. In the results we present below, response characteristics of DS units and non-DS units are compared and DS responses are analyzed in detail to gain insight into the stimulus cues that are used to obtain directional information. The data strongly suggest that, apart from the spike density coding of sound direction, the temporal characteristics of the response are essential for sound source localization.

METHODS

Surgical preparation

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] of 25- to 30-cm body length were anesthetized initially with 250 mg/l MS 222 (Sandoz, Switzerland) and transferred to the experimental set-up. Fixation of the skull was achieved by clamping the upper jaw between a tight-fitting mouthpiece and a nosepiece. The trunk was fastened with Velcro strips. Most of the fish body was wrapped in wet tissue to prevent dehydration of the skin. Water temperature was kept at 8–14°C, depending on the season. Through the mouthpiece artificial respiration was provided by water (0.4 l/min) saturated with fresh air. During the surgical procedures, the water contained 50 mg/l MS 222. With a dental drill, an opening in the skull was made to expose the optic tectum. All wounds inflicted were treated with lidocaine ointment. After finishing the surgical procedures, the fish was immobilized with 0.15 mg pancuronium bromide (Pavulon, Organon, The Netherlands) injected intraperitoneally, and the MS 222 was removed from the system by replacing the circulating water. Because MS 222 (reversibly) affects the activity of CNS neurons (Späth and Schweickert 1977), recordings

started 2 h after removal of this anesthetic. Animals were killed at the end of an experiment. Animal treatment was in accordance with the Dutch law.

Stimulus generation

To the fish inner ear, the underwater acoustic stimulus is essentially a motion stimulus. The contribution of the swimbladder, which functions as a pressure-to-motion transducer, to the compound motion vector in the inner ear (see INTRODUCTION) has been estimated theoretically (de Munck and Schellart 1987; Schuijff 1976a,b). However, the swimbladder's contribution has never been verified experimentally by physical measurements. In our experiments, a two-dimensional vibrating platform was used to apply a stimulus to the fish inner ear. The water level was kept below the fish body, and thus the swimbladder did not contribute to the stimulation of the inner ear. Control experiments with a hydrophone (Brüel and Kjær, type 8103) in the stomach of the fish revealed that the pressure/motion ratio inside the fish body was 44 dB less than in the far field. This implies that for the smallest stimulus amplitudes, pressure was subthreshold (Abbott 1973). In our experiments, therefore, the motion vector stimulating the inner ear was well defined and under control. Keeping the water level low also has the advantage that the lateral line system is not, unintentionally, stimulated. This sensory system also projects to the TS (Schellart and Kroese 1989) and might, otherwise, have hampered the interpretation of the responses.

The mechanical properties of the vibrating platform and its performance have been reported before (Schellart et al. 1995). Briefly, the stimulus device comprises a square vibrating platform suspended by eight springs to an outer frame (50 × 50 cm). The platform is driven, independently in the x and y direction, by eight electromagnetic coils between the platform and the frame. The fish tank is mounted on this platform. The entire construction itself is mounted on top of an air-damped experimental table, resulting in an ambient mechanical noise level at the position of the fish of 0.36 mm s^{-2} RMS for 50–300 Hz.

With a miniature three-dimensional accelerometer (weight 1.7 g, sensitivity 1.5 mV/m.s^{-2}), attached with three small screws, the motion of the fish skull was measured. We suppose these measurements reflect the movements of the sensory maculae and thus are a reasonable estimate of the deflection of the hair bundles (de Vries 1950). It appeared that the fish head did not exactly copy the platform motion. This problem was solved by measuring the skull motion, before an experiment, as a function of the (known) driving signal in different directions in the horizontal plane. With this information, the input signal necessary to obtain the desired skull motion was calculated (Goossens et al. 1995).

The standard stimulus in our experiments was rectilinear motion applied as tone bursts (rise time 80 ms, stimulus on 640 ms, fall time 130 ms, stimulus off 590 ms). Both at stimulus onset and offset, the response, which often has a transient component originating from the neuron's physiological characteristics, also is influenced by the relatively long time constant of the vibrating platform's damping properties (Schellart et al. 1995). To minimize transient response components at stimulus offset, the fall time was made to last longer than the rise time, thus allowing a reasonable estimate of spontaneous activity during the silent interval. During an experiment, the motion vector was rotated continuously anticlockwise in the horizontal plane with each consecutive tone burst 7.2° (occasionally 3.6°) apart. For this standard stimulus, cross-talk perpendicular to the stimulus direction was always $>20 \text{ dB}$ smaller than the stimulus itself. Cross talk in the vertical direction (which depended on the stimulus direction in the horizontal plane; Fig. 2d from Goossens et al. 1995) could not be compensated for, but care was taken that ambiguous responses (i.e., spike density

histograms that coincided with the cross talk in the vertical direction) were not taken into account in the results presented below.

