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

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Abbreviations:

AM	amplitude modulated
AMD	amplitude modulation depth
AMF	amplitude modulation frequency
CCL	crest cell layer of MON
CF	carrier frequency
CNS	central nervous system
CON	nucleus caudalis octavolateralis
DCN	dorsal cochlear nucleus
DNp	deep neuropil layer of MON
DON	dorsal octavolateralis nucleus
ELLL	electrosensory lateral line lobe
iA	iso-amplitude
iF	iso-frequency
JAR	jamming avoidance response
LLN	lateral line nerve
MD	modulation depth
ML	molecular layer of MON
MON	nucleus medialis octavolateralis
OT	optic tectum
PGI	nucleus praeglomerulosus
PLLn	posterior lateral line nerve
PPa	peak to peak amplitude
PSTH	peri-stimulus-time-histogram
RF	receptive field

SAM	sinusoidal amplitude modulation
TS	torus semicircularis
TZ	transitional zone of MON
Trg	secondary gustatory tract
Trv	descending tract of the germinal nerve
VIIn	sensory root of the facial nerve
VIIIn	eights nerve

I. Introduction

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.

2002; Kröther et al. 2002, 2004; Plachta et al. 2003; Engelmann and Bleckmann 2004; Bleckmann 2006, 2008).

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 praeeminentialis, 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 preglomerular 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 praeglomerulosus (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 praeeminetialis 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 (*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 ℃ and 18 ℃.

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

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-

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.

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).

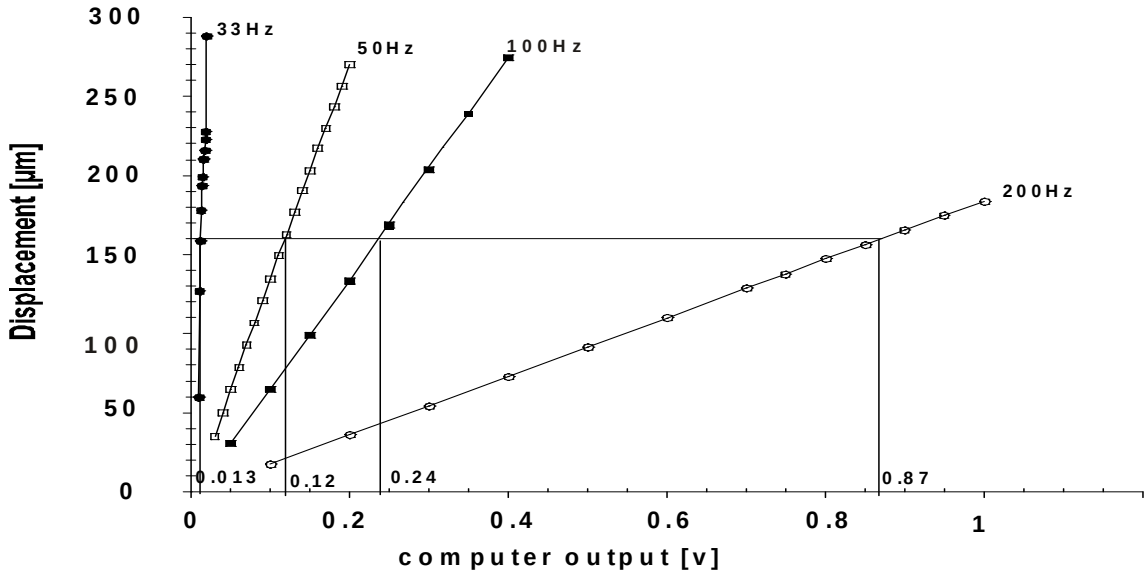

Fig. 1 Voltage delivered to the mini shaker (x-axis) and displacement amplitude of the dipole (y-axis), the Carrier Frequencies were 33 Hz, 50 Hz, 100 Hz and 200 Hz. At 160 µm p-p displacement of the dipole, voltages delivered to the mini shaker were 0.013v, 0.12v, 0.24v, 0.87v for the Carrier Frequencies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

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

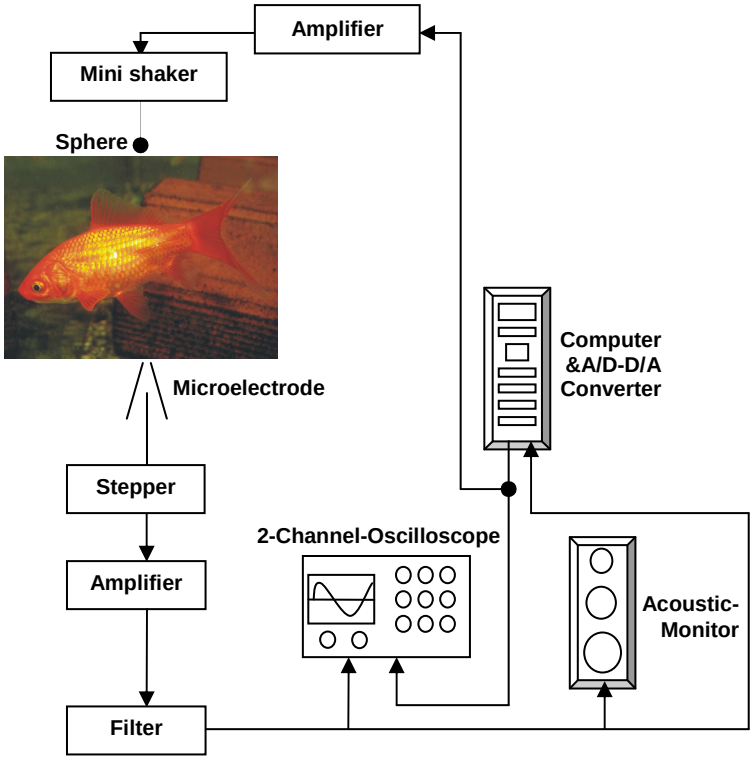

Fig. 2 Experimental setup

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

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)

$$\mathbf{R}=\sqrt{(\sum_{xi})^2+(\sum_{yi})^2\ \ /4}$$

with: $xi=\cos\phi,\quad yi=\sin\phi$ **and:** $\phi=\text{phase of individual spike}$

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

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:

$$Z=R^2*n_s,$$

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-aminobenzoat 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.

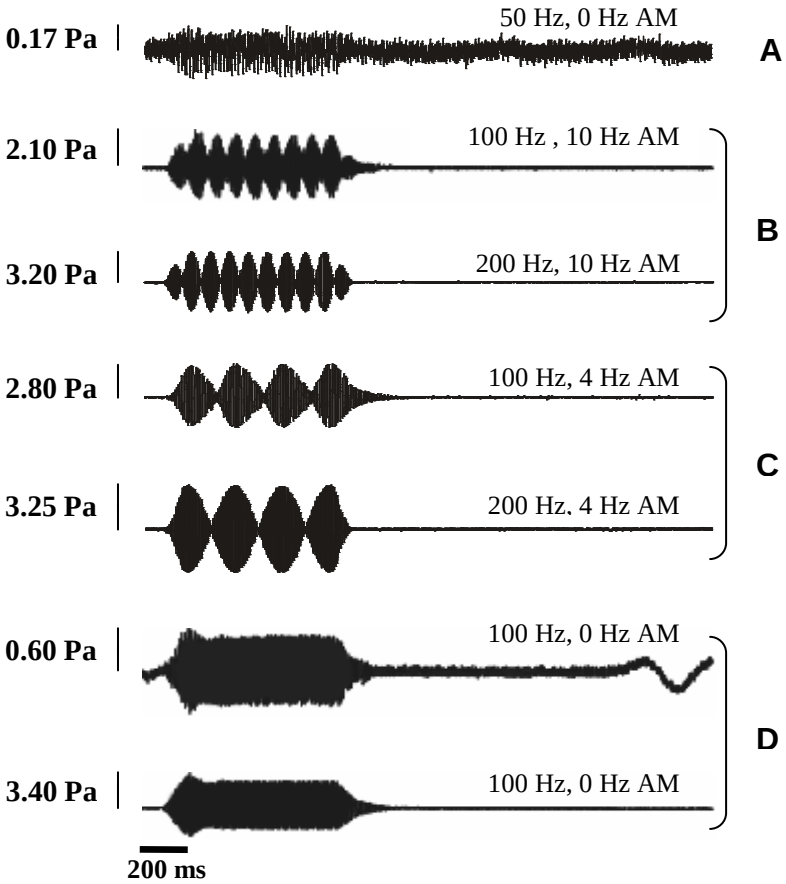

Fig. 3 Pressure waves (measured with a submerged hydrophone, scaled in Pa) generated by the vibrating sphere**. A.** A constant-amplitude sine wave stimulus (CF 50 Hz). **B and C.** An amplitude-modulated sine-wave stimulus [CF 100 Hz and 200 Hz, AMF 10 Hz and 4 Hz, respectively]. **D.** A constant amplitude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

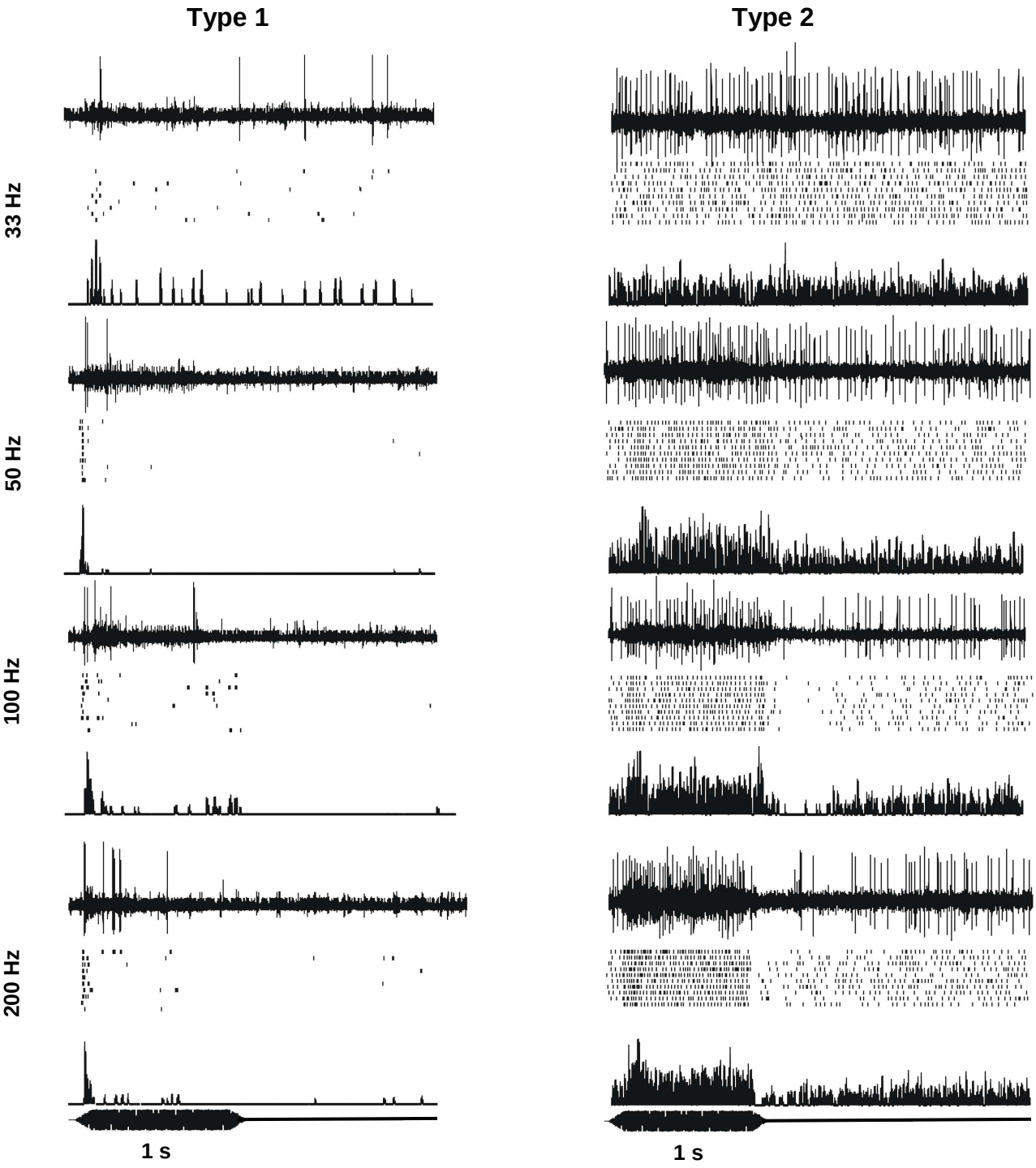

Fig. 4 Examples of unit responses to a stationary sphere vibrating with either 33, 50,100 or 200 Hz. In each graph of this figure the top trace shows original recording ,spikes activity over time is illustrated by dots displays (middle) and peri-stimulus time histograms (PSTHs) (down). Stimulus traces 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

