Response of the Medial Octavolateral Nucleus (MON) in the Goldfish, *Carassius auratus*, to constant-amplitude and amplitude-modulated water wave stimuli

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Ramadan Ali

aus

Tubrug-Libya

Bonn 2008
Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn

1. Referent: Prof. Dr. Bleckmann
2. Referent: PD Dr. Mogdans
Tag der Promotion: 8.07.2008
# Table of contents

## I Introduction

1.1 The mechanosensory lateral line system ............................................................1
1.2 The ascending lateral line pathway .................................................................2
1.3 Efferent projections.........................................................................................4
1.4 Level response functions..............................................................................5
1.5 Hydrodynamic stimulation with amplitude modulated water waves.............6

## II Materials and methods

2.1 Animals........................................................................................................9
2.2 Animal preparation.......................................................................................9
2.3 Stimulation..................................................................................................10
   2.3.1 Hydrodynamic stimulation.................................................................10
   2.3.1.1 Vibrating sphere stimulus.........................................................10
   2.3.2 Stimulus measurement ......................................................................12
       2.3.2.1 Displacement measurements .............................................12
       2.3.2.2 Pressure measurements ....................................................12
   2.4 Stimulus protocol.....................................................................................13
2.5 Data acquisition and analysis......................................................................14
   2.5.1 Experimental setup............................................................................14
2.6 Histology....................................................................................................17

## III Results

3.1 Summary of recorded units........................................................................19
3.2 Response of MON units to dipole stimuli..................................................20
   3.2.1 Characteristics of medullary unit responses to sinusoidal hydrodynamic
       stimuli..................................................................................................20
   3.2.2 Frequency response ..........................................................................23
   3.2.3 Characteristics of medullary unit responses to amplitude modulated
       hydrodynamic stimuli...............................................................27
Table of contents

3.2.4 Phase coupling to constant-amplitude and amplitude modulated sine wave stimuli

3.2.5 Effects of amplitude modulation depth

3.3 Level response function of medullary units to pure sine wave stimuli and to amplitude modulated water motions

3.3.1 Input-output functions to pure sine wave stimuli

3.3.2 Input-output functions to amplitude modulated sine wave stimuli

3.4 Receptive fields

3.5 Anatomy

IV Discussion

4.1 Response to pure sine wave stimuli

4.2 Frequency - response characteristics

4.3 Response to amplitude modulation sine wave stimuli

4.4 The encoding of amplitude and phase information

4.5 Level response functions

4.6 Comparison with electrosensory units

4.7 Histology

V Summary

5. Summary

Literature

Curriculum vitae
Acknowledgments

I would like to express my gratitude to my supervisor Prof. Dr. Horst Bleckmann, whose expertise, understanding, and patience created the best possible post graduate experience. His vast knowledge and skills in many areas, and his assistance in writing this thesis, were invaluable.

A very special thanks goes out to Dr. Joachim Mogdans, whose motivation and encouragement facilitated tremendously my post graduate career in neurobiology. He provided me with direction, technical support and became more of a mentor and friend than a co-supervisor.

Thanks also goes to Dr. Michael Hofmann who provided me with histological advice at times of critical need.

I want to express sincere gratitude to Dr. Boris Chagnaud for the experimental training during the beginning of my Ph.D. study at the institute.

I would also like to thank my friends in the neurobiology lab, particularly Björn Scholze, Arne Rüter, Dr. Jill Ebert, Silke Fest, Gunnar Meyer, Ines Nauroth and Volker Hofmann for our philosophical debates, exchange of knowledge and skills, and venting of frustration during my post graduate program, which helped to enrich the experience.

I am grateful for suggestions, comments, and contributions from Prof. Randy Zelick during his work in the lab of Prof. Bleckmann.

I additionally thank Mrs. Dung and Mrs. Sassen for their daily assistance which has also contributed to my work.

I would also like to thank my parents for their support, and provision through my entire life.