Although our method of stimulating the fish inner ear is a guarantee for a clearly defined and controlled stimulus, it also has its limitations. Resonances of the system at frequencies >240 Hz (Schellart et al. 1995) and the (time consuming) necessity to calculate the required compensation of the driving signal at the selected stimulus frequency (Goossens et al. 1995) made it impossible to characterize each neuron in terms of best frequency (but see Nederstigt and Schellart 1986). Generally, recordings lasted 5–15 min (see DISCUSSION). Characterization of a neuron in terms of directional selectivity at a single stimulus amplitude required ~2 min.

The frequency of the standard stimulus was 172 Hz. The –3-dB width of the audiograms of salmonids as the rainbow trout (Abbott 1973) and the salmon (Hawkins and Johnstone 1978) is ~50–250 Hz. The range of applied stimulus intensities matches the behaviorally determined dynamic range (Abbott 1973). In a minority of experiments, the applied stimulus was not rectilinear but elliptical or circular (i.e., the fish's head performs an elliptical or circular orbit). For elliptical orbits, the long axis was parallel to the previously determined direction of preference of the neuron. The responses to a positive and a negative direction of revolution of the orbit's motion vector were compared. Sometimes, it was possible to repeat the experiment at another frequency.

Recording procedure

Glass microelectrodes filled with 3 M KCl (40–80 M Ω) were advanced with a stepping motor at an angle of ~75° through the optic tectum and the midbrain ventricle to penetrate the (right-hand) TS, where (single-unit) recordings were made. Arrival of the electrode tip at the TS surface was recognized easily, as a jump of the recorded DC signal, on the oscilloscope. This depth served as a reference for recording depth in the TS. The position of the electrode tip in the horizontal plane was calculated from the penetration point on the tectum, penetration depth, and angle. Spikes, recorded either extracellularly or intracellularly (WPI Intra 767 amplifier), were converted to standard transistor transistor logic pulses and fed into a computer along with signals marking the start of the tone burst and the zero-crossings of the stimulus. Also the amplitude and phase of the three accelerometer signals were stored. When, during an experiment, a unit was encountered, the first objective was to establish whether it was a DS unit or not.

Data analysis

Spike activity was inspected on-line by means of a dot display. Dot displays were split in two parts, one showing the spikes while the tone burst was on (*left half* in Fig. 2) and the other showing the spike activity while the tone burst was turned off and the subsequent silent interval (*right half* in Fig. 2). From this dot display, the spike density histogram, as a function of the stimulus direction, was calculated (at the right of each dot display in Fig. 2). From the histograms below each dot display [poststimulus time histograms (PSTHs)] the (global) transient/sustained nature of the response (irrespective of stimulus direction) could be evaluated.

Firing of auditory neurons was registered with respect to the positive zero-crossings of the sinusoidal stimulus with a temporal resolution of 40 μ s. From sections of the dot display, period histograms (presented as polar plots) were computed, showing whether spikes were synchronized to the stimulus or not (Fig. 3). From the Fourier-transformed spike density of the period histogram, the synchronization index (SI) and the phase were calculated, $SI = 0.5 \cdot F_1 / F_0$ (F_0 : mean activity, F_1 : first harmonic), ranging from 0 to 1. A χ^2 version of the Rayleigh test (Mardia 1972) was used

to determine whether the spike distribution of the period histogram deviated from a uniform distribution.

From the spike density histogram, the preferred direction (at the maximum of the histogram), and a measure of the directional selectivity were obtained. This is the directional selectivity range, defined as the –3 dB width of the peaks in the spike density histogram. By definition, a cosine-shaped direction response relation has a directional selectivity range of 90°. Such a cosine relationship is shown by hair cell responses (Shotwell et al. 1981) and by eighth nerve fibers of fish (Fay 1984). When the –3 dB points were >150° apart or absent, a unit was considered as not directionally selective.