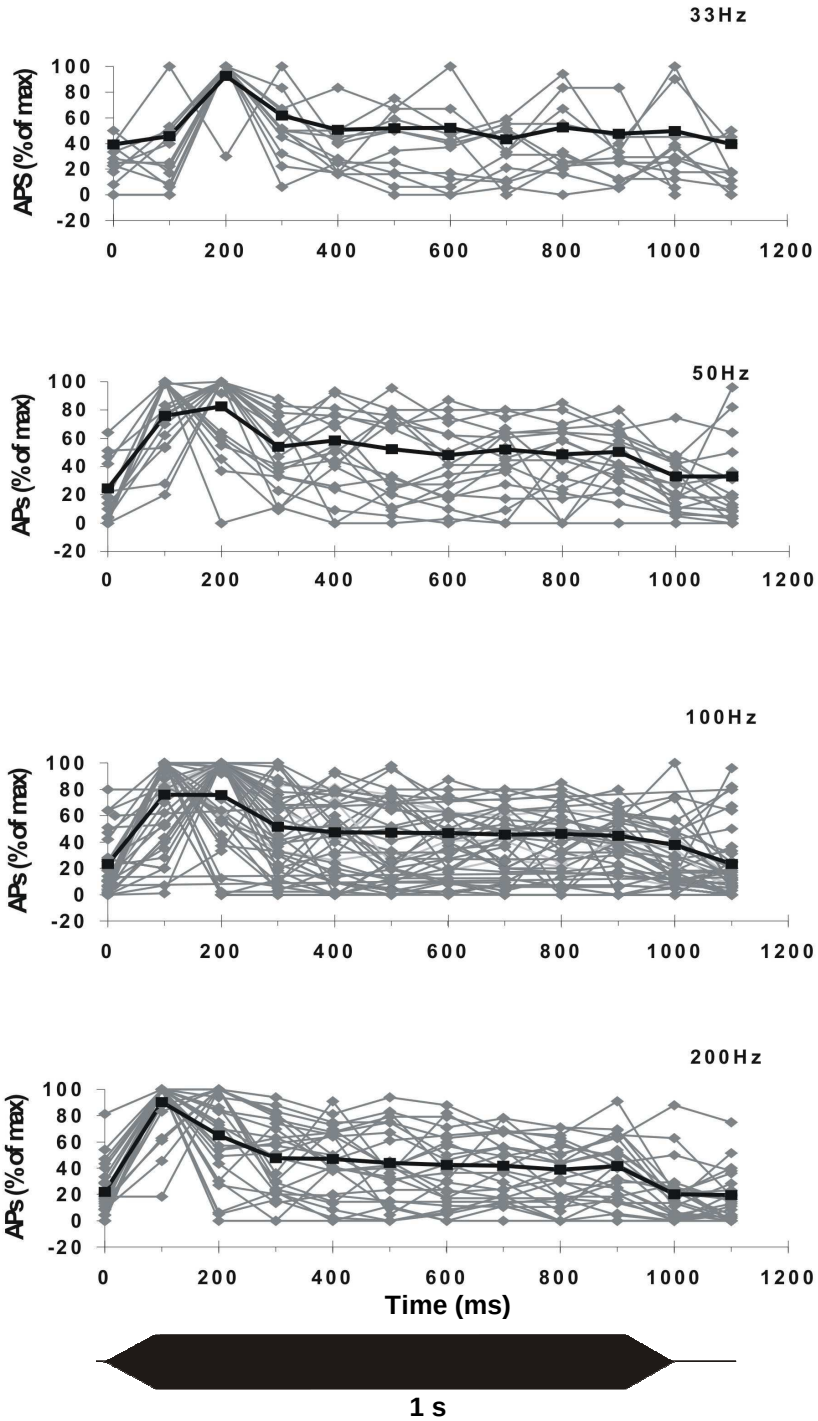

Fig. 5 Percentage of spikes per bin (binwidth 100 ms) as function of time. For each neuron and CF, respectively, the highest number of spikes per bin was set equal to 100%. Data are shown for the stimulus frequencies 33 Hz (upper) to 200 Hz (lower). Stimulus trace at the bottom, p-p displacement was 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%).

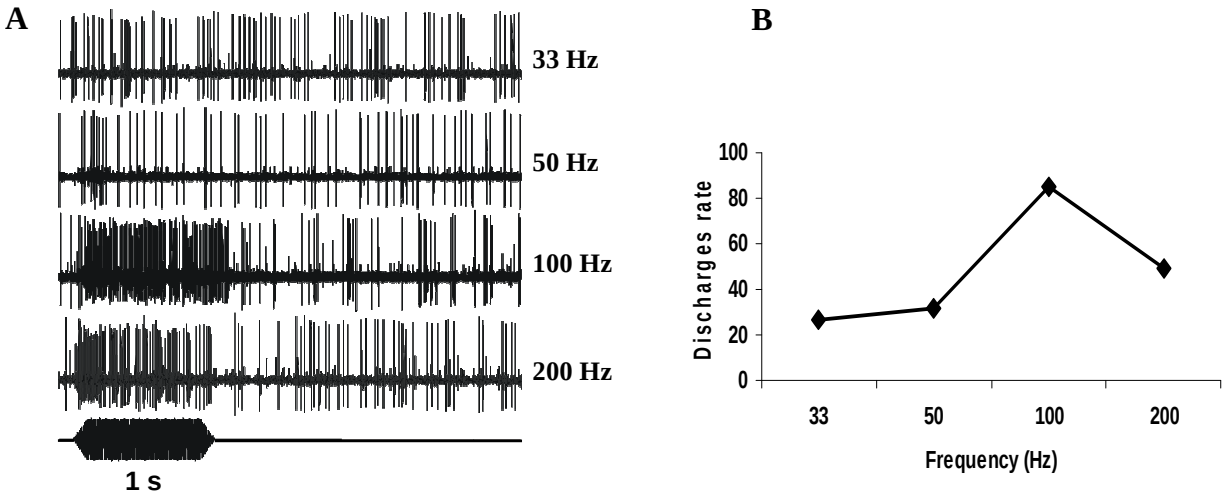

Fig. 6 A, B. A Example of the responses (original recording) of a single unit as function of stimulus frequency (33, 50, 100 and 200 Hz). **B** Responses were quantified by counting the number of spikes elicited during the time of stimulation. The unit responded with the highest number of action potentials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

III. Results

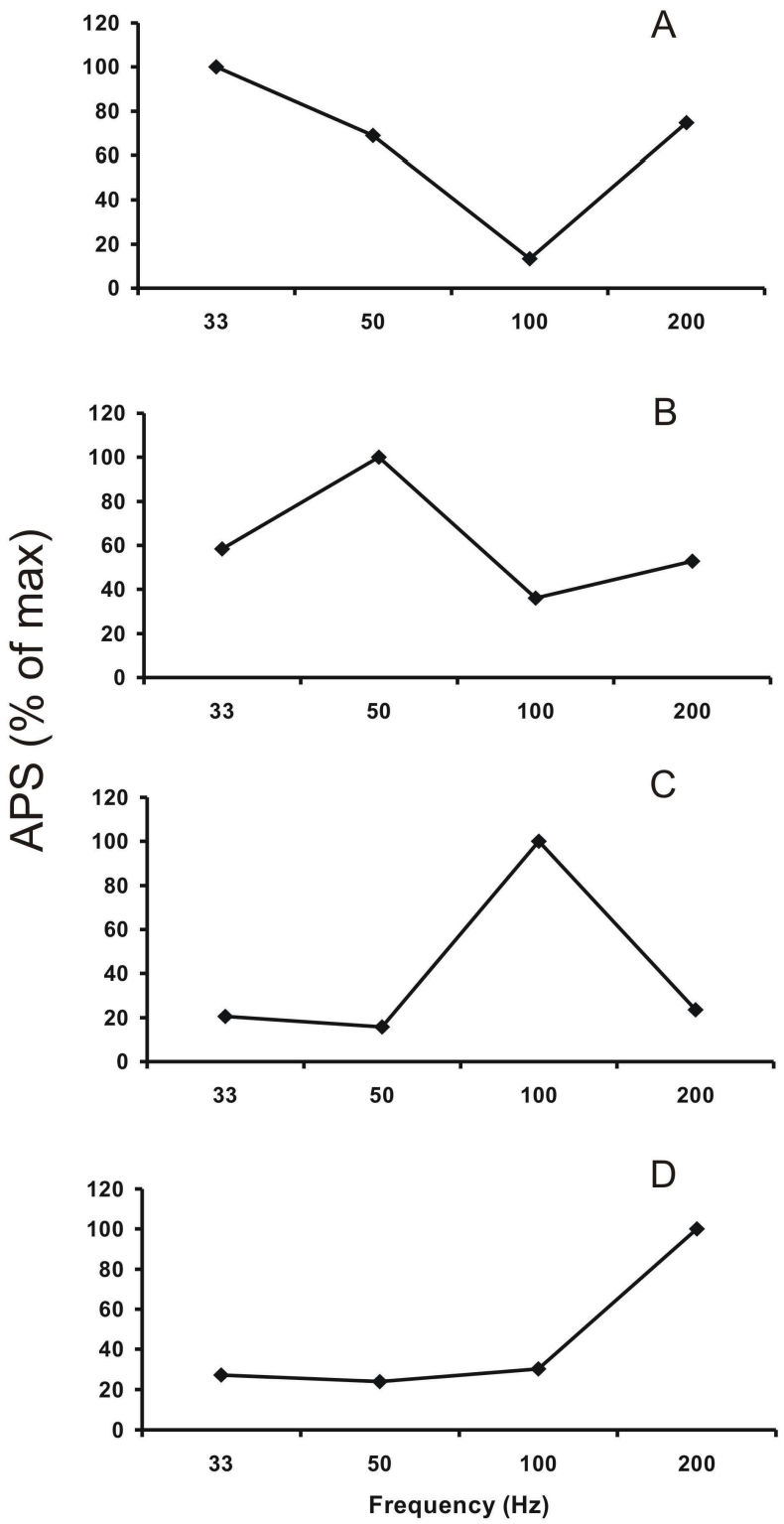

Fig. 7A-D Responses (percentage of maximum) of 4 single units as function of stimulus frequency. Units showed low-pass (A), band-pass (B, C) or high-pass characteristic (D). P-p displacement amplitude always was 160 µm.

III. Results

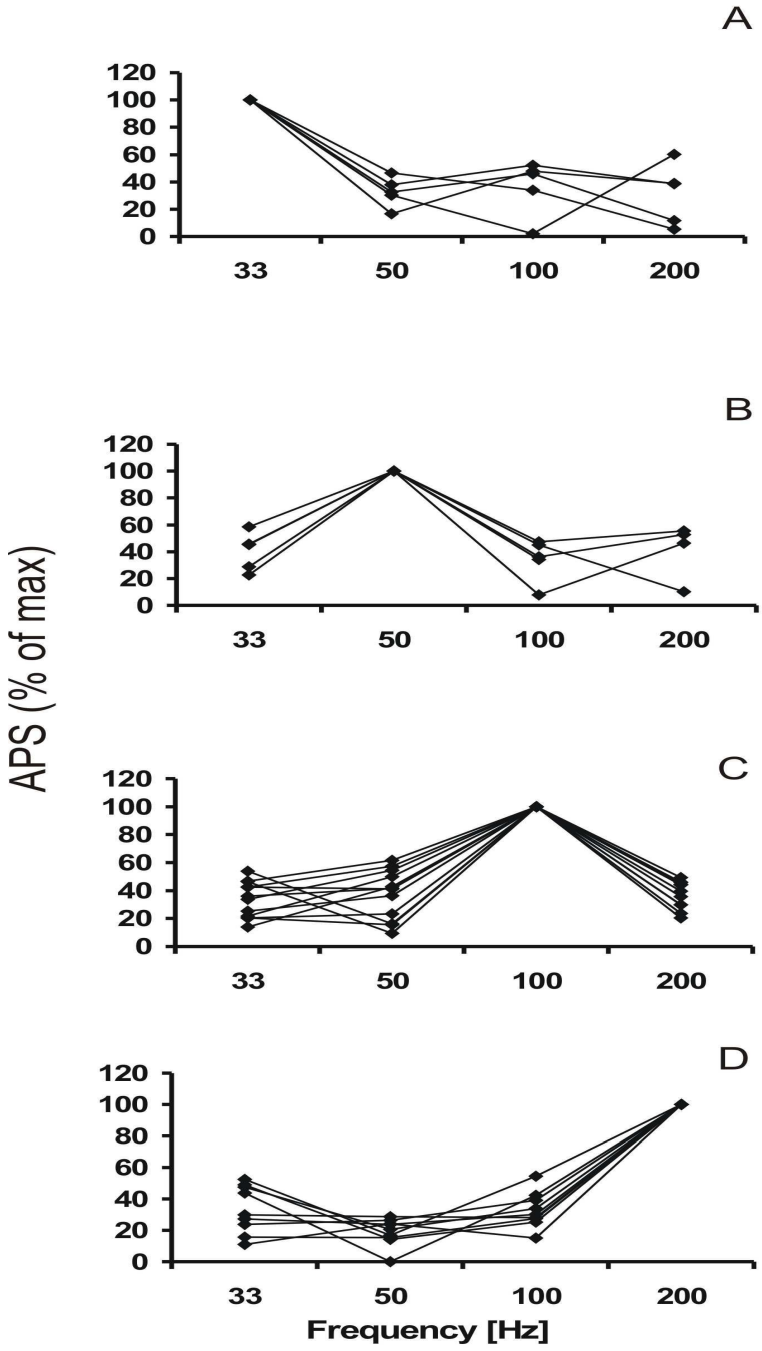

Fig. 8A - D Responses (percentage of maximum) of MON units as function of stimulus frequency. Units were grouped with respect to the maximum discharge rate elicited at a certain frequency. Units showed best responses to 33 Hz (A), 50 Hz (B), 100 Hz (C), or 200 Hz (D). Note that units responded to all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

III. Results

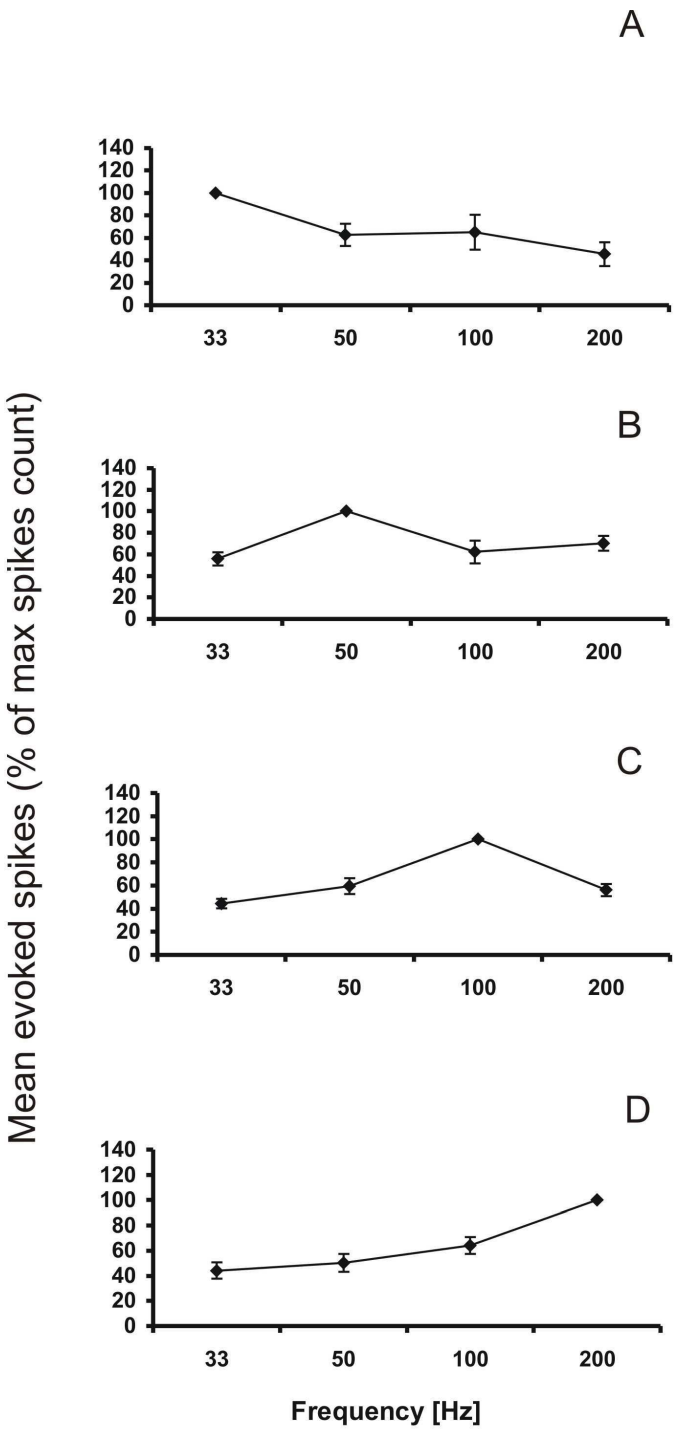

Fig. 9A - D Neural responses as function of stimulus frequency. Responses were quantified by counting the average number of spikes elicited during the time of stimulation and setting the highest number obtained for a given unit and bin, respectively, equal to 100%. Data were averaged across the units 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.