The most special thanks to my wife, Alia and best friends, Mahfouz and Nasser. Without their love, encouragement and editing assistance, I would not have finished this thesis.
In conclusion, I recognize that this research would not have been possible without the financial assistance of the Ministry of Higher Education in Libya represented by Libyan bureau in Berlin, and express my gratitude to those agencies for the health insurance during my post graduate program.
**Abbreviations:**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>amplitude modulated</td>
</tr>
<tr>
<td>AMD</td>
<td>amplitude modulation depth</td>
</tr>
<tr>
<td>AMF</td>
<td>amplitude modulation frequency</td>
</tr>
<tr>
<td>CCL</td>
<td>crest cell layer of MON</td>
</tr>
<tr>
<td>CF</td>
<td>carrier frequency</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CON</td>
<td>nucleus caudalis octavolateralis</td>
</tr>
<tr>
<td>DCN</td>
<td>dorsal cochlear nucleus</td>
</tr>
<tr>
<td>DNp</td>
<td>deep neuropil layer of MON</td>
</tr>
<tr>
<td>DON</td>
<td>dorsal octavolateralis nucleus</td>
</tr>
<tr>
<td>ELLLL</td>
<td>electrosensory lateral line lobe</td>
</tr>
<tr>
<td>iA</td>
<td>iso-amplitude</td>
</tr>
<tr>
<td>iF</td>
<td>iso-frequency</td>
</tr>
<tr>
<td>JAR</td>
<td>jamming avoidance response</td>
</tr>
<tr>
<td>LLN</td>
<td>lateral line nerve</td>
</tr>
<tr>
<td>MD</td>
<td>modulation depth</td>
</tr>
<tr>
<td>ML</td>
<td>molecular layer of MON</td>
</tr>
<tr>
<td>MON</td>
<td>nucleus medialis octavolateralis</td>
</tr>
<tr>
<td>OT</td>
<td>optic tectum</td>
</tr>
<tr>
<td>PGI</td>
<td>nucleus praegglomerulosus</td>
</tr>
<tr>
<td>PLLn</td>
<td>posterior lateral line nerve</td>
</tr>
<tr>
<td>PPa</td>
<td>peak to peak amplitude</td>
</tr>
<tr>
<td>PSTH</td>
<td>peri-stimulus-time-histogram</td>
</tr>
<tr>
<td>RF</td>
<td>receptive field</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>SAM</td>
<td>sinusoidal amplitude modulation</td>
</tr>
<tr>
<td>TS</td>
<td>torus semicircularis</td>
</tr>
<tr>
<td>TZ</td>
<td>transitional zone of MON</td>
</tr>
<tr>
<td>Trg</td>
<td>secondary gustatory tract</td>
</tr>
<tr>
<td>Trv</td>
<td>descending tract of the germinal nerve</td>
</tr>
<tr>
<td>VIIn</td>
<td>sensory root of the facial nerve</td>
</tr>
<tr>
<td>VIIIin</td>
<td>eights nerve</td>
</tr>
</tbody>
</table>
1. Introduction

1.1 The mechanosensory lateral line system

Fish and aquatic amphibians have a mechanosensory lateral line. The sensory organs of the lateral line are called neuromasts. Lateral line neuromasts can be distributed over the entire fish and amphibian body (Northcutt, 1989). Each neuromast consists of a patch of hair cells underneath a gelatinous cupula. In fish two types of neuromast can be distinguished: superficial neuromasts (SN), which occur freestanding on the surface of the skin, and canal neuromasts (CN), which are recessed in subepidermal canals (e.g. Münz, 1979; Webb, 1989; Song and Northcutt, 1991). CN communicate with the outside water by means of small pores (Münz 1979). The lateral line system of fish shows a high morphological diversity that causes a functional divergence in the perception of certain qualities of water motion (Coombs et al. 1992). Up to a frequency of about 70 Hz (stimulation with a constant volume vibrating sphere), SNs are sensitive to water velocity whereas CNs are more sensitive to water acceleration (e.g. Coombs et al. 1988; Webb 1989b; Kroese and Schellart 1992). Until the last 20 years relatively little work has been done on the central physiology of the lateral line system of fishes and aquatic amphibians, especially with respect to higher brain centres (reviews see Bleckmann and Bullock 1989; Claas et al. 1989; Schellart and Kroese 1989). However, over the last 20 years the number of studies in which the physiology of the central lateral line has been studied has markedly increased (e.g. Bleckmann et al. 2001; Engelmann et al. 2002; Kirsch et al.)
I. Introduction


1.2 The ascending lateral line pathway

Mechanosensory lateral line information is transferred to the central nervous system (CNS) through the lateral line nerves (LLN). At least three LLNs, the anterior, middle and the posterior, innervate the head and the trunk lateral lines (McCormick 1982; Northcutt 1989, 1997). The LLNs terminate in two major areas of the CNS, the dorsal part of the medulla oblongata and the eminentia granularis of the cerebellum (McCormick 1982). In some species additional projections reach the corpus cerebelli and the valvula cerebelli (Wullimann et al. 1991b). The dorsal part of the medulla oblongata receiving LLN input is separated into two nuclei, the medial octavolateralis nucleus (MON) and the nucleus caudalis octavolateralis (CON, not found in all fish species)(McCormick and Hernandez 1996). Efferent fibres of the MON join the lateral longitudinal fasciculus and project into the ipsi- and contralateral nucleus ventrolateralis of the torus semicircularis (TSv1), with strong contralateral predominance. Moreover, the MON also projects bilaterally to the nucleus praeminentialis, to the sensory trigeminal nucleus (STN) and sparsely to certain areas in the optic tectum (OT) (McCormick and Hernandez 1996). The TSv1 projects to the nucleus praeglomerulosus lateralis (PG1) of the diencephalon (Echteler 1984; Murakami et al. 1986a, b; McCormick 1989; Striedter 1991). Finally, the PG1 projects in a species-dependent manner
to regions of the dorsal part of the area dorsalis telencephali. In cyprinids, for instance, the PG1 projects to the area dorsalis pars medialis, to the area pars lateralis and to the area pars centralis telencephali, while in gymnotoids, only the caudal part of medial nucleus of area dorsalis of the telencephalon has such heavy reciprocal interconnections with the lateral pregglomerular nucleus (Murakami et al. 1986a, b; Striedter 1992).