RESULTS

General

In the TS of 47 trout, we found 227 neurons responding to the standard, slowly rotating rectilinear, acoustic stimulus of 172 Hz. About 75% of the auditory neurons in the trout's TS respond at this frequency (Schellart 1990), and thus a fair sample was taken to investigate the directional dependency of the response. The responses of 36 units (16%) were too weak (or recordings were too short) to determine their directional dependency. The spike rate of 106 units (47%) did not depend on stimulus direction (Fig. 2A). The spike rate of 85 units (37%), however, varied with stimulus direction over an angle of 180°, after which the response was repeated (Fig. 2, B–F). This reflects the 180° directional ambiguity of the fish inner ear response (see INTRODUCTION). DS units mainly occur in the upper 400 μ m of the TS (Wubbels et al. 1995). The response of nine neurons, stimulated at 172 Hz and another frequency (between 108 and 207 Hz), depended on stimulus direction irrespective of frequency. One non-DS unit (at 172 Hz) also displayed a direction-independent response at two other stimulus frequencies.

Almost all the responses of DS units and non-DS units were excitatory, i.e., an increase of the discharge rate in response to the tone burst, and can be characterized as “primarylike” (see PSTHs in Fig. 2, A, B, and D) or “onset” (Fig. 2C) type responses (Rhode and Greenberg 1992). Sometimes, “chopper” (Fig. 2E), “pauser”, or “buildup” response behavior (Rhode and Greenberg 1992), as has been observed in the goldfish TS (Lu and Fay 1993), also was encountered in the trout. However, it should be noted that in fish the distinction between these response types appears to be less obvious than in mammals. Inhibitory responses, i.e., a decrease of the discharge rate in response to the tone burst, were encountered in non-DS units only (14%). Occasionally, an OFF response, i.e., a transient increase of discharge rate after cessation of the tone burst, was present in non-DS units (5%).

The spontaneous activity of auditory neurons in the TS usually is “irregular”, i.e., the interspike interval distribution is exponential (Fig. 2). Spontaneous activity in the TS (Fig. 4) is significantly less than in the medulla of the same species (Wubbels et al. 1993) and the auditory nerve of, for instance, the goldfish (Fay 1981) or the cod (Horner et al. 1981). Other reports also mention that spontaneous activity of auditory neurons in the TS is low (Crawford 1993; Lu and Fay 1993). On average, the spon-

FIG. 2. Auditory responses from neurons in torus semicircularis (TS) of rainbow trout, each dot representing a spike. Motion direction is rotated continuously with consecutive tone bursts 7.2° (A, B, C, and F) or 3.6° (D and E) apart. Vertical scales, indicating stimulus direction, are identical. For each recording, rostro-caudal direction (0°) is indicated; a complete rotation (360°) is indicated for last recording only (F). Tone bursts (frequency 172 Hz) and subsequent silent intervals are indicated below dot displays. Vertical histograms (scale bar: 20 spikes) show number of spikes during a single tone burst and during subsequent silent interval, respectively. Former shows directional dependency of response. Below each dot display, poststimulus time histogram (PSTH; scale bar: 20 spikes), adding response irrespective of stimulus direction, is given. Histograms in *bottom right corner* show interspike interval distribution. An example is given of a response that was independent of stimulus direction (A). Spike rate of 37% of units appeared to depend on stimulus direction (B–F). In these directional-selective responses, maxima are 180° apart (see text). Stimulus amplitude (mm s^{-2}): 30 (A and C), 1.2 (B), 60 (D and E), and 75 (F).

taneous activity of DS units is less than non-DS units' spontaneous activity (Fig. 4). Most DS units appeared to be "silent" units (spontaneous activity < 1 spikes/s). Response latencies (measured with respect to stimulus onset) of DS units, which generally did not depend much on intensity (see below), and non-DS units were of the same magnitude (≥ 11 ms). The response latency distribution is shown in Fig. 5. Latencies of DS and non-DS units appear to be the same. The mode of the distribution is found at 18 ms, which is 4 ms longer than for medullar auditory neurons (Wubbels et al. 1993).

Spike density

Regarding the spike density as a function of stimulus direction, a diversity of responses are observed. More or less cosine-shaped histograms were found (Fig. 2B), and units that were either less direction selective (Fig. 2C) or, on the other hand, very direction selective (Fig. 2D). Also, asymmetric histograms were obtained (Fig. 2, E and F). The spike density of these units varies linearly with stimulus direction, whereas spike activity sometimes diminishes below the spontaneous level (Fig. 2F). The directional selec-

FIG. 3. Dot display of a direction-dependent response (A). From sections of this recording, polar period histograms were calculated (B). Mean phase angle of spikes within period histogram is indicated ($\rightarrow$). For this unit (same as depicted in Fig. 2B), phase angle is independent of stimulus direction.

tivity range of eighth nerve fiber responses is 90° (Fay 1984). It should be realized, therefore, that auditory units in the TS with a directional-selectivity range that is not equal to 90° (this includes the non-DS units), receive input from hair cells with different orientations on the sensory maculae.