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

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 ≥ 4.6 (Batschelet, 1981).

III. Results

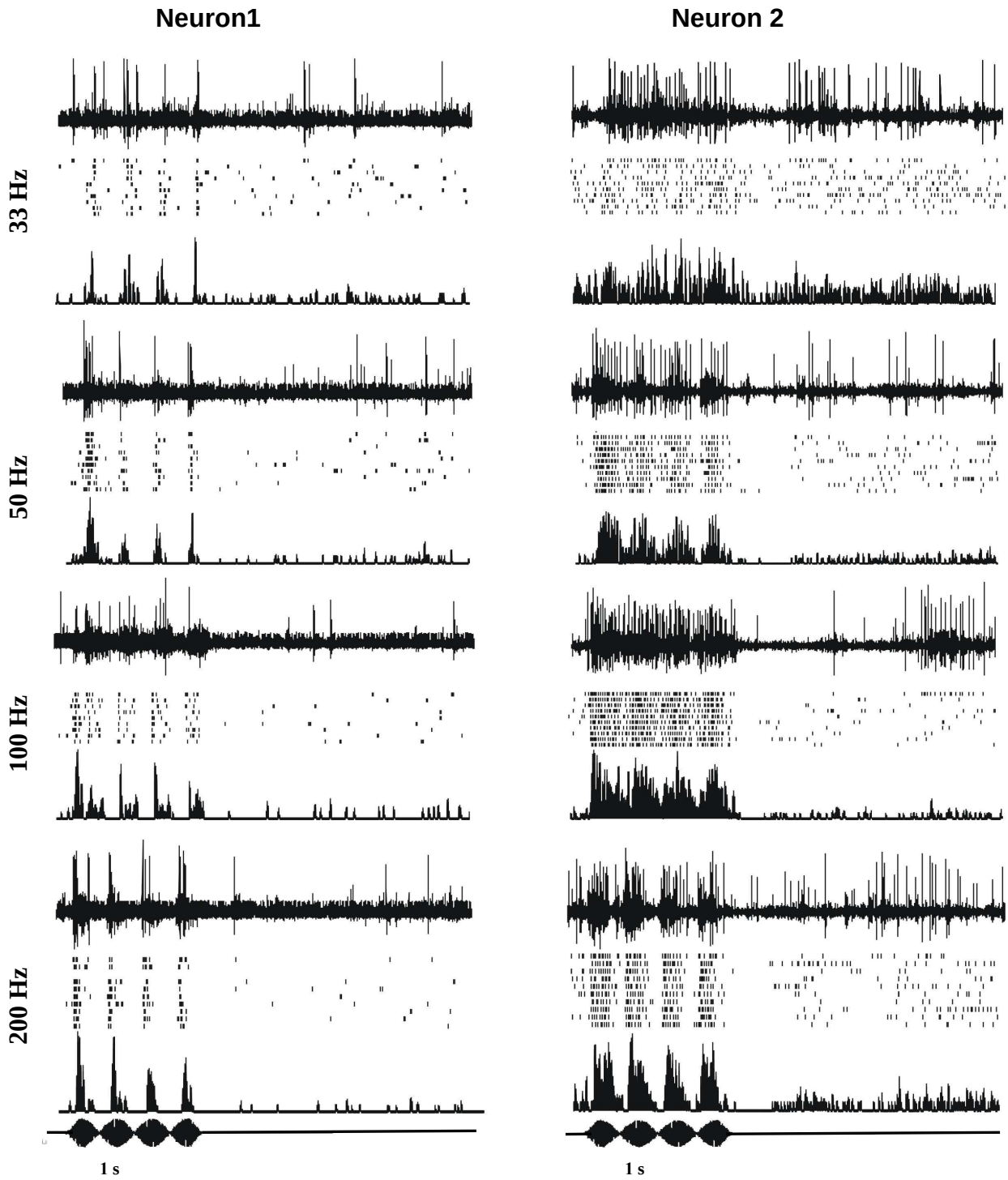

Fig. 10 Discharge patterns of two **MON** units in response to a 4 Hz AM stimulus. Note that the two units responded phasic-tonically or tonically to amplitude-modulated stimuli and phase coupled to the 4 Hz AM frequency. In each graph the top trace shows the original recording. Below each raster plot 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.

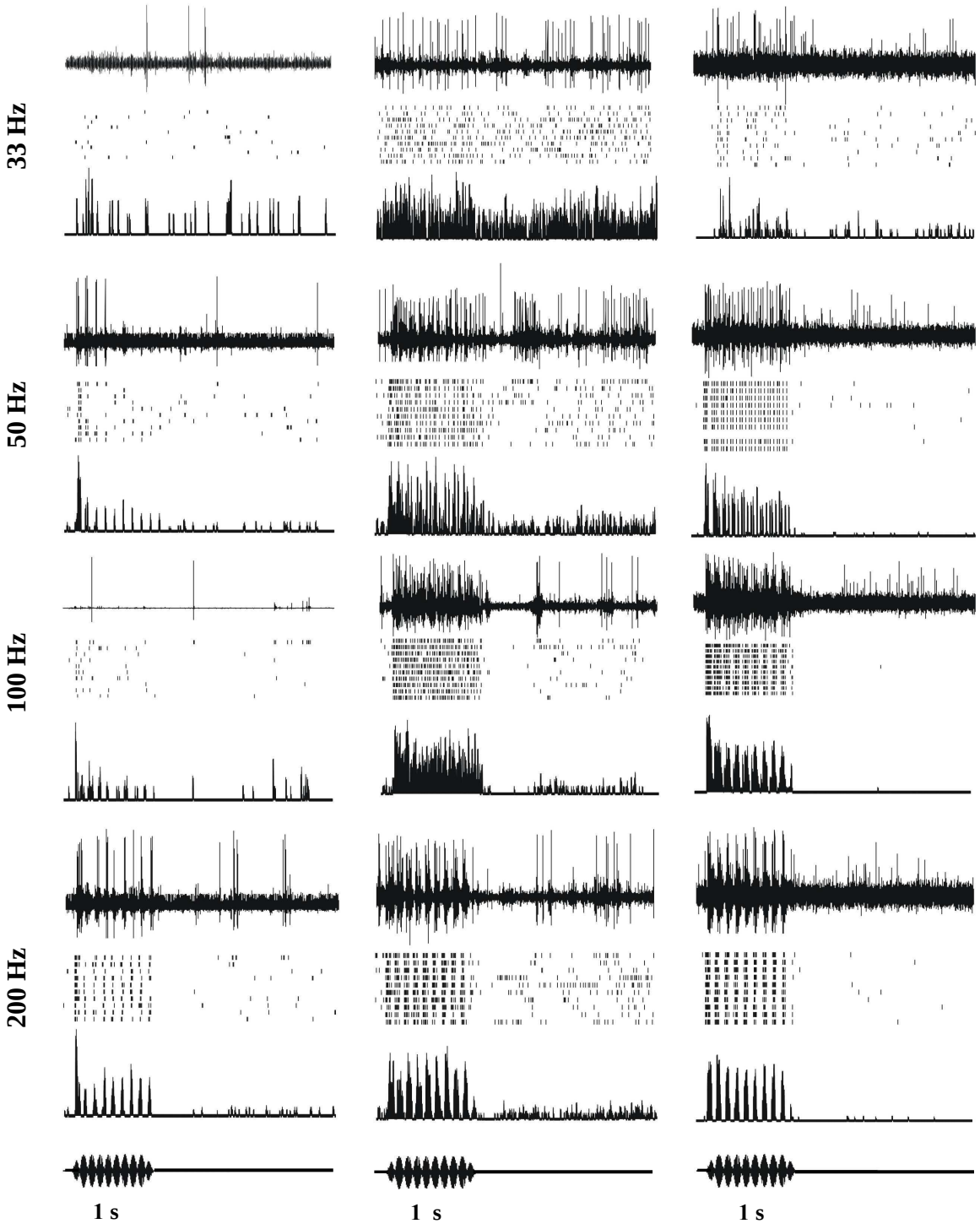

Fig. 11 Discharge patterns of three **MON** units (from left to right) in response to a 10 Hz AM stimulus. Note that all units responded tonically to the amplitude-modulated stimuli and clearly phase coupled to the 10 Hz AM frequency. In each graph the top trace shows the original recording which corresponds 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.

III. Results

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

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

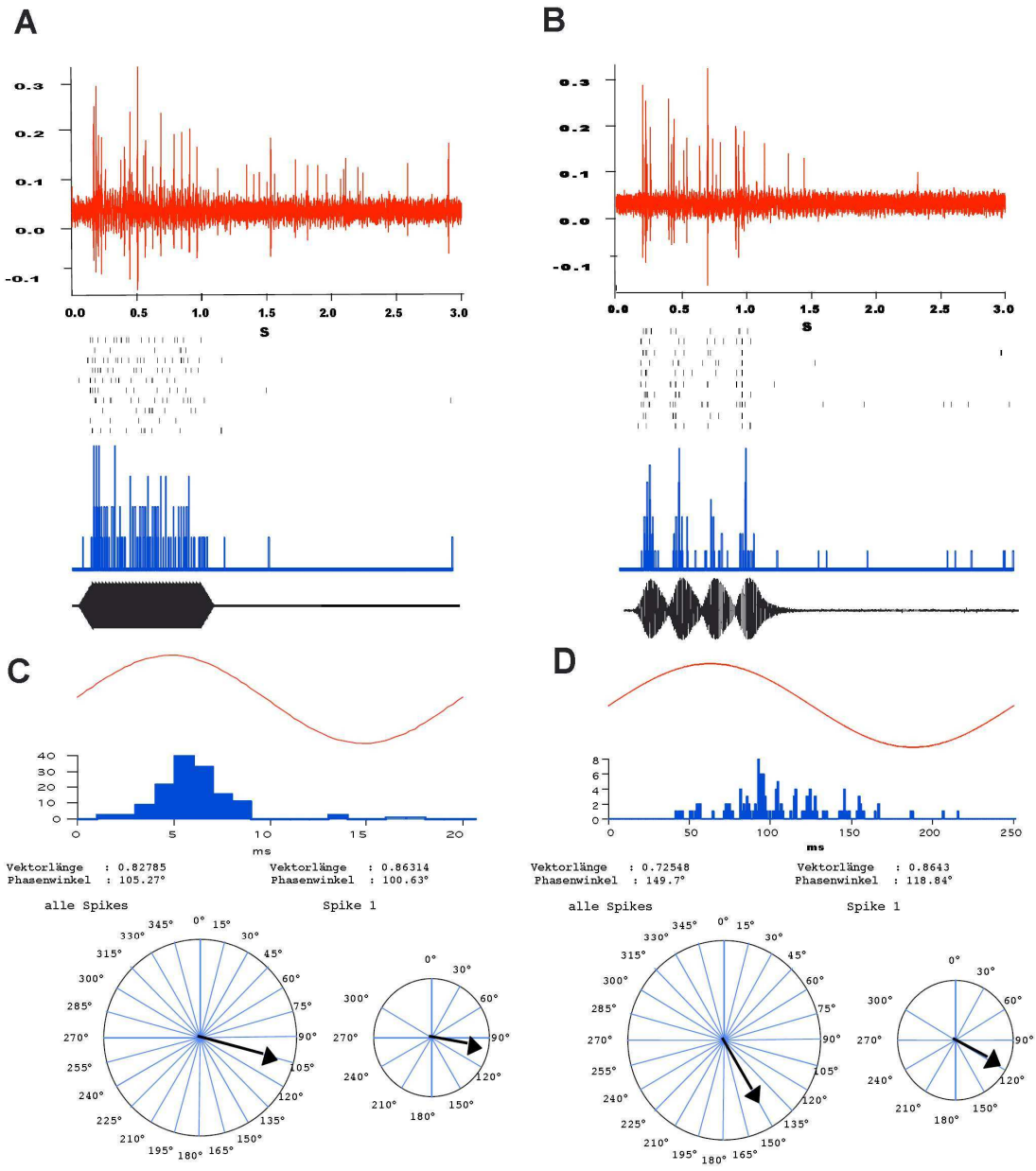

Fig. 12 A-D Data reduction and calculation procedure from single unit activity to vector strength (R). A, B examples of a few unit response to a 50 Hz CF stimulus at 0 Hz AMF (A) and 4 Hz AMF (B). From top to bottom: few unit response, dot display of 10 successive trials, PSTH (peri-stimulus-time histogram) 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