The sensory information that is represented by the activity of primary lateral line afferent fibres is processed in the MON of the fish brainstem (Puzdrowski 1989; New et al. 1996). Studies using vibrating sphere stimuli have shown that many MON units exhibit primary-like responses and receptive fields (Coombs et al. 1998). Receptive fields that are completely unlike those of primary afferents can also be found among MON units (Mogdans and Kröther 2001). Studies in which the lateral line was stimulated with water motions generated by a moving object indicate that some MON neurons integrate the information of many neuromasts, and these neuromasts may be distributed across large portions of the lateral line periphery (Mogdans et al. 1999; Mogdans and Goenechea 2000). Thus, there are at least two pathways in the lateral line brainstem, one that processes local hydrodynamic information generated, for example, by a small stationary vibrating source, and another that processes more complex water motions such as those generated by a moving source (Mogdans and Goenechea 2000).
1.3 Efferent projections

Descending recurrent projections are common in sensory systems. In the lateral line system descending projections exist from the telencephalon to the nucleus praegglomerulosus (e.g. Wullimann 1996). The primary processing station of lateral line information, the MON, receives at least two kinds of recurrent, descending input (McCormick and Hernandez 1996; Striedter 1991). One input comes from the nucleus praeeminentialis pars ventralis, which gets input from the MON and the torus semicircularis. The descending input to the MON terminates in the two most dorsal layers of this structure, the dorsal and ventral molecular layers (also called the cerebellar crest). The ventral molecular layer consists primarily of axons projecting directly from the ipsi and contralatera nucleus praeeminentialis. The dorsal layer, on the other hand, receives input from the nucleus praeeminentialis indirectly. This layer is composed of parallel fibres originating in a mass of cerebellar granule cells lying immediately dorsal to the MON. This granule cell mass, the posterior eminentia granularis, receives its input mainly from the ipsi and contralateral nucleus praeeminentialis. In some teleosts, the MON receives additional input from the ipsilateral sensory trigeminal nucleus (McCormick and Hernandez 1996).

As already mentioned our knowledge of the neural mechanisms that underlie the central processing of lateral line information is far from
sufficient. Despite the fact that over the last 20 years the number of studies on the central physiology of the lateral line has markedly increased (reviews see Bleckmann 1994: Bleckmann and Bullock 1989; Coombs et al.1998; Kröther et al 2002; Edds-Walton and Fay 2005; Bleckmann 2008) we still have only a vague idea of the central processing of lateral line information. All studies done so far have shown, however, that the following physiological changes occur along the ascending lateral line pathway: from primary afferents to the telencephalon there is a striking decrease in spontaneous (ongoing) activity, an increase in response decrement to a repetitive stimulus regime, and a decrease in phase coupling to a sinusoidal stimulus.

Sinusoidal water motions generated by a stationary vibrating sphere have been used in many physiological studies of the central lateral line. These studies revealed that many central lateral line units do not respond to sinusoidal water motions. Those who do may respond in a phasic, phasic-tonic or tonic fashion and they may or may not phase lock to the stimulus (for review see Bleckmann and Bullock 1989). In most cases the responses of central lateral line neurons to a sine wave stimulus are substantially different from those of primary afferents (e.g. Coombs et al. 1998; Kröther et al. 2002).

1.4 Level Response Function

Recordings from primary lateral line afferents in goldfish show that both the degree of phase-locking and the discharge rate increases with
increasing displacement amplitude of a vibrating sphere. At stimulus levels just above threshold, units respond to the stimulus with a modulation of the ongoing discharge rate, i.e., they exhibit phase-locking without a substantial increase in discharge rate. With increasing displacement amplitude, the degree of phase-locking increases and usually reaches a plateau at stimulus levels about 20 dB above threshold (e.g. Mogdans and Bleckmann 1999). Some medullary units encode stimulus amplitudes up to 150 µm, while other medullary units already show saturation at a peak-to-peak (p-p) displacement amplitude of 6 µm (Bleckmann et al. 1989b). Thus in terms of the upper stimulus amplitude which can be encoded there is some range fractionation. In some central lateral line units an increasing stimulus amplitude past the point at which saturation occurs leads to a decrease of neural response (Bleckmann et al. 1989b; Schellart and Kroese 1989).

1.5 Hydrodynamic stimulation with amplitude modulated water waves

Historically, the analysis of the discriminatory abilities of the lateral line systems was based on electrophysiological and behavioural experiments employing relatively simple, reproducible stimuli (e.g. Bleckmann et al. 1981; Münz 1985; Coombs et al. 1996; Vogel and Bleckmann 1997; Mogdans et al. 1999). Such basic experiments can clarify many of the fundamental signal processing steps that occur in the periphery and in the CNS. Simple stimuli often do not however, elicit responses from units in higher brain centers (e.g. Bleckmann and Bullock 1989). Thus to shed
light on the signal processing mechanisms in higher lateral line areas, it is essential to use more complex stimuli, i.e., stimuli which may be more natural (Müller et al. 1996; Wojtenek et al. 1998).