For DS units, 19% of the responses were exclusively transient (e.g., Fig. 2C), and 34% of the responses were sustained exclusively (e.g., Fig. 2B). The response of 47% of the DS units, however, consists of a sustained component together with a transient component, which is at least twice as large as the sustained component (Fig. 2, D–F). For non-DS units, transient response components apparently occur even more frequently. With respect to the transient/sustained nature of the response of auditory neurons in the TS, our results are similar to the results obtained in two mormyrid species (Crawford 1993) and the goldfish (Lu and Fay 1993). In the catfish, no differences were observed between adaptive behavior of medullar and mesencephalic auditory

neurons (Plassmann 1985). In the acoustic nuclei in the medulla of the trout, however, transient response components are observed less frequently than in the TS (Wubbels et al. 1993).

Spike synchronization

A well-known feature of primary auditory afferents, in general, is that their spikes become synchronized to the stimulus frequency. Most of the units (95%) of the acoustic nuclei of the hindbrain of the trout also synchronize (Wubbels et al. 1993). In the TS, a segregation occurs. Only 23% of the non-DS units synchronize, whereas 75% of the DS units do (Fig. 6), which is a very significant difference (χ^2 test, $P \leq 0.001$). In general, when synchronization of the spikes occurs, this is observed in the transient as well as in the sustained part of the response. Almost all synchronizing units that we encountered discharged at only one preferred

FIG. 4. Spontaneous activity of directionally selective (DS) and non-DS units (logarithmic binwidth).

phase of the period histogram. Only two (DS) units were found that discharged at two preferred phases of the period histogram (i.e., a half-cycle of the stimulus apart). In the TS of the goldfish, this appears to be a more common phenomenon (Lu and Fay 1993).

From dot display sections at consecutive stimulus directions, polar period histograms were calculated showing the synchronization of the response (Fig. 3). For some units, the phase angle of this synchronization is practically constant (Figs. 3B and 8A). Because the period histograms A and F of Fig. 3B contain relatively more spontaneous activity (see Fig. 3A), their SI is lower and the estimate of the instant of firing is less precise. The jumps of 180° were to be expected, because the point of reference (the positive zero-crossings

of the sinusoidal stimulus) for calculating the phase angle of the synchronization is reversed with respect to the polarity of the hair cells, which provide the input to this unit. The phase angle of the synchronization of other units, however, changes, almost linearly, with rotation of the stimulus direction (Figs. 7 and 8B). Obviously, the occurrence of both types of responses theoretically provides the fish with a temporal code for the stimulus direction. All DS units that synchronized were examined with respect to their phase shift for a (standard) rotation of stimulus direction of 90° . The spike density of the response differed for each unit, and the directional selectivity showed much variation (e.g., compare the responses shown in Fig. 2, B and C). Therefore the number of sections of the response (see Fig. 3) that were

FIG. 5. Latency of auditory neurons. Center value of bins (binwidth 4 ms) is plotted below horizontal axis. No latency differences were found between populations of DS ($n = 85$) and non-DS units ($n = 28$). Because most non-DS units display spontaneous activity, their latency could not be determined accurately. Stimulus amplitude: 30 mm s^{-2} .

FIG. 6. Synchronization of DS and non-DS units (stimulus amplitude 30 mm s^{-2}). NS, synchronization not significant according to the Rayleigh test.

used, and the extent to which extrapolation proved necessary, for estimating the phase shift for the standard rotation of the stimulus (90°) could be very different.

The result is a very asymmetric distribution (Fig. 9). Half of the units show a negative phase shift (less than -15°), i.e., spike generation is delayed, when the stimulus direction is rotated anticlockwise, whereas 42% show no phase shift ($<|15^\circ|$). The occurrence of a phase shift as a function of stimulus direction in the response of fish midbrain neurons has been shown previously in a preliminary report (Wubbels et al. 1994). This type of phase shift also was observed in the response of one synchronizing non-DS unit (for which the classification "non-DS" appears to be inappropriate). A direction dependent phase shift also has been reported for the amphibian auditory system, although in the auditory nerve fibers (Jorgensen and Christensen-Dalsgaard 1994).

Stimulus intensity

The data presented in Fig. 8 were obtained for different intensities (range 30 dB). It is clear that at a higher intensity spikes are generated ($\leq 0.9 \text{ ms}$) earlier (positive phase shift). In general, however, the phase shift as a function of stimulus direction does not change with intensity (the shape of the curves remains unaffected). Also, the latency (Fig. 5) hardly is affected by this phase shift. We examined the response parameters, i.e., response latency, mean spike density, synchronization index, directional selectivity range, phase of the period histogram at optimal stimulus direction, and phase shift per 90° rotation of stimulus direction, as a function of stimulus intensity. An example, which is typical for DS units, is shown in Fig. 10. In general, most response parameters hardly depend on intensity.