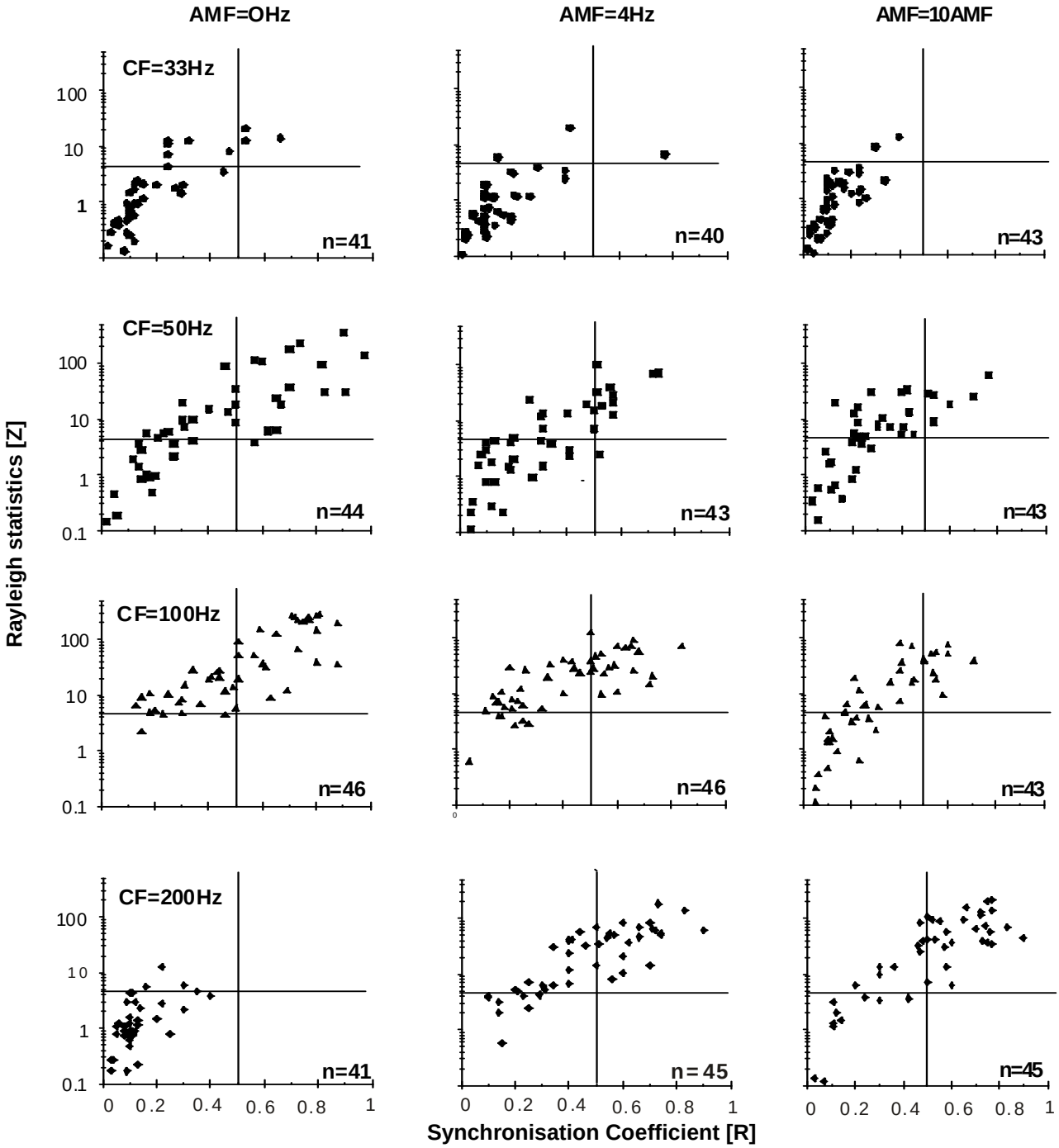

Fig. 13 Scatter plots of Z values obtained from Rayleigh test versus coefficients of synchronization (R). Phase locking is plotted with respect to the CF (left column) or with respect to the AMF (middle and right columns: 4 Hz and 10 Hz, respectively).The horizontal lines indicate the critical value 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.

III. Results

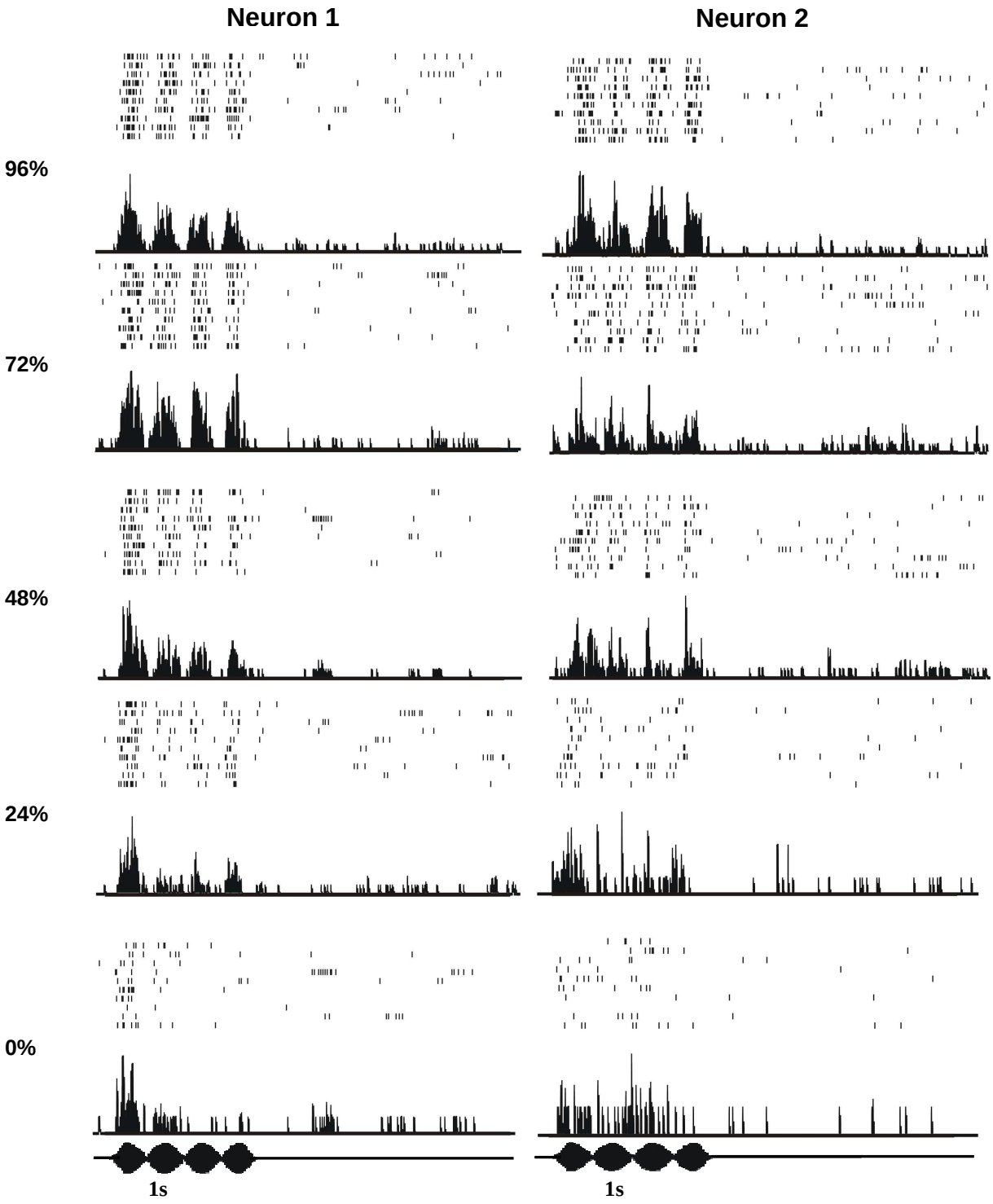

Fig. 14 Responses of two **MON** units to sinusoidal (CF 100 Hz) water motions. Amplitude modulation varied between 96% (top) and 0% (bottom). In each graph shows a raster plot and the corresponding peristimulus time histogram (PSTHs) of the responses. Stimulus traces are at the bottom. AMF was 4 Hz, 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

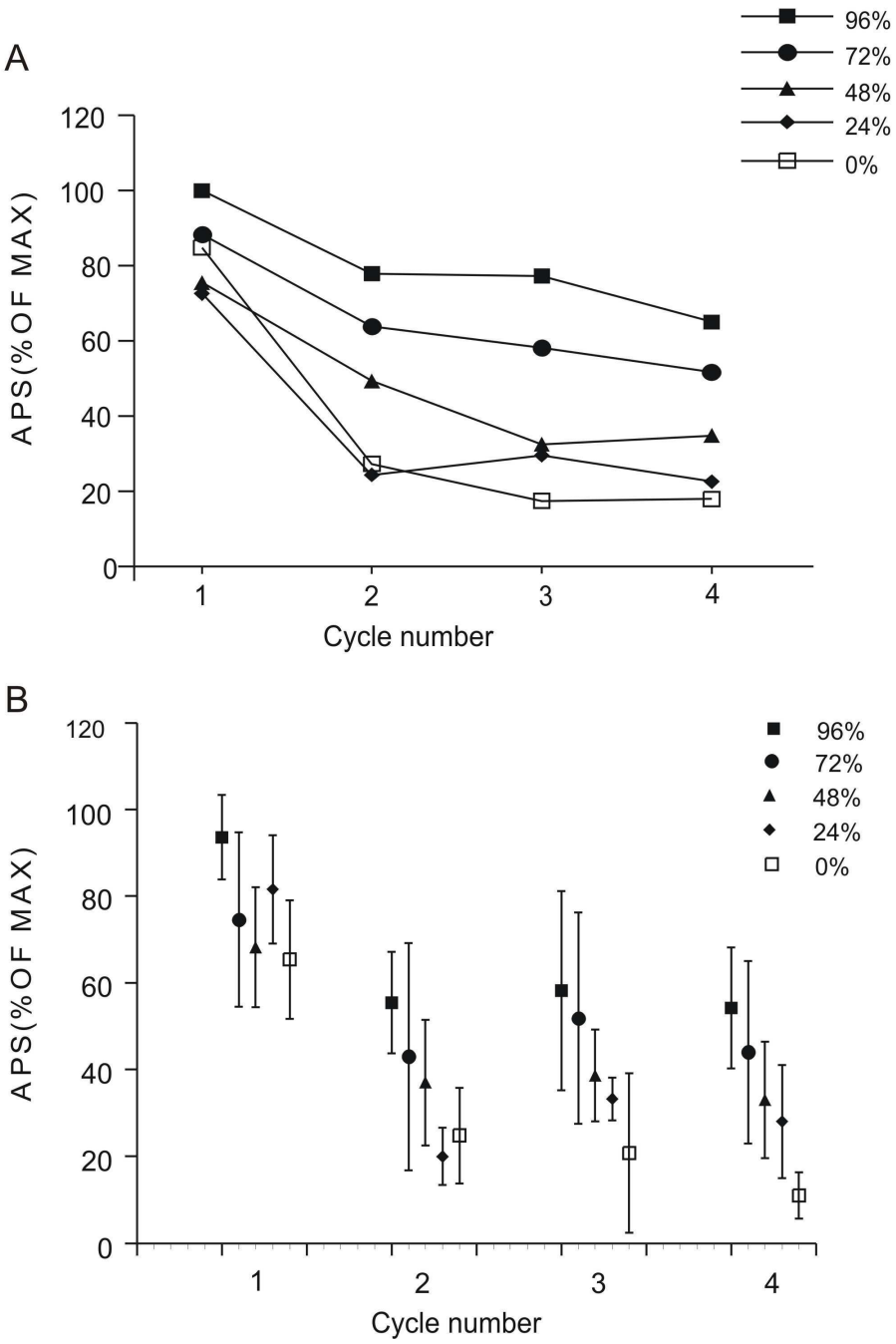

Fig. 15 A Example of the responsiveness (percentage of maximum) of a single unit as function of the number of AM cycles (AMF 4 Hz). CF was 100 Hz. Different symbols show data obtained with different amplitude modulation depths (AMDs) which varied between 0 and 96%. **B** Mean number of action potentials (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.

3.3 Level response functions 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

Extracellular recordings were made from 42 single units in 19 goldfish. As already mentioned (see above), many MON units stimulated with pure sine wave water motions phase locked to the CF of the stimulus. In general, in many units discharge rates and the degree of phase-locking increased with increasing displacement amplitudes of the sphere (Figs. 16-19). In many units, however, discharge rates reached a plateau at stimulus levels above 160 µm (Fig. 17A, 19A) (for an exception see Fig. 16). In most units the degree of phase-locking also reached a plateau at stimulus levels greater than 160 µm (Figs. 17 B, C and 19 B, C). In general, threshold amplitudes of the units varied between 25 µm and 100 µm p-p sphere displacement (Figs. 16-19). In some units level response functions were steep, i.e., in these units discharge rates increased over a narrow range of displacements (50 µm to 160 µm) (c.f. Fig. 17A).

III. Results

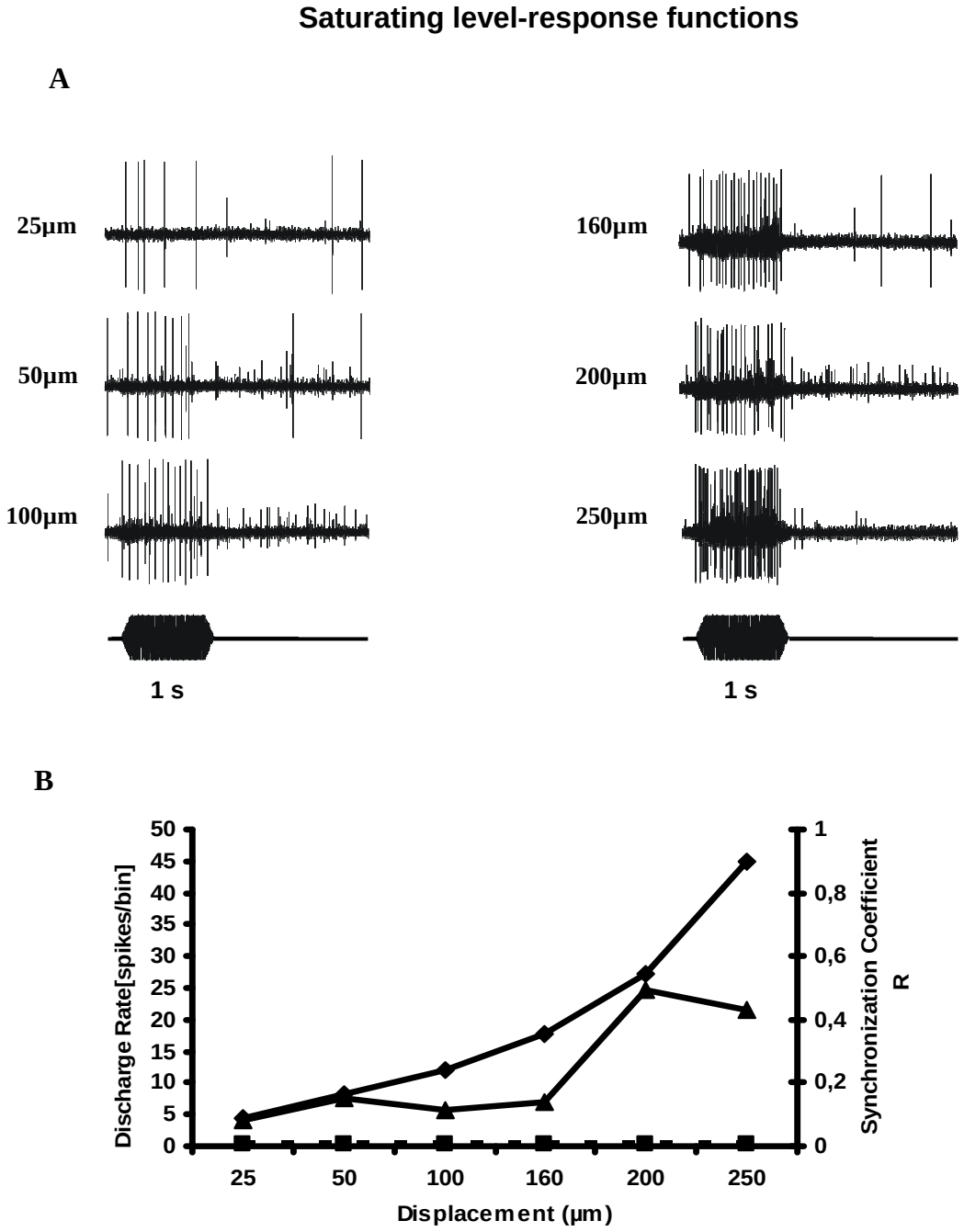

Fig. 16 A, B. A. Responses of a MON unit to a pure 100 Hz sine wave stimulus. Sphere displacement amplitudes varied between 25 and 250 µm. **B**. Level-response function of the unit shown in A. Discharge rates (diamonds, left-hand axis) and synchronization coefficients R (triangles, right-hand axis) and mean of spontaneous rates (squares, left-hand axis) are plotted as function of sphere displacement. Note that in the amplitude range tested this unit did not saturate.