The majority of lateral line research has used single-frequency stimuli, typically generated by a stationary sinusoidally-vibrating sphere (e.g. Münz 1985; Coombs et al. 1996; Bleckmann 1994; Plachta et al. 1999). These stimuli enabled scientists to answer a number of questions, such as the encoding of the carrier frequency (CF), the amplitude, the amplitude modulation frequency (AMF) and the amplitude modulation depths (AMD) at different levels of the lateral line pathway. Although these stimuli may be still far from being similar to natural water motions, many electrophysiological studies of the peripheral (Münz 1985; Coombs and Montgomery 1992; Coombs et al. 1996; Mogdans et al. 1999) and central lateral line (Bleckmann et al. 1989; Coombs et al. 1998) have established that a large number of central lateral line units are driven by such stimuli. Iso-frequency (iF) and iso-amplitude (iA) water motions are probably rare in natural aquatic environments. Rather, shifts in frequency and/or amplitude of acoustic and hydrodynamic sensory stimuli are common in natural habitats (Bleckmann 1994; Bodnar and Bass 1997; McKibben and Bass 1998; Bleckmann et al. 2001). Therefore it is likely that the lateral line system of fishes and aquatic amphibians is especially sensitive to such stimuli.
I. Introduction

Recent studies have revealed that temporal discharge patterns of primary lateral line afferents reflect both the CF and the AMF of sinusoidal water motions (Mogdans and Bleckmann 1999). In particular AM stimulation causes more prominent and phase-locked responses in the midbrain than constant amplitude stimuli (Plachta et al. 1999). The MON is the first site of central processing of lateral line information (see also above), but we do not know whether and how medullary lateral line units respond to amplitude modulated water motions. Therefore, the aim of the present study was to investigate how MON units respond to constant frequency, amplitude modulated sinusoidal water motions. The stimulus variables examined were the CF, the AMF, and the depth of amplitude modulation.
II. Materials and methods

2. Materials and methods

2.1 Animals

Data for this study were obtained from a total of 41 goldfish \textit{(Carassius auratus)}, ranging from 6 to 13.5 cm in body length (measured from snout to base of tail), and between 11 and 29 g. Fish were obtained from a local supplier and kept in tanks (250 liter) with water plants and on a daily 14/10 h LD cycle. Water temperature varied between 15 °C and 18 °C.

2.2 Animal preparation

Fish were anesthetized either with 2.5 % MS222 (3-Aminobenzoic Acid Ethyl Ester, Sigma) or with ice water before surgery. The anesthetized fish were injected with Pancuronium bromide (Organon Teknika, 0.3 - 0.8 µl/g body weight) into the dorsal back musculature to immobilize them for the experiment. Fish then were transferred to a surgical setup and a small area of the skin at the site of the surgery was infused with the local anesthetic Xylocaine (ASTRA Chemicals). Using a dental drill (Minimot 40/E, Proxon), a small (ca. 4 x 4 mm) opening was made in the skull above the medulla. Excess fatty tissue and fluids were removed to uncover the cerebellum. The cerebellum was deflected forward with a small cylinder of tissue paper to expose the surface of the medulla. The fish were moved to
II. Materials and methods

the experimental tank (40 x 48 x 25 cm), which was filled with aged tap water. Room temperature was 20° ± 2°C. The experimental tank was placed on a custom-fabricated vibration-isolated table to minimize background vibrations. To prevent the exposed brain from drying, a physiological salt solution was dropped into the opening of the cranium (Oakley and Schaefer 1978). The immobilized fish was artificially respirated with aerated freshwater passed over the gills at a rate of 60 - 200 ml/min by means of polyethylene tubing inserted into the fish’s mouth.

2.3 Stimulation

2.3.1 Hydrodynamic stimulation

Hydrodynamic stimulation was performed with a solid plastic sphere (diameter 8 mm) mounted to a Ling mini-shaker (Ling Dynamic Systems, model V 106) with a small brass rod (diameter 3 mm). The shaker was installed on a movable ball-bearing base which allowed linear movements of the shaker parallel to the side of the fish. The movable ball-bearing slide was attached to a gantry mechanically isolated from the fish and experimental tank.

2.3.1.1 Vibrating sphere stimulus

The mini-shaker was driven by the analog output of a computer and a DA-
II. Materials and methods

converter (Apple Macintosh PPC 7300, 14-Bit AD/DA-Converter; Instrunet 100B, Software SSII GW Instruments). The computer-generated signals were D/A-converted at a rate of 16 kHz, band-pass filtered at 0.3-2000 Hz (custom built filter) and power amplified (Amplifier PA25E, Ling Dynamic Systems). Carrier frequencies (CF) were 33, 50, 100 and 200 Hz; amplitude modulation frequencies (AMF) were 0, 4 and 10 Hz. The amplitude modulation depth (AMD) was set between 0 and 96 % in 24 % steps. The AM stimuli were generated by multiplying the sinusoidal CF signal with the sinusoidal AM frequency at a given AMD. To obtain level response functions, a stationary vibrating sphere (diameter 8 mm), displacement amplitudes 25 - 250 µm) generated constant-amplitude and amplitude-modulated sine wave stimuli (duration 1 s, CF 100 Hz, AMF 10 Hz).

The resulting stimuli had a duration of 1000 ms including the rise and fall times of 100 ms. The oscillatory axis of the sphere was rostro-caudal, parallel to the trunk of the fish. To avoid boundary layer effects, the distance between the sphere and the fish was at least 5 mm and at most 8 mm.