Twelve DS units were found with a strongly synchronized response at low amplitudes (1 unit: SI = 0.9, amplitude 0.6 mm s^{-2} ; 3 units: SI = 0.9, amplitude 1.2 mm s^{-2} ; 8 units: SI > 0.75 amplitude 3 mm s^{-2}). However, recordings did not last long enough for measurements at the lowest amplitudes for all units.

Topography

Previously presented evidence that the sound direction in the horizontal plane is topographically mapped in the trout TS (Wubbels et al. 1995) is supported by additional data. In Fig. 11, the preferred direction for each DS unit (or to be more precise, the projection of the preferred direction on the horizontal plane) and the horizontal projection of its recording site in the TS is shown from a dorsal view. In the medial part of the TS, the direction of preference of almost all neurons is approximately parallel to the rostro-caudal axis of the fish. Twenty out of 26 DS units encountered there have a preferred direction of $0 \pm 20^\circ$. In the lateral part of the TS, all preferred directions are about equally present.

In the medial part of the TS, all DS units have a directional-selectivity range of $\geq 60^\circ$, whereas in the lateral part $\sim 25\%$ show better spatial tuning than 60° (Fig. 12A). Synchronization of the response occurs less frequently in the medial TS (χ^2 test, $P < 0.05$). A pronounced phase shift of synchronized spikes, as a function of stimulus direction, is observed only for (a minority of) lateral DS units (Fig. 12B). For these reasons, it appears that DS units in the medial part of the TS and DS units in the lateral part represent two functionally distinct populations of neurons.

Nonrectilinear stimuli

After characterization of the auditory neurons in terms of directional selectivity with respect to rectilinear stimuli, we applied nonrectilinear stimuli to see whether the parameters of the stimulus orbit affected the response. In general, reversal of the revolution of the orbit hardly

FIG. 7. From sections of a recording of a direction-dependent response, polar period histograms were calculated. Phase angle of these period histograms changes as a function of stimulus direction. Stimulus amplitude: 90 mm s^{-2} . When stimulating this unit at 3 mm s^{-2} ($=2.6 \text{ nm}$), almost identical SI and phase shift were observed.

FIG. 8. Phase shift in period histograms, as a function of stimulus direction, of response of 2 different units (like the ones shown in Figs. 3 and 7) at various stimulus intensities. Phase angle of synchronization of unit (A) is independent of stimulus direction. Jumps of 180° are caused by an alternating reference point (see text for explanation). Phase angle of synchronization of unit (B) changes almost linearly with rotation of stimulus direction.

changed the unit's response. The most striking exception, however, is shown in Fig. 13. The sustained component of the response to a circular stimulus orbit (positive direction, Fig. 13A) disappears for the opposite direction of revolution (Fig. 13B), and spontaneous activity is even suppressed (compare *left-* and *right-hand panels* in Fig. 13B). The short transient at the onset of the tone burst, however, remains unchanged.

For 20 units that were stimulated with elliptical or circular orbits, we examined the response ratio for positive and negative direction of revolution. A response ratio of ≥ 2 , or alternatively ≤ 0.5 , was chosen to indicate that orbit parameters may be encoded in these responses. Of course this is an arbitrary measure, but because the response hardly depends on stimulus amplitude, it does not seem unreasonable to choose a ratio of 2 as indicative of a significant response difference. The response ratio of four units exceeded this criterion (Fig. 14A).

For the same 20 units, and 3 more, the response to elliptical or circular stimuli was compared with the response to rectilinear stimuli. The response of 19 units to an elliptical (long axis in preferred direction, amplitude equal to the rectilinear stimulus) or circular stimulus was the same as for a rectilinear stimulus (in the preferred direction). The response ratio of four units exceeded the criterion (Fig.

14B). The response ratio of only one out of these four units also had exceeded the criterion for positive and negative direction of revolution (i.e., the unit of Fig. 13).

DISCUSSION

Electrode selectivity

In many respects, the response properties of the auditory neurons that we encountered in the TS differ from primary afferent or medullar neurons (Wubbels et al. 1993). Our impression is that auditory neurons in the TS are small, because it proved difficult to keep recordings >5 – 15 min. The visual neurons that we also encountered in the TS (Wubbels et al. 1995) were held much more easily. Moreover, auditory neurons were found mainly in the upper part of the TS (Wubbels et al. 1995), comprising two layers of neurons with small somata (Cuadrado 1987).