III. Results

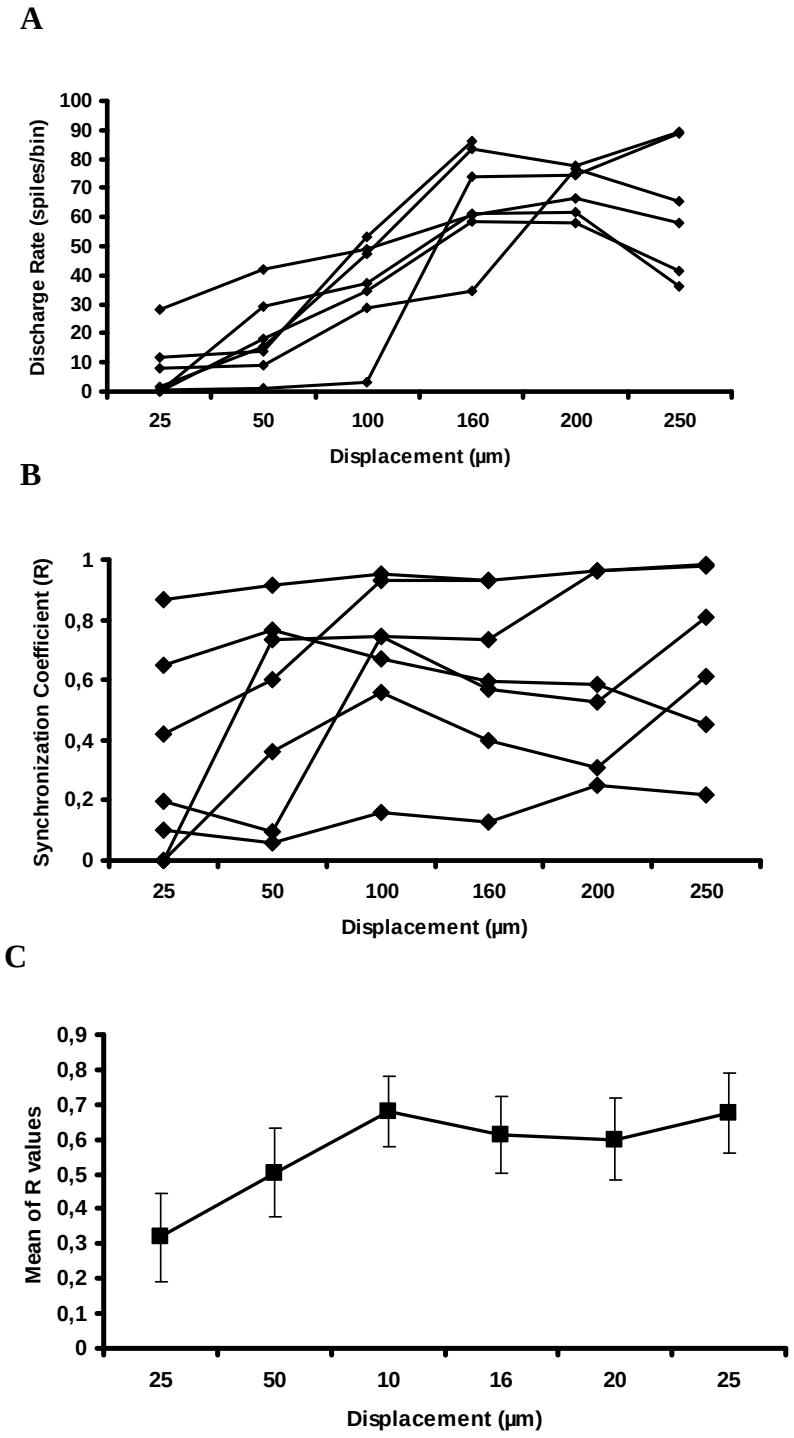

Fig. 17 A-C. A. Level response function of all (n = 7) units that showed saturating response functions to a 100 Hz sine wave stimulus. Sphere displacement amplitudes varied between 25 and 250 µm. **B.** Synchronization coefficient R of MON units that showed saturating response functions (n = 7). **C.** Mean R values of the units shown in A.

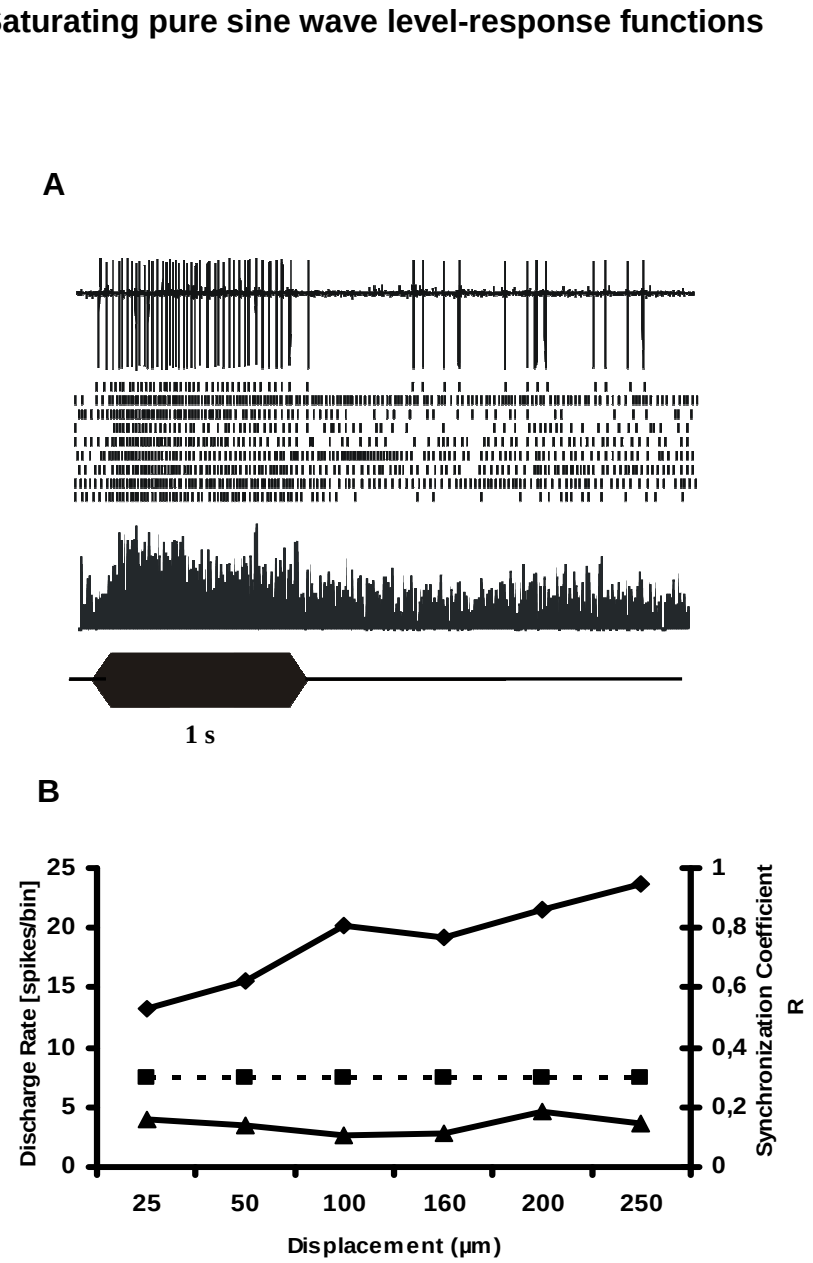

Fig. 18 A, B. A. From top to bottom: Spike trace, raster diagram and peri-stimulus-time-histogram (binwidth 2 ms) (sphere displacement 100 µm peak-to-peak). **B.** Level-response function of a MON unit. Discharge rate (diamonds, left-hand axis) and synchronization coefficient R (triangles, right-hand axis) and mean of spontaneous rates (squares, left-hand axis) are plotted as function of sphere displacement.

III. Results

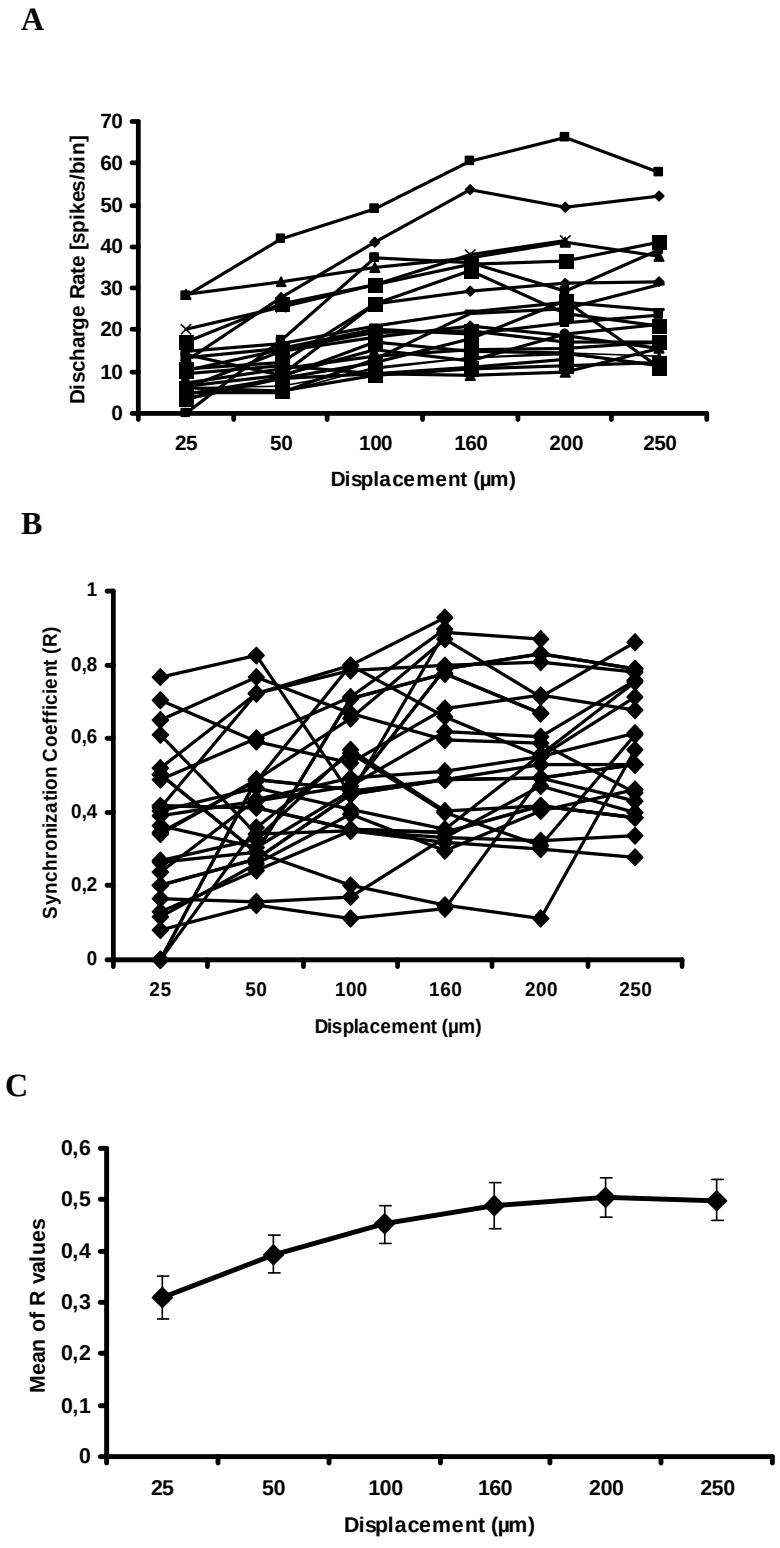

Fig. 19 A-C. Responses of a MON units to a pure sine wave stimulus (100 Hz). Displacement amplitudes varied between 25 and 250 µm. **A.** Level response function of all (n = 32) units that showed strong saturating response function. **B.** Synchronization Coefficient R of the MON units showed in A. **C.** Mean of R values of units showed in A.

3.3.2 Input-output functions to amplitude modulated sine wave stimuli.

In these experiments the mean amplitude of the AM sine wave stimuli (amplitude modulation depth 96%) also varied between 25 and 250 µm. Like to pure sine wave stimuli, the units responded to amplitude modulated stimuli also with saturating level-response functions. This was true for all 42 units tested (for examples see Figs. 20-23), i.e. discharge rates of most units reached a plateau at higher stimulus amplitudes. In 3 units the discharge rates decreased at higher stimulus amplitude (Figs. 21A, 22B). At stimulus levels just above threshold, many units responded to the amplitude modulated stimuli with a modulation of ongoing discharge rate, i.e., these units exhibited phase-locking to the AMF without substantially increasing their discharge rates (for an example see Fig. 22B). Provided a unit phase locked to the AMF the degree of phase-locking increased with increasing stimulus amplitude and often reached a plateau at stimulus levels above 200-250 µm (Figs. 22 and 23).