To test whether a vibration-sensitive unit responded also to a moving source, the sphere (diameter 8 mm) was moved manually along the side of the fish in an anterior-to-posterior or posterior-to-anterior direction without applying sinusoidal vibrations. The same test was done when a unit was encountered which did not respond to the vibrating sphere stimulus.
II. Materials and methods

2.3.2 Stimulus measurement

2.3.2.1 Displacement measurements

In the amplitude range 25 to 250 µm, the peak-to-peak displacement of the vibrating sphere was calibrated for the CFs 33 Hz, 50 Hz, 100 Hz, and 200 Hz and for the AMFs 4 Hz and 10 Hz by using a capacitive displacement sensor (model 4810, L.O.T.ORIEZ). Measurements were made in air in the absence of a fish (Fig. 1). The time waveforms of the sensor output were digitized (GWI, Instrunet and SuperScope II, sampling rate 10 kHz) and stored on a computer (Apple Power Macintosh 7300).

![Displacement vs. Computer Output](image)

**Fig. 1** Voltage delivered to the mini shaker (x-axis) and displacement amplitude of the dipole (y-axis), the Carrier Frequencies were 33 Hz, 50 Hz, 100 Hz and 200 Hz. At 160 µm p-p displacement of the dipole, voltages delivered to the mini shaker were 0.013v, 0.12v, 0.24v, 0.87v for the Carrier Frequencies 33 Hz, 50 Hz, 100 Hz and 200 Hz respectively.

2.3.2.2 Pressure measurements
II. Materials and methods

The pressure waves generated by the vibrating sphere were measured with a hydrophone (Brüel and Kjaer 8103) positioned at the location in the experimental tank where normally the fish would reside. The hydrophone was connected to a charge amplifier (Brüel and Kjaer 2635). Measurements were made for all stimuli that were used in the physiological experiments.

2.4 Stimulus protocol

The search for units always started with two stimuli, the moving (velocity of sphere movement was about 5 cm/s) and vibrating (CF 50 Hz or 100 Hz, p-p displacement amplitude 160 µm) sphere. If a unit was encountered that responded to the moving/vibrating sphere stimulus, it was assumed to be a lateral line unit. The receptive field (RF) of the unit was determined by comparing the number of spikes per stimulus evoked at different positions of the sphere at the side of the fish. Response strength was judged by listening to the acoustic monitor or by counting the number of spikes on the digital storage oscilloscope. If the unit responded to the vibrating sphere, the sphere was placed at the position with the largest response. At this position the amplitude of the sphere was adjusted such that it was in the middle of the dynamic range of the unit. In all cases a unit was first stimulated with a CF stimulus, then with an AMF stimulus and finally with AMF stimuli that differed with respect to their modulation depths. For control, this stimulation protocol was also performed for units which also responded to airborne sound, i.e., neurons were tested for
II. Materials and methods

acoustic sensitivity with a hand clap. Due to the long duration of the stimulation protocol not all stimuli could be executed before the unit was lost. This is the reason for the discrepancies in the sample sizes for the different stimulus conditions.

2.5 Data acquisition and analysis

2.5.1 Experimental set up

For recordings, indium electrodes (impedance ≤1 MΩ; Dowben and Rose 1953) or glass micropipettes filled with 3 M KCl (impedance 50–90 MΩ) were used. Action potentials recorded with indium electrodes or glass micropipettes were amplified (DAM-80, WPI), bandpass filtered (300-3000 Hz), displayed on oscilloscope (HM 205-3) and monitored with a loudspeaker. The spike signals were digitized by a computer (Apple Macintosh PPC 7300, AD/DA-Converter instrunet 100B, GWI; Superscope II, GWI sampling rate 10000pts/sec) for final analysis (c.f Fig. 2). In most cases, the neuronal activity was analyzed off-line. To isolate the response of a unit from background noise and to reduce the amount of data for analysis, the traces subsequently were analyzed with a computer (Apple Macintosh, Power PC 7300 superscope II, GWI). To distinguish
II. Materials and methods

between single units and multi unit recordings, the characteristics of the spikes were inspected visually (slope, peak amplitude, and duration).

The ongoing activity of each unit was calculated prior (> 100 ms) to stimulus onset and presented as spikes per second (Superscope II, GWI, J. Mogdans custom macro) or (Igor, CED-System, M.Hofmann custom script). Spike trains of ten repetitions were expressed as peri-stimulus-time-histograms (PSTHs). In general, PSTHs were triggered 100 ms before the start of stimulation. For analysis, the average firing rate (spikes/s), the average phase angle (degrees) of each spike with respect to the voltage that was fed into the vibrator, the degree of phase-locking (synchronization coefficient R) and the Rayleigh statistic Z were calculated across all presentations of a particular stimulus burst. To
II. Materials and methods

calculate these measures, elapsed spike times across the 10-20 stimulus bursts were added together and collapsed into a single cycle's worth of time (= period histogram). Average firing rate was determined from the numbers of spikes elicited during the 10-20 stimulus bursts and expressed in spikes/s.