Synchronization of the response was observed for 40% of all 227 auditory units in the TS of the rainbow trout. This figure is considerably higher than previously reported for the same subject (Nederstigt and Schellart 1986; Schellart and Kroese 1989; Schellart et al. 1987). A similar difference has been encountered in the TS of the goldfish. Page (1970) found no synchronization to an acoustic stimulus in the TS,

FIG. 9. Phase shift of period histograms, for a rotation of stimulus direction of 90° (see text for explanation), for all synchronized DS units.

whereas Lu and Fay (1993) observed that $>50\%$ of the responses were synchronized. The latter suggest that the probability to isolate synchronizing auditory units depends on the type of electrode that was used (Lu and Fay 1993), which is thought to be related to the size of the neurons. This also could explain the different results in the TS of the trout, because the impedance of the glass microelectrodes that were used in this study was ~ 10 times higher than in the previous studies on trout (Nederstigt and Schellart 1986; Schellart et al. 1987).

Sensitivity

At the lowest stimulus amplitudes, the indirect contribution to the stimulus from the swimbladder was subthreshold (METHODS). Because, in general, response parameters are independent of intensity, we suppose the response was not seriously affected by a contribution from the swimbladder at any stimulus amplitude.

Many DS units retain a strong synchronization at very small stimulus intensities, whereas the spike density still is a function of stimulus direction. The “neurophysiological threshold,” being an arbitrary measure because it is related to the amount of data recorded and the statistics that are used, has been estimated for medullar neurons (Wubbels et al. 1993). The response of the 12 most sensitive DS units to a stimulus that was -27 dB (1 unit), -21 dB (3 units), and -13 dB (8 units) relative to this medullar neurophysiological threshold was strongly synchronized (see RESULTS), suggesting a strong convergence at the midbrain level of VIIIth nerve input. An increase of the sensitivity of auditory neurons in the TS (23 dB compared with saccular nerve fibers) also has been reported for the goldfish (Lu and Fay 1993).

Otolith organs

In salmonids, the lagena has its hair cells oriented in the vertical direction and the saccule in both the vertical and the rostro-caudal direction (Popper 1977). The utricle of most fish has a fanlike hair cell pattern almost in the horizontal plane (e.g., Dale 1976). The utricle has been considered as a mainly gravistatic receptor (e.g., Platt and Popper 1981). For instance, in Schuijff's model (1976a,b) binaural input from both saccular organs was hypothesized for the vectorial weighing, in the horizontal plane, of the model's motion system. The utricle, however, seems a more “natural” detector system for this function. Therefore the orbit model (Schellart and de Munck 1987) was based on the orbits of the utricular otolith. Nowadays, most investigators appear to agree on the auditory function of all three otolith organs (Popper and Fay 1993). Gravistatic and auditory functions probably need not exclude each other in the fish otolith systems because the adaptive capability of hair cells can be very robust and fast, i.e., within tens of milliseconds (Assad and Corey 1992). In the lateral part of the TS, DS units have different directions of preference in the horizontal plane (Fig. 11) and different directional selectivity ranges (Fig. 12A), indicating interaction between input from hair cell groups with different orientation patterns. This also supports the idea that the utricle has an auditory function.

Spatial representation

It has been shown that auditory space is represented in the midbrain of terrestrial vertebrates (Irvine 1992; Knudsen and Konishi 1978; Middlebrooks and Knudsen 1984). This representation can be based on either a “place” code (i.e., a sharp spatial tuning of single neurons) as has been shown for some owl species, or on an “ensemble” code (i.e., the

FIG. 10. Response parameters as a function of stimulus intensity of a DS unit. Synchronization index ($\times 200$) and latency (ms; resolution 4 ms) do not change with intensity. Directional selectivity range (degrees) shows some variation. Phase (degrees) of period histogram, i.e., instant of firing at optimal stimulus direction, is practically constant. Phase shift (degrees) for a rotation of 90° of stimulus direction and mean spike density of response (spikes per 10 s) appear to be intensity dependent. Usually, however, latter parameter is rather invariant with stimulus strength.

relative amount of activation of a population of more broadly tuned place coding neurons) as has been proposed for less-specialized birds and for mammals (see Irvine 1992). Another alternative of spatial representation is a "panoramic" code (i.e., the temporal pattern of the firing of many neurons) as has been conjectured for auditory neurons in a cortical area of the cat (Middlebrooks et al. 1994). We have presented evidence that the sound direction in the horizontal plane is mapped topographically in the fish midbrain as well (Wubbels et al. 1995) (Fig. 11). Most units, however, responded over a relatively wide range of angles in the horizontal plane, and thus spatial tuning of the neurons in the TS of the trout appears to be rather coarse. Given the characteristics of the observed directional responses, we think that temporal information also plays an important role in the auditory localization process. Choosing between an ensemble and a panoramic code, for the representation of auditory space in the trout TS, is a matter of conception, because the former does not necessarily exclude a temporal contribution to the spatial representation.