III. Results

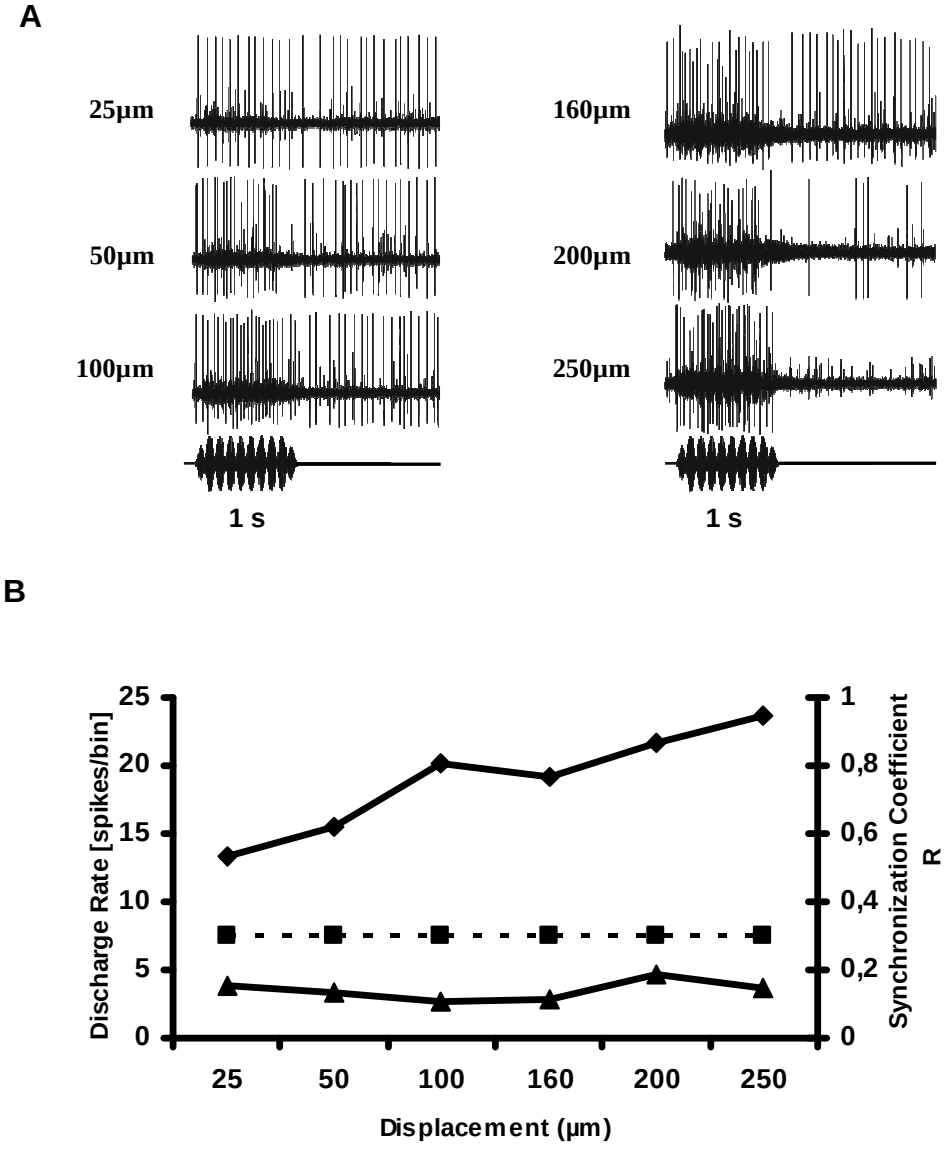

Fig. 20 A, B. A. Responses of the MON unit already shown in Fig. 16 A to an amplitude modulated sine wave stimulus (CF = 100 HZ, AMF = 10 Hz, amplitude modulation depth 96%). P-p sphere displacements varied between 25 µm and 250 µm. **B.** Level-response function of the MON unit shown in A. Discharge rate (diamonds, left-hand axis) and synchronization coefficient R (triangles, right-hand axis) and mean spontaneous rate (squares, left-hand axis) are plotted as function of p-p sphere displacement.

III. Results

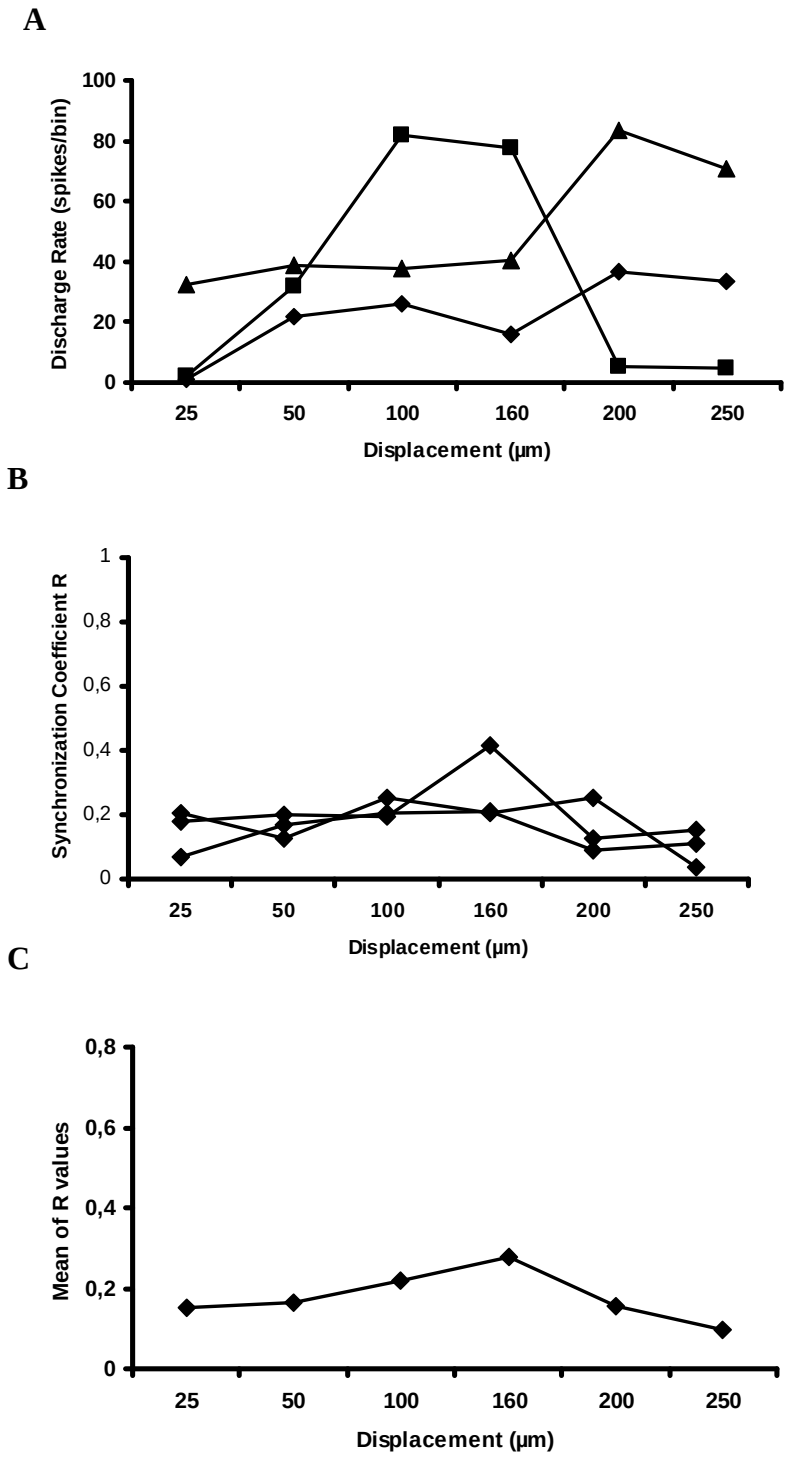

Fig. 21 A-C. Responses of three MON units to an amplitude modulated sine wave stimulus (CF = 100 Hz, AMF = 10Hz, amplitude modulation depth 96%). Mean displacement amplitudes varied between 25 and 250 µm. **A.** Level response functions. **B, C.** Synchronization coefficient R of the MON units shown in A.

III. Results

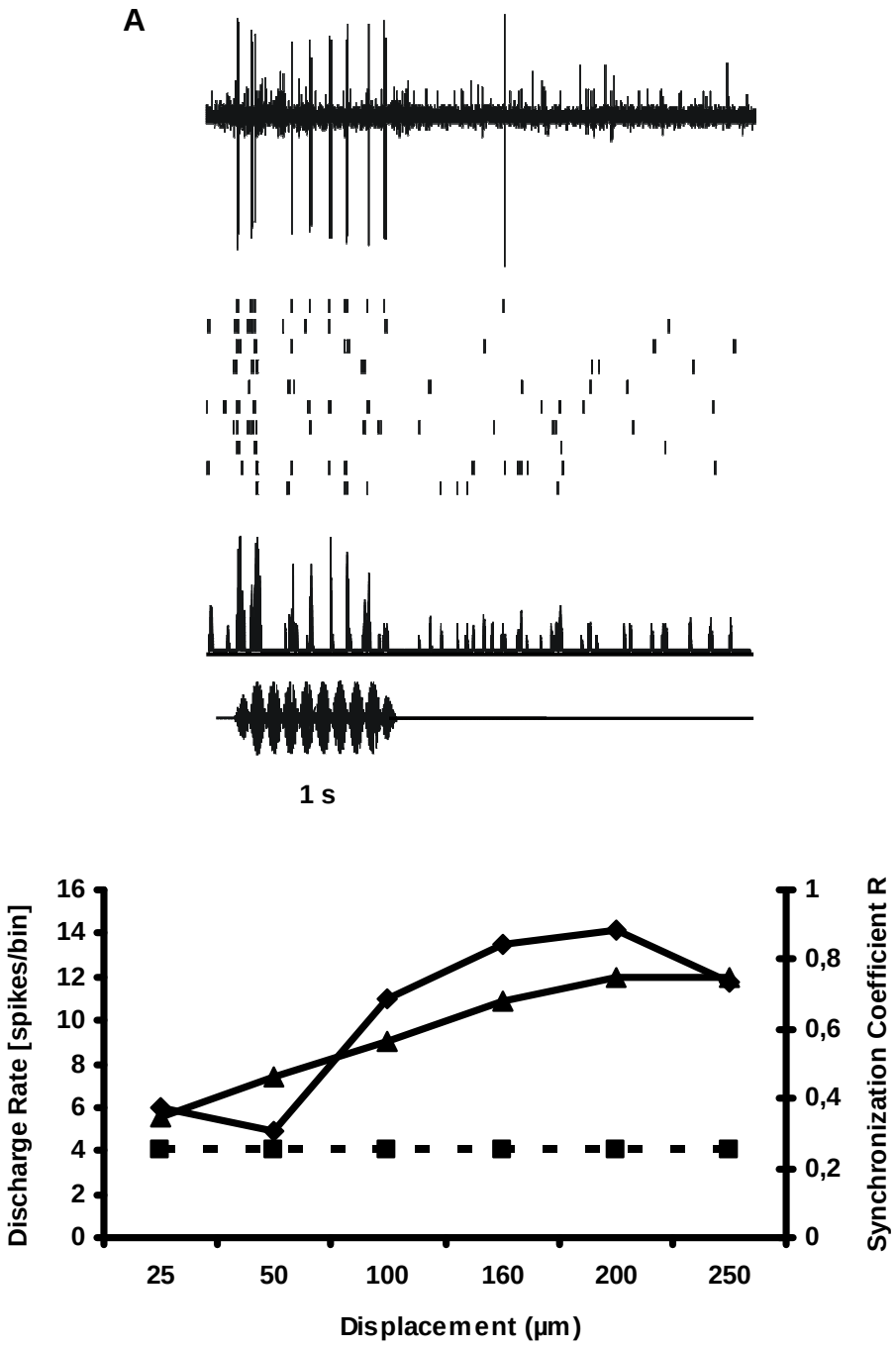

Fig. 22 A, B. A. From top to bottom: Spike trace, raster diagram and peri-stimulus-time-histogram (binwidth 2 ms) of the responses. The bottom trace shows the stimulus (sphere displacement was 160 µm peak-to-peak). **B.** Level-response functions of an MON unit. Discharge rate (diamonds, left-hand axis) and synchronization coefficient R (triangles, right-hand axis) and mean ongoing activity (squares, left-hand axis) are plotted as function of sphere displacement.