The dynamic range of a unit was defined as that part of the input/output (IO) function for which the response rate (average ongoing activity subtracted) was between 10% and 90% of the maximum response rate measured. To describe the selectivity of a unit for a particular phase of the stimulus, a synchronization coefficient (vector strength R, after Goldberg and Brown 1969) was calculated. The direction of the vector describes the average phase angle to which a unit responds and its magnitude describes the strength of phase-locking. The Rayleigh statistic Z was used to determine whether or not measures of vector strength were statistically significant. The phases of each spike relative to the CF or the AMF were calculated (Goldberg and Brown, 1969)

\[
R = \sqrt{\left(\sum x_i\right)^2 + \left(\sum y_i\right)^2} / 4
\]

with: \( x_i = \cos \phi, \quad y_i = \sin \phi \) and: \( \phi = \) phase of individual spike

The strength of phase coupling (R) was calculated with a program using circular statistics (Igor Pro, Wavemetrics) (Batschelet 1981). An R value of
II. Materials and methods

1 indicates perfect phase coupling, i.e., all spikes occur at the same phase angle, whereas an R of 0 represents no phase coupling, i.e., spikes occur at random phase angles. The R value is analogous to the vector strength used in auditory physiology (Goldberg and Brown 1969). The Rayleigh test was used to find out whether phase coupling was significant (Batschelet 1981). The Rayleigh test results in a Z value, with:

\[ Z = R^2 \times n_s, \]

where \( n_s \) = total number of spikes.

For a result to be significant at the 0.01 level, \( Z \) must be \( \geq 4.6 \) (Batschelet, 1981).

2.6 Histology

In fifteen specimens of goldfish an electrolytic lesion was made at the end of a recording session at the location at which single unit responses were detected. The brains of these fishes were serially sectioned in the transverse plane and stained with cresyl violet. This tissue was either paraffin-sectioned (15 µm) or frozen-sectioned (50 µm). Fish were deeply anesthetized in a concentrated solution of Ethyl 3-aminobenzoate methanesulfonate and perfused intracardially with 50 ml of Ringer’s solution followed by 4% saline fixative (2% glutaraldehyde / 2% paraformaldehyde) in 0.1 M phosphate buffer (PB; pH 7.4). Brains were then removed from the skull, and postfixed for 1 hour in the same fixative and stored in 30% sucrose in 0.1 M PB overnight for cryoprotection.
II. Materials and methods

Brains were sectioned frozen on a sliding microtome at 50 µm in the transverse plane. Sections were then counterstained with cresyl violet, dehydrated through a graded series of alcohol, and cover slipped. The sections were analyzed with a microscope and reconstructed using Adobe Photoshop 6.0 (Adobe Systems, Inc., San Jose, CA) on a laptop computer.
III. Results

3. Results

3.1 Summary of recorded units

116 recordings were made from the medulla of goldfish *Carassius auratus*. Of these recordings, 86 were single unit recordings. Thirty recordings were multi unit. All of these units responded to the vibrating sphere stimulus (CF 33, 50, 100 and 200 Hz, AMF 4 Hz and 10 Hz, p-p displacement amplitude 160 µm). Fig. 3 shows examples of the pressure waves caused by the sphere vibrating with various CFs and AMFs.

![Fig. 3](image)

**Fig. 3** Pressure waves (measured with a submerged hydrophone, scaled in Pa) generated by the vibrating sphere. **A.** A constant-amplitude sine wave stimulus (CF 50 Hz). **B and C.** An amplitude-modulated sine-wave stimulus [CF 100 Hz and 200 Hz, AMF 10 Hz and 4 Hz, respectively]. **D.** A constant amplitude sine wave stimulus (CF 100 Hz). The displacement amplitude of the sphere was 160 µm in **A, B and C** and 50 respectively 250 µm in **D**.
III. Results

3.2 Responses of MON units to dipole stimuli

3.2.1 Characteristics of medullary unit responses to sinusoidal hydrodynamic stimuli

Sinusoidal water motions generated by a stationary vibrating sphere have been used in many physiological studies of the central lateral line (e.g. Kröther et al. 2002; Plachta et al. 2003; Engelmann et al. 2002). When stimulated with a vibrating sphere two types of medullary units could be distinguished. Type 1 units (n=22) showed phasic responses and fired only a few action potentials at the beginning of the stimulus. Type 2 units (n=23) showed a sustained discharge if stimulated with CF water motions, but sometimes responses were especially pronounced if high-frequency stimuli were applied (for two examples see Fig. 4).

A summary plot showing the number of spikes per bin (binwidth 100 ms) as function of time after stimulus onset for all MON units tested is given in Fig. 5. Only 40% to 50% of all cells reached the maximum discharge rate within 100-200 ms after stimulus onset when CF was 33 Hz or 50 Hz, whereas 72% to 74% of all cells had their strongest responses within 100-200 ms after stimulus onset when high-frequency stimuli (100 or 200 Hz) were applied.
III. Results

Fig. 4 Examples of unit responses to a stationary sphere vibrating with either 33, 50, 100 or 200 Hz. In each graph of this figure the top trace shows original recording, spikes activity over time is illustrated by dots displays (middle) and peri-stimulus time histograms (PSTHs) (down). Stimulus traces at the bottom, p-p displacement amplitude of the sphere was 160 µm. The figure shows a type 1 unit response (left) and a type 2 unit response (right).
III. Results

**Fig. 5** Percentage of spikes per bin (binwidth 100 ms) as function of time. For each neuron and CF, respectively, the highest number of spikes per bin was set equal to 100%. Data are shown for the stimulus frequencies 33 Hz (upper) to 200 Hz (lower). Stimulus trace at the bottom, p-p displacement was 160 µm. Note that in most units the strongest responses occurred within 100-200 ms after stimulus onset. Individual traces shown in gray; dark line shows the mean response of all units.
III. Results

3.2.2 Frequency response

The frequency characteristics of MON units was determined by measuring iso-displacement curves. The distance between the surface of the fish and the surface of the sphere was 5-8 mm, CFs were 33 Hz, 50 Hz, 100 Hz and 200 Hz.