Directional hearing I: the orbit model

If directional hearing is based on the analysis of orbit parameters, considerable differences between the responses to rectilinear stimuli and elliptical or circular stimuli are to be expected, because the axes ratio is an important parameter in this model (Schellart and de Munck 1987). Choosing an arbitrary criterion of 2 for the ratio of the response amplitudes (see RESULTS), just four units ($n = 20$) responded differently to rectilinear and elliptical (or circular) stimuli

(Fig. 14B). Discrimination between the direction of revolution within the orbit is essential to the orbit model to resolve the directional ambiguity. Only one unit was found with dramatically different responses (and 3 more that just exceeded the criterion; $n = 20$) for opposite directions of revolution (Fig. 14A). Most units for which the direction of revolution was reversed did not change their response at all.

Apparently, support for the orbit model is limited to a small number of units indeed. Furthermore, this difference was observed mostly in the sustained part of the response. The transient part of the response was affected less. As a consequence, the orbit model, when depending on the sustained part of the response, would be a rather slow detection system. A different response to opposite directions of revolution (Fig. 13) also may be the result of an analysis of the temporal relation between the orthogonal motion components (van den Berg 1985) and thus is not necessarily in conflict with the phase model.

The orbit model has been hypothesized for pure tone stimuli originating from monopole sources. In principle, the effects of another spectral composition of the stimulus signal on its performance can be overcome by the introduction of neuronal band-pass filters. Thus this model would allow localization of fish that use their swimbladder (approximately a monopole) for conspecific communication (Schellart and Popper 1992). For nonmonopole sources, however, for which the motion component itself generally describes an elliptical orbit, it is difficult to conceive how reliable directional information can be obtained. The phase model does not suffer from this drawback.

Directional hearing II: the phase model

Contrary to the orbit model, the phase model depends on the preservation of temporal information about the motion

FIG. 11. Dorsal view of location of recording site of DS units in TS and their preferred direction. Center and orientation of each line segment indicate unit's location and direction of preference, respectively. . . . (arbitrarily) divides TS in a medial and lateral part. Most DS units in medial part are tuned spatially in rostro-caudal direction. In lateral part, all preferred directions are present uniformly.

and pressure components of a sound field to solve the 180° ambiguity (Schuijf 1976a,b; van den Berg and Buwalda 1994). This model also allows directional discrimination of nonmonopole sources by making use of the phase relation between the pressure and motion component (van den Berg and Buwalda 1994). Psychophysically, it has been shown that cod are able to discriminate between sound fields in which the only difference is this phase relation between pressure and motion component (Wubbels and van den Berg 1996). Theoretically, analysis of the spectral relation between the pressure and motion components provides information about the source distance (van den Berg and Buwalda 1994).

A precondition of directional hearing models, in general, is that orthogonal hair cell groups in the inner ear, comprising this model's hypothetical motion system, have to be innervated independently by primary afferents. Anatomic evidence (Saidel and Popper 1983) and physiological evidence (Fay 1984) has been obtained that this is indeed so. Motion of the swimbladder wall can be transmitted to the otolith organs acoustically through the intervening tissue, as in the nonspecialized trout, or via structures like the Weberian ossicles in e.g., carp-like and catfish species. In case of acoustical transmission through the intervening tissue, attenuation of the pressure induced motion will depend on the distance of the otolith organs to the apex of the swimbladder (Schellart and Popper 1992). Irrespective of the type of transmission, however, the coupling magnitude

will be different for lagena, saccule, and utricle. In the phase model, therefore, it is hypothesized that independent pressure information is available to the auditory system by some form of neurological comparison of the response of the three maculae. There is some evidence that the pressure and motion component of the sound stimulus are not processed homogeneously in the goldfish midbrain (Fay et al. 1982).