III. Results

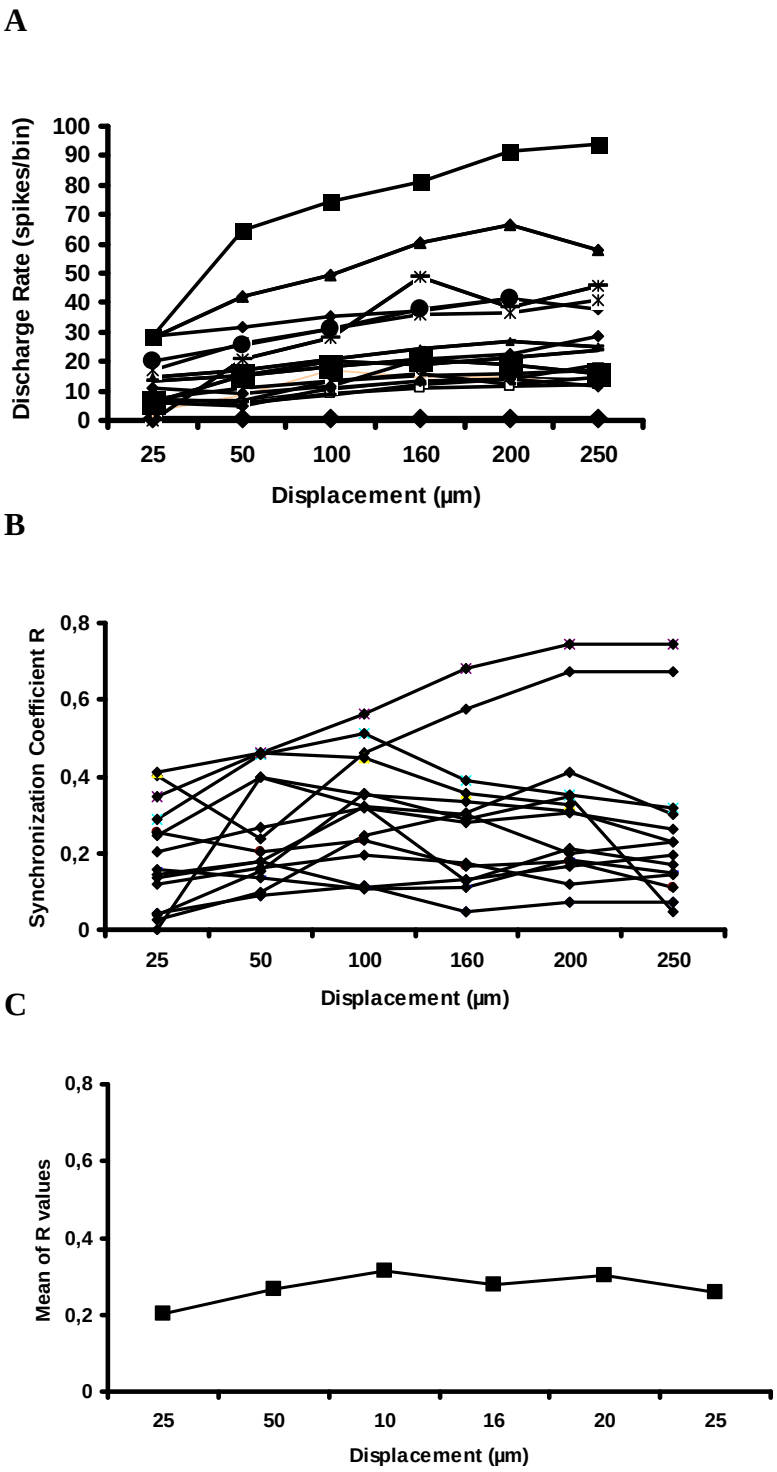

Fig. 23 A-C. Responses of MON units to an amplitude modulated sine wave stimulus (CF =100 Hz, AMF = 10Hz, amplitude modulation depth 96%). Displacement amplitudes varied between 25 and 250 µm. **A.** Level response function of all (n = 25) units that showed saturating response function. **B, C.** Synchronization coefficient R of the units shown in A.

3.4 Receptive fields

Sensory systems must operate over a wide range of spatial scales, and single neuron receptive field (RF) organization may contribute to the ability of a neuron to encode information about stimuli having different spatial characteristics (Coombs et al 1996; Curcic-Blake and van Netten 2006; Goulet et al. 2008). A crude analysis revealed that many MON units of goldfish had RFs that consisted of a region from which stimulation with the vibrating sphere caused an increase in discharge rate (excitatory region) and another region from which stimulation with the vibrating sphere caused a decrease in discharge rate (inhibitory region) (Mogdans and Kröther, 2001). In the present study, the RFs of 7 units were determined in some detail. Sphere vibration amplitude always was 160 µm, applied with a frequency of 100 Hz. The sphere was positioned close (5 to 8 mm) to the fish at the height of the trunk lateral line canal and moved in 1cm steps along the side of the fish until the most anterior part of the head and the most posterior part of the tail, respectively, was reached. All units responded with an increase in discharge rate to the vibrating sphere. With the exception of the unit shown in Fig. 24 D, i.e. of a unit with a small RF, all units had brought RFs which often appeared to be double peaked (Fig. 24 B, C, E).

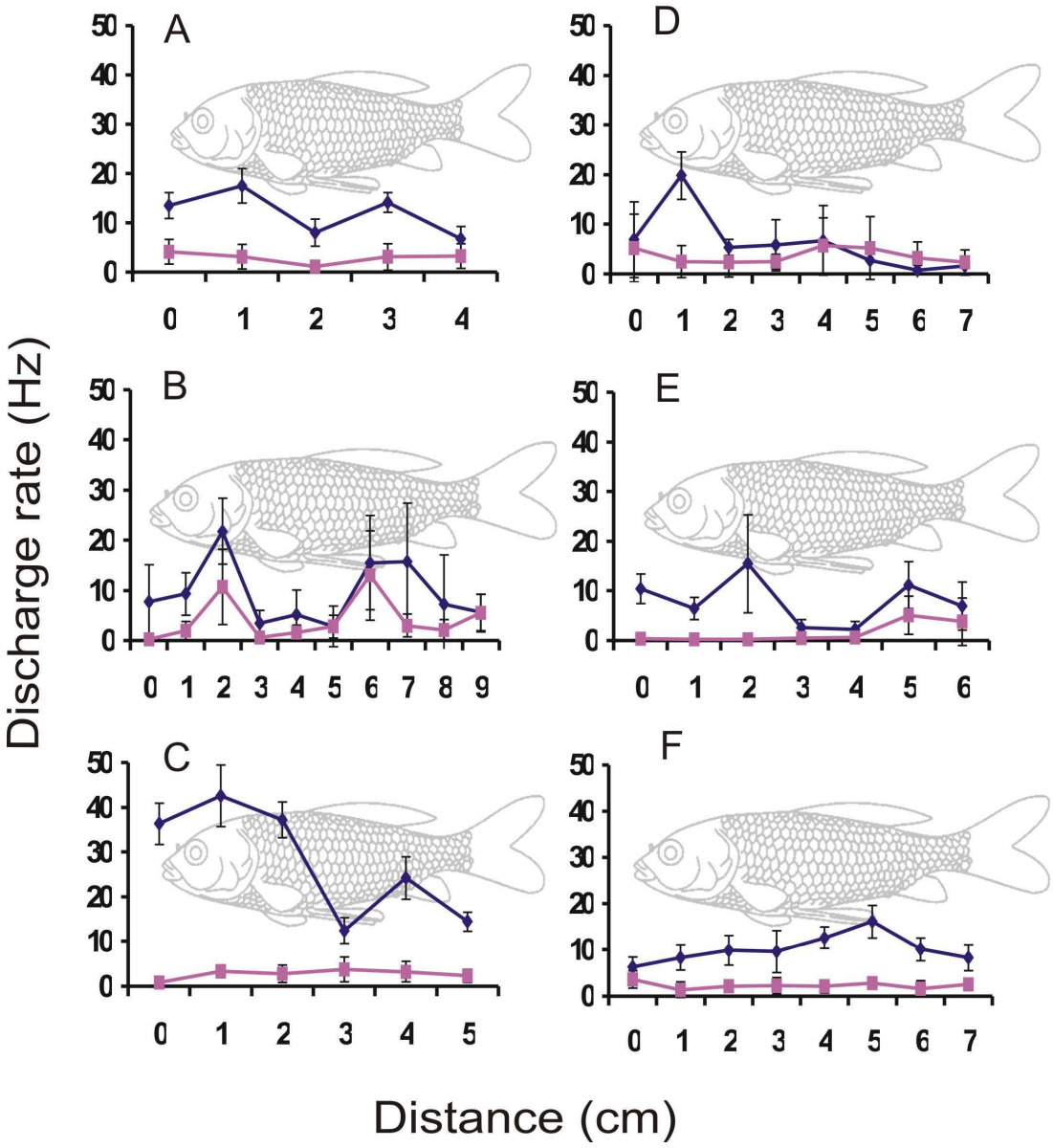

Fig. 24 A-F. Responses of MON units of goldfish to a100 Hz vibrating sphere stimulus. Peak-peak displacement amplitude was 160 µm. Blue line: evoked discharge rate, pink line: ongoing discharge rate. Vertical bars equals one SD. The size of the fish is to scale. Direction of sphere vibration always was parallel to the long axis of the fish.

Fig. 25 shows that the responses of the 6 out of the 7 units whose RF were investigated were not phase locked to the vibrating sphere stimulus. irrespective of sphere position, Z-values usually were below 4.6, i.e. Z-

values did not reach the significance level. One unit did phase lock to the stimulus, but only at the sphere positions 0, 1 and 2 cm (c.f. Fig. 25C).

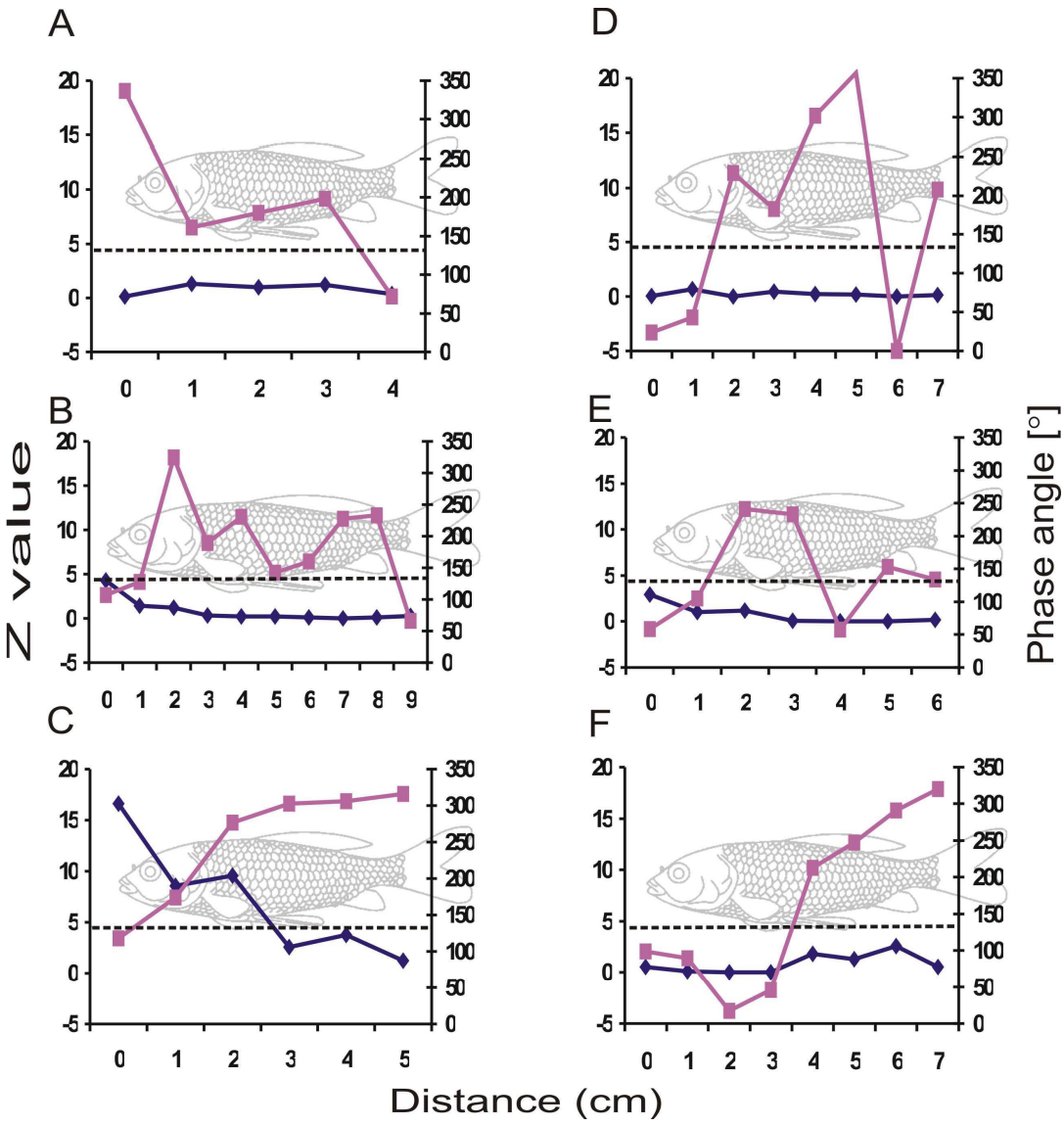

Fig. 25A-F. Responses of MON units of goldfish to a100 Hz vibrating sphere stimulus. Displacement amplitude was 160 µm. Phase angles (red lines re: right hand axis) and synchronization coefficients (Z-values) (blue lines re: left hand axis) are plotted as function of sphere position. **A-F** Data from the units already presented in **Fig. 24.** With the exception of the unit shown in C none of the units phase locked to the stimulus.

III. Results

3.5 Anatomy

In fifteen animals electrolytic lesions were applied at the end of an experiment. From these animals, 11 lesions were recovered. Eight lesions were in the molecular layer or in the crest cell layer of the MON, i.e. in areas which are known to receive lateral line input (Montgomery et al.1996; New et al. 1996). The remaining three lesions were close to the ventral border of the deep neuropil layer of the MON (c.f. Fig. 26). The molecular layer is the most superficial layer of the MON comprising three discrete cell types. Cristal cells, stellate cells and granule-like cells as well as the crest cell layer comprise the largest and most visible class of neurons in the MON; crest cells segregate into two distinct classes based upon dendritic morphology and soma shape: basilar crest cells and non-basilar crest cells (New et al. 1996). Fig. 27 shows a representative section through the brainstem of a goldfish with a lesion recovered in the MON.