To compare the frequency responses of MON units, the p-p displacement of the sphere was set to 160 µm for all CF applied. Units showed heterogeneous weak tuning characteristics including low-pass, band-pass and high-pass (for an example see Fig. 6). The majority of units (Fig. 7 and 8) had their best-frequency (highest number of spikes per stimulus) at 100 Hz (45%), followed by 200 Hz (42%), 50 Hz (13%), and 33 Hz (11%).

Fig. 6 A, B. A Example of the responses (original recording) of a single unit as function of stimulus frequency (33, 50, 100 and 200 Hz). B Responses were quantified by counting the number of spikes elicited during the time of stimulation. The unit responded with the highest number of action potentials at a CF of 100 Hz. P-p displacement amplitude was 160 µm.
III. Results

Fig. 7A-D Responses (percentage of maximum) of 4 single units as function of stimulus frequency. Units showed low-pass (A), band-pass (B, C) or high-pass characteristic (D). P-p displacement amplitude always was 160 µm.
III. Results

Fig. 8A - D Responses (percentage of maximum) of MON units as function of stimulus frequency. Units were grouped with respect to the maximum discharge rate elicited at a certain frequency. Units showed best responses to 33 Hz (A), 50 Hz (B), 100 Hz (C), or 200 Hz (D). Note that units responded to all other frequencies with less than 60% of the maximum discharge rate.
III. Results

Fig. 9A - D Neural responses as function of stimulus frequency. Responses were quantified by counting the average number of spikes elicited during the time of stimulation and setting the highest number obtained for a given unit and bin, respectively, equal to 100%. Data were averaged across the units shown in Fig. 8. In terms of displacement, units showed a weak low-pass characteristic (A), band-pass characteristic (B, C) or high-pass characteristic (D). P-p displacement amplitude was 160 µm.
### III. Results

3.2.3 Characteristics of medullary unit responses to amplitude modulated hydrodynamic stimuli

Responses of MON units evoked by AM (4 Hz and 10 Hz) water motions differed from those evoked by a single frequency sine wave stimulus. A comparison of Fig. 4 with the Figs. 10 and 11 shows that the responses of MON units changed when the pure sine wave stimulus was amplitude modulated. The units responded with a burst of discharge to each modulation cycle. Thus, the response profiles were phasic to constant amplitude pure sine wave stimuli, but changed to tonic for amplitude modulated pure tone stimuli.

3.2.4 Phase coupling to constant-amplitude and amplitude modulated sine wave stimuli

To learn the degree to which medullary lateral line unit activity reflects the AMF or the CF of a stimulus, the phase angle of each spike was determined with respect to the CF and AMF, respectively. Thereafter the vector strength \( R \) (as described in chapter 2.5) was calculated. The procedure for the data conversion and calculation of the phase locking is shown in Fig. 12. The resulting \( R \)-values were further analysed with the Rayleigh-statistics (see material and methods).

The responses of primary lateral line afferents reflect both the AMF and the CF of a stimulus (Mogdans and Bleckmann 1999). In contrast, the
III. Results

responses of toral lateral line units reflect only the AMF but not the CF of a sinusoidal stimulus (Plachta et al. 1999). The responses of most medullary lateral line units reflected the CF of 50 and especially of 100 Hz (see Fig. 13, left column). At these CFs the responses of most units also reflected the AMF of the stimulus (AMF 4 and 10 Hz) (see Fig. 13 middle and right column). If the CF was 200 Hz the responses did not reflect the CF (Fig. 13, lower left). However, at 200 Hz the responses of nearly all units reflected the AMFs (4 Hz and 10 Hz) (see Fig. 13 lower row).

Medullary units generally responded with short bursts to the onset of a vibrating sphere stimulus. These bursts contained only a few spikes, therefore artificially high R-values to the CF often were calculated. Few spikes accidentally occurring at the same phase lead to high R-values if the entire population of spikes is low. Avoiding this weakness of the R-values, the statistically more reliable Z-value was calculated. Both values were transferred into a plot (c.f. Fig. 13). At a significance level $p \leq 0.01$ the Z-value must be $\geq 4.6$ (Batschelet, 1981).
**III. Results**

*Fig. 10* Discharge patterns of two MON units in response to a 4 Hz AM stimulus. Note that the two units responded phasic-tonically or tonically to amplitude-modulated stimuli and phase coupled to the 4 Hz AM frequency. In each graph the top trace shows the original recording. Below each raster plot is a peristimulus time histogram (PSTHs). Stimulus traces (voltage input to the vibrator) are at the bottom, p-p displacement amplitude of the sphere was 160 µm.
III. Results