Our results suggest that in the trout two functionally different types of DS units occur, located in the medial and lateral part of the TS, respectively (Fig. 11). Possibly, this also is related to the separate processing of pressure and motion information. Because we stimulated the inner ear with a motion component (see METHODS), it is to be expected that in this experimental set-up, neurons of both the "motion system" and the "pressure system" will display a direction-dependent response. Thirty-seven percent of all auditory responses we encountered were direction dependent. The directional-selectivity distribution, which was measured in the horizontal plane, shows a sharp peak at 0° in the medial part of the TS. This is the spatial tuning one might expect of a pressure system, because it corresponds with the direction of the indirect stimulus vector, after bilateral neuronal addition, from the swimbladder apex. In the lateral part of the TS, the directional-selectivity distribution appears to be uniform, which in turn might be expected for a motion system representing all possible directions in the horizontal plane.

The relative amounts of synchronization of non-DS units (23%) and DS units (75%) were significantly different. In

FIG. 12. Response characteristics of DS units in medial and lateral part of TS. Directional selectivity range (A) and phase shift of response for 90° rotation of stimulus direction (B) as function of preferred direction.

FIG. 13. Dot displays showing response to a circular stimulus with positive (A) and negative (B) direction of revolution. SI is 0.95 and 0.81, respectively. Transient part of response appears to be unaffected, but sustained part is considerably different. Stimulus amplitude: 30 mm s^{-2} . Scale bars represent 8 spikes.

the phase model, phase (i.e., temporal) information is necessary for directional hearing, and thus we are inclined to conclude that nonsynchronizing units (DS units included) are not involved in directional hearing. Because the mean spike density and the index of synchronization hardly change with stimulus strength, phase information is preserved independent of intensity information. The instant of firing (the phase of the period histogram) itself may depend on stimulus strength (curves shifted along y axis; Fig. 8), but the phase shift, as a function of stimulus direction, hardly depends on intensity (curves keep their shape; Fig. 8). The phase shift varies with the unit recorded, and its distribution among DS units is very asymmetric (Fig. 9).

The response strength of adjacent populations of hair cells, with slightly different directional sensitivity in the horizontal plane (as in the utricle), will be a function of stimulus direction. Converging input from these hair cell populations on a TS neuron, with correspondingly different delays, may be the cause of the phase shift, as a function of stimulus direction, that has been observed for DS units like the one in Fig. 8B. This would be an inversion of the "coincidence detection" model (Jeffress 1948) in which delay lines were introduced to convert a peripheral temporal cue (the interaural time difference) into a central directional code. Delay lines have been demonstrated in the owl's auditory system (Carr and Konishi 1988) and have been hypothesized

FIG. 14. Response ratio for positive and negative direction of revolution of stimulus orbit (A). Response ratio for elliptic or circular stimuli and rectilinear stimuli (B). Response to a rectilinear stimulus in unit's preferred direction is compared with response to an elliptical stimulus with its long axis in preferred direction (equal amplitudes). Bin width along horizontal axis is 0.5 octave. Bars indicated (x) have a ratio >2 or <0.5 .

for the electrosensory system of *Eigenmannia* (Carr et al. 1986). Also experimental data from the auditory system of mammals are consistent with the concept of delay lines (Rose et al. 1966; see also Irvine 1992). In the rainbow trout, a peripheral directional cue (although ambiguous) is converted into a central temporal code, which in turn may be used, in combination with a temporal reference code, to encode the sound source direction unambiguously (see below).

The (synchronizing) DS units may be part of either the motion system or the pressure system of Schuijf's phase model (1976a,b). Although, the results presented here suggest a functional difference between medial and lateral DS units, identification as motion neuron or pressure neuron was not possible in our experimental set-up. However, the occurrence of two types of units with a phase shift behavior as shown in Fig. 8, A and B, provides a simple basis for a temporal code for the stimulus direction, because the phase difference between the two units is proportional to direction. The differential character of the proposed mechanism guarantees its independence of stimulus amplitude. The stimulus we applied with the vibrating platform can be compared with an external sound source slowly circulating around the fish, with a constant motion component, but without the coupled pressure component occurring under natural circumstances. Because the orientation of the motion vector depends on the source position with respect to the fish, toral neurons with input from equally oriented hair cells are expected to display a phase jump of 180°, as was found for the unit of Fig. 8A, whether they belong to the motion system or to the pressure system. In the natural situation, however, when a pressure component accompanies the motion component in a sound field, putative pressure system neurons will have their spikes synchronized to the stimulus at a constant phase angle (without the 180° jump), because the indirect stimulus from the swimbladder does not depend on source position. Hypothesizing that neurons belonging to the pressure system (Schuijf 1976a,b) behave like the unit of Fig. 8A, and (at least some) neurons belonging to the motion system behave like the unit of Fig. 8B, then the phase difference between the two unequivocally encodes the stimulus direction (0–360°) for any intensity. We conclude that, in general, the results presented in this report strongly favor the phase model.

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