Fig. 11 Discharge patterns of three MON units (from left to right) in response to a 10 Hz AM stimulus. Note that all units responded tonically to the amplitude-modulated stimuli and clearly phase coupled to the 10 Hz AM frequency. In each graph the top trace shows the original recording which corresponds to the first trace in the respective raster plot (middle). Below each raster plot is a peristimulus time histogram (PSTHs). Stimulus traces at the bottom, p-p displacement amplitude of the sphere was 160 µm.
The calculated Z-values (c.f. Fig. 13, first row) show that there was neither
a phase coupling to the CF (33 Hz) nor to the AMF (4 Hz and 10 Hz), i.e.
most Z-values of the first row (CF=33 Hz) were located in the lower
quadrants. This indicates that phase locking was not significant. Fig. 12A
shows the responses of medullary units to an unmodulated constant
frequency stimulus. These units phase coupled to the CF (Fig. 12C). In
addition these units phase coupled to the AMF (see Fig. 12B, D, right). The
phase-coupling to the AMF was not homogenous. The phase-locking to
the AMF depended on both the CF and the AMF of the stimulus. This is
depicted in Fig. 13 for the CF 50 Hz and the AMF 4 Hz and also for the CF
100 Hz and the AMF 4 Hz. In both cases the Z-values with respect to the
phase coupling to the AMF varied between moderate significant to strong
significant. If the ratio between the CF and the AMF is relevant, this
should become evident by comparing the number of spikes per stimulus
for one CF (for each unit) at different AMFs. As long as the unit can follow
the AMF at a certain CF, the number of spikes should increase with
increasing AMF. This only held true within certain limits.

3.2.5 Effects of amplitude modulation depth.

To quantify the sensitivity of medullary units to stimuli which where
amplitude modulated, amplitude modulation depth (AMD) was varied in
24% steps (c.f Chapter 2.3).

Responses to AM stimuli depended on modulation depth. When
modulation depth was maximal (96%), units responded to each of the 4
III. Results

modulation cycles. With decreasing modulation depth units tended to discharge less and less to increasing numbers of AM cycles (for two examples see Fig. 14). On average the responses to the fourth cycle were only about 60% of the responses to the first cycle (c.f. Figs. 15B). When modulation depth was 24% or smaller, discharge patterns resembled the responses to unmodulated stimuli, i.e. units responded with an on-response to the first AM cycle but responded only weakly to successive AM cycles (Fig. 15A, B). In A and in B (of Fig. 15) the responses to the second amplitude modulation cycle of the stimulus already may fall below 50% of the maximum at an AMD of 24%.
**III. Results**

**Fig. 12 A-D** Data reduction and calculation procedure from single unit activity to vector strength (R). A, B examples of a few unit response to a 50 Hz CF stimulus at 0 Hz AMF (A) and 4 Hz AMF (B). From top to bottom: few unit response, dot display of 10 successive trials, PSTH (peri-stimulus-time histogram) of responses and time course of the stimulus applied. C Coefficient of phase-coupling (R) for a unit that was stimulated with a pure tone of 50 Hz. The top inset shows the distribution of spikes with respect to the CF (50 Hz). The sine wave symbolises one cycle of the CF. The wheel charts (below) show the phase angle and the R value calculated for all spikes (left wheel) and only for the first spike of each trial (right wheel). In D the 50 Hz pure tone was amplitude modulated (modulation depth 96%) with 4Hz. The top inset shows the distribution of spikes for each spike with respect to the AMF. The sine symbolises one cycle of the AMF.
III. Results

Fig. 13 Scatter plots of Z values obtained from Rayleigh test versus coefficients of synchronization (R). Phase locking is plotted with respect to the CF (left column) or with respect to the AMF (middle and right columns: 4 Hz and 10 Hz, respectively). The horizontal lines indicate the critical value for the Rayleigh test ($Z = 4.6$, at $p = 0.01$), dividing units into those without significant phase locking (below) and with significant phase locking (above). The vertical line at $R = 0.5$ divides the phase locking units into weakly phase locking (left) and strongly phase locking (right). From top to bottom CF was 33 Hz, 50 Hz, 100 Hz and 200 Hz.
Fig. 14 Responses of two MON units to sinusoidal (CF 100 Hz) water motions. Amplitude modulation varied between 96% (top) and 0% (bottom). In each graph shows a raster plot and the corresponding peristimulus time histogram (PSTHs) of the responses. Stimulus traces are at the bottom. AMF was 4 Hz, p-p displacement amplitude of the sphere was 160 µm. Note that the two units responded less and less with decreasing amplitude modulation depth.
III. Results

**Fig. 15 A** Example of the responsiveness (percentage of maximum) of a single unit as function of the number of AM cycles (AMF 4 Hz). CF was 100 Hz. Different symbols show data obtained with different amplitude modulation depths (AMDs) which varied between 0 and 96%. **B** Mean number of action potentials (percentage of maximum) of lateral line units as function of the number of AM cycles and AMD. CF was 100 Hz. Data were averaged across five units. Vertical bars indicate one SD.