III. Results

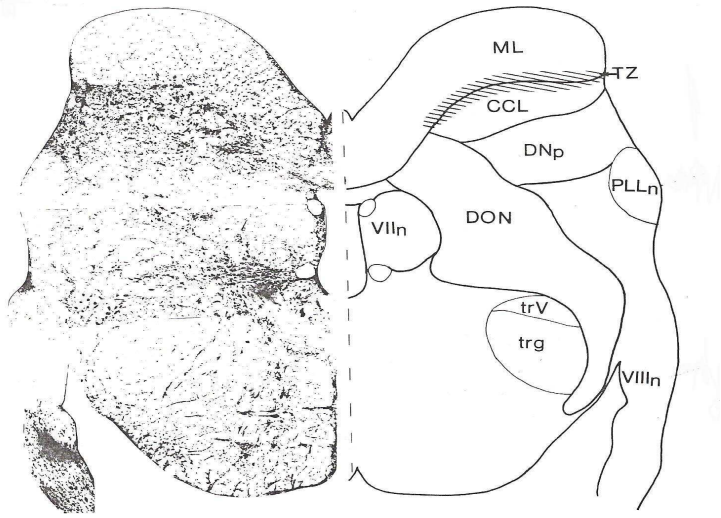

Fig. 26 Transverse section through the brain of a goldfish. The section is at the level of the eighth nerve. On the right is a mirror image line drawing of the cresyl violet-stained hemisection shown on the left. The figure was taken from New et al. (1996). ML: molecular layer of MON, CCL: crest cell layer of MON, DON dorsal octavolateralis nucleus, Plln posterior lateral line nerve, TZ: transitional zone of MON, DNp: deep neuropil layer of MON, VIIIn eights nerve, trv descending tract of the trigeminal nerve, trg secondary gustatory tract, VIIn sensory root of the facial nerve

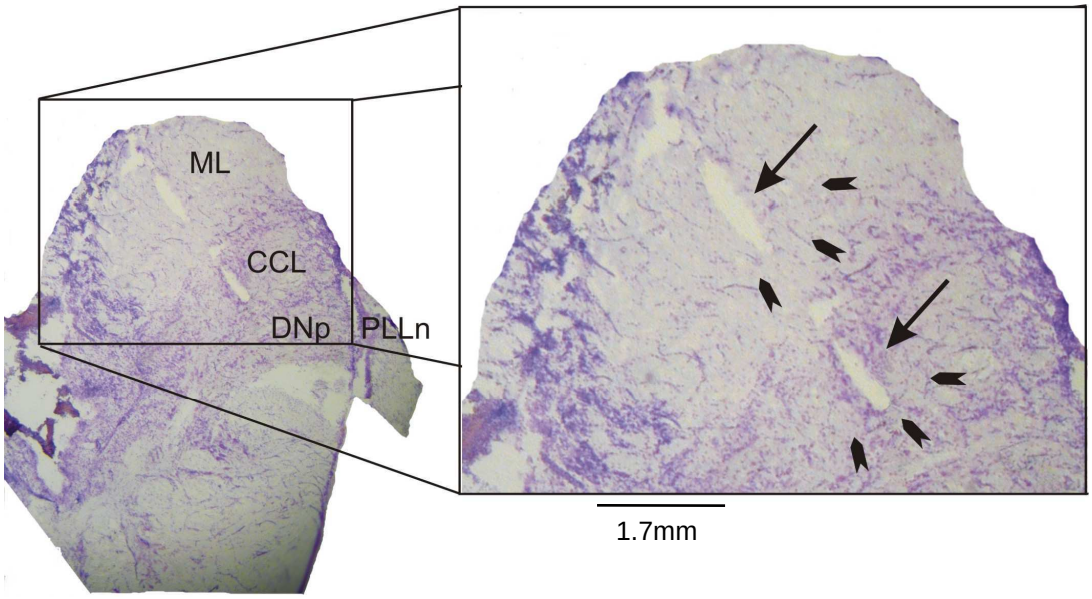

Fig. 27 Cresyl violet-stained brain section of a goldfish. The lesioned recording site was recovered in the right MON. Traces of the electrode track are seen (see arrows). The end of the electrode track is in the crest cell layer of the MON: Top arrow heads point to the border of the molecular layer, bottom arrow heads to the border of the crest cell layer. Scale bars 1.7 mm. ML molecular layer, CCL crest cell layer, DNp deep neuropil, PLLn posterior lateral line nerve.

IV. Discussion

4. Discussion

4.1 Responses to pure sine wave stimuli

Afferent lateral line fibres travel to the brain in at least three distinct nerves which innervate the neuromasts on the fish head and trunk (Northcutt 1989; Puzdrowski 1989; Song and Northcutt 1991). The first site of lateral line integration is the medial octavolateralis nucleus (MON) in the fish brainstem (McCormick and Hernandez 1996; New et al. 1996). Compared to afferent fibers, MON units have much lower spontaneous and evoked rates of activity. The responses to sine wave stimuli exhibit greater degrees of adaptation and greater heterogeneity both in terms of the response patterns and in terms of phase-coupling. Moreover, most MON units are substantially less sensitive to sine wave stimuli than primary afferents (e.g. Paul and Roberts1977; Caird 1978; Wubbels et al. 1993; Montgomery et al. 1996; Coombs et al. 1998). All these findings have been confirmed in the present study. In goldfish, about 30% of the units in the MON do not respond to a stationary vibrating sphere, even when tested with displacement amplitudes of up to 800 µm (Mogdans and Goenechea 2000). Such displacement amplitudes are substantially higher than the amplitudes causing rate saturation in lateral line afferents. On the other hand, many of these seemingly insensitive units readily respond well to the water motions generated by a moving sphere (Mogdans et al. 2003a).

IV. Discussion

Most midbrain (torus semicircularis) lateral line units of goldfish show no ongoing activity. In contrast to primary lateral line afferents most toral lateral line units neither encode the temporal envelope nor the CF of a dipole stimulus. In addition most toral lateral line units also do not encode the duration of a pure sine wave stimulus because of strong adaptation (Plachta et al. 1999).

In all species that have been studied so far, the responses of medullary lateral line units to dipole stimuli range from primary-like to completely primary unlike, suggesting that considerable transformation of primary afferent input already takes place in the brainstem, and that the degree of transformation is variable (Coombs et al. 1998). For instance, the post-stimulus time histograms (PSTHs) of MON crest cells in the mechanosensory lateral line system of the dwarf scorpionfish show some interesting differences from those of primary afferents. Whereas most lateral line afferents have phase-locked tonic responses, only some crest cells phase-lock to the stimulus. Most crest cells show more phasic responses than primary afferents, particularly at higher stimulus frequencies (Montgomery et al.1996). This was also found in the present study.

One distinctive property of crest cell responses to single frequency sine wave stimuli is the suppression of activity by higher frequencies. Frequencies of 100 Hz, which produce maximal activity of afferents, may

produce a strong suppression of activity in the crest cells of the mechanosensory lateral line system (Montgomery et al.1996). The data from the present study are in agreement with these findings (c.f chapter 4.1) in that (1) medullary lateral line units had less spontaneous activity than primary lateral line afferents, (2) medullary lateral line responses in goldfish to pure sine wave stimuli were both, adapting or sustained (type 1 and 2, Fig. 4) thus only some units encoded stimulus duration, and (3) medullary lateral line units exhibited phase-locking which decreased at higher stimulus frequencies due to the fact that units start to discharge more than one spike per stimulus cycle (Fig. 13, first column).

4.1.1 Comparison with the acoustic and the electrosensory system

The above differences in MON responses have also been noted for other, similarly organized octavolateralis systems, including the dorsal cochlear nucleus (DCN) of the mammalian auditory system, the dorsal octavolateralis nucleus (DON) of the ampullary-electrosensory system of cartilaginous fishes, and the electrosensory lateral line lobe (ELLL) of weakly electric Gymnotids and Mormyrids (Montgomery et al. 1996). In agreement with MON responses the responses of the principal cells of the DON, ELL and DCN also tend to be more phasic than those of the primary afferents of the respective system.

IV. Discussion

As already mentioned above, we still do not know the information processing that takes place in the central lateral line pathway. The apparent loss of sensitivity to vibrating sphere stimuli by many central lateral line units (e.g., Paul and Roberts 1977; Caird 1978; Wubbels et al. 1993; Montgomery et al. 1996; Coombs et al. 1998), the contrasting high sensitivity of many of such units to a moving object (Mogdans and Goenechea 2000) and the fairly large and complex receptive fields of many central lateral line units (e.g. Mogdans and Kröther 2001 and this study) suggest that the central lateral line system is not particularly well suited for the detection and analysis of pure sine wave stimuli. Instead, many central (e.g. MON) units appear to be integrating inputs from large parts of the lateral line periphery. The present study shows, that the responses of MON units that were sensitive to sine wave stimuli may be similar to the responses of primary lateral line afferents, i.e. these units respond to pure sine wave stimuli with sustained and phase-locked discharges and discharge rates that are modulated according to the amplitude modulation frequency.

4.2 Frequency - response characteristics

The responses of primary lateral line afferents of the dwarf scorpionfish, *Scopeana papillosus*, to sine wave water motions are relatively homogeneous, with the responses increasing over the mid frequency range at a rate consistent with an acceleration-sensitive system. The responses of MON crest cells in the mechanosensory lateral line of the

dwarf scorpionfish are again consistently different from those of primary afferents; frequencies above 50 Hz often produce a suppression of the response. In some units, onset of the stimulus 'tone burst' produced only a single spike that was followed by suppression. This suppression was clearly seen in the frequency-response curves. of the crest cells. Up to a frequency of approximately 50 Hz the sensitivity of the units increased, thereafter it declined with increasing frequency (Montgomery et al. 1996). One interpretation of this finding is that the stimulus used activated not only the excitatory receptive field but also an inhibitory surround. Inhibitory postsynaptic potentials are longer-lasting (typically 20 ms or so) than excitatory postsynaptic potentials, so that strong activation of inhibitory inputs onto crest cells could reduce their responses at frequencies above 50 Hz to all but the first spike (Montgomery et al. 1996).

The majority of toral lateral line units of goldfish responded to CFs ranging from 33 to 200 Hz. If stimulated with identical displacement amplitudes, units showed low-pass, band-pass and high-pass characteristics (Plachta et al. 1999). In contrast midbrain lateral line units of trout yielded only two peaks of increased sensitivity which coincide with the best frequencies (in terms of displacement) of superficial and canal neuromasts (Schellart and Kroese 1989).

In the present study the frequency characteristics of MON units was also determined by measuring iso-displacement curves (Figs. 6 to 9). P-p

displacement amplitudes of the sphere always were 160 µm, the distance between the surface of the fish and the surface of the sphere was 5-8 mm. Carrier frequencies (CFs) were 33 Hz, 50 Hz, 100 Hz and 200 Hz. Under these conditions many units had flat frequency response curves. However, a few units showed some tuning characteristics including low-pass, band-pass, and high-pass (Figs. 7, 8 and 9 D). The majority of MON units responded best (highest umber of spikes per stimulus) at 100 Hz (45%). Other units responded best at 200 Hz (42%), 50 Hz (13%), or 33 Hz (11%). In general frequency tuning was absent or weak in most units. This agrees with peripheral (review see Münz 1989) and other central lateral line studies (review see Bleckmann and Bullock 1989).

4.3 Response to amplitude modulation sine wave stimuli.

The detection of amplitude modulations is a fundamental capability of many sensory systems. Amplitude modulated stimuli are more typical for a natural hydrodynamic environment than constant amplitude sine wave stimuli (Montgomery 1989; Satou et al. 1991; Bleckmann et al. 1991). Consequently teleost fish have the ability to discriminate stimulus amplitudes. In *Cottus bairdi*, amplitude discrimination limens were independent of sensation level, source distance, and frequency over most of the detection bandwidth (10-100 Hz), with mean values ranging from 5 to 6 dB (10- 50 Hz) and 8-9 dB (100 Hz), respectively (Coombs and Fay 1993). The surface-feeding fish *Aplocheilus lineatus* also discriminates stimulus amplitudes if confronted with single frequency surface wave

stimuli. At 40 Hz *Aplocheilus* distinguishes a 4 µm (0.15 µm) stimulus from a 21 µm (1 µm) stimulus (Waldner 1981). These values correspond to amplitude differences of 14.4 and 16.4 dB, respectively.

All posterior lateral line nerve fibers of goldfish, *Carassius auratus*, when stimulated with amplitude-modulated sine waves, respond with strong phase-locking to the CF and, in addition, discharge rates are modulated according to the amplitude modulation frequency (Mogdans and Bleckmann 1999). However, phase- locking to the AMF was weaker than phase-locking to the CF. This indicates that the discharges of primary lateral line afferents encode both the CF and the AMF (Mogdans and Bleckmann 1999). As expected amplitude modulated sinusoidal stimuli caused also more prominent lateral line response in the midbrain of goldfish than constant amplitude stimuli. In addition toral lateral line units of goldfish phase locked to the AMF (Plachta et al.1999).

In the present study the responses of MON units evoked by amplitude modulated sinusoidal water motions also differed from those evoked by the unmodulated carrier. The responses of medullary lateral line units were characterized by a burst of discharge to each modulation cycle (Figs. 10, 11). Midbrain lateral line units of goldfish have similar response properties (Plachta et al. 1999) Thus, in both the MON and in the midbrain of goldfish response profiles tend to be phasic to constant amplitude sine

wave stimuli but tonic for amplitude modulated single frequency water motions.

4.3.1 Comparison with central acoustic and electrosensory units

Toral acoustic units of goldfish represent the CF and the AMF of stimulus (Lu and Fay 1996). Furthermore central acoustic units may show a sharp frequency tuning to distinct CFs (Lu and Fay 1996). Nesting male plainfin midshipman fish, *Porichthys notatus*, produce unmodulated, long duration calls known as hums. Hums are spectrally simple signals, consisting of a fundamental frequency (80–120 Hz) and several higher harmonics. Although an individual hum has an essentially flat envelope, midshipman acoustic communication does include at least two distinct types of envelope modulation. First, nesting males emit trains of short (50–200 ms) pulsed sounds, known as grunts.

Grunts are associated with agonistic contexts and presumably signal aggression. While only nesting males produce grunt trains, both sexes also produce isolated single grunts. The second type of envelope modulation results from the frequent occurrence of overlap between the hums of nearby males. When two hums with slightly different fundamental frequencies overlap, the acoustic waveforms interfere to produce beats at their difference frequency. Behavioral experiemnts have shown that the midshipman is sensitive to beat modulations from 0.5 to 10 Hz, with fewer

fish approaching the beat than the pure tone. Reducing the degree of modulation increased the effectiveness of beat stimuli. Hence, the lack of modulation in the midshipman's advertisement call corresponds to the importance of envelope modulation for the categorization of communication signals even in this relatively simple system (Mckibben and Bass 2001). The temporal envelope modulation may be important for the discrimination of different types of species specific acoustic signals.

For weakly electric fish both amplitude and phase cues are important. For instance, the South American fish *Eigenmannia* sp. is very sensitive to electrosensory signals that contain amplitude and phase modulations (Heiligenberg 1991). The same holds true for the African wave-type electric fish, *Gymnarchus*, both types of fish perform a jamming avoidance response (Kawasaki and Guo 1998). Consequently the electrosensory lateral line of these fish encodes the amplitude and the phase of a stimulus to a high precision.

The MON units in the present study were much less sensitive to amplitude modulations than acoustic or electrosensory units. For example midbrain electrosensory units of the weakly electric fish *Eigenmannia* have an amplitude modulation threshold of only 0.05 % (Rose and Heiligenberg 1986).

IV. Discussion

4.4 The encoding of amplitude and phase information

Mogdans and Bleckmann (1999), while studying the peripheral lateral line of goldfish, found that when amplitude modulation depth was varied between 0% and 96%, discharge rates elicited by the AM stimuli were reduced by only 1- 24% with respect to the discharge rates elicited by the unmodulated stimuli. This effect was comparable for AMFs of both 4 Hz and 10 Hz. The synchronization coefficients of individual fibres (peripheral lateral line) were high with respect to the CF (between 0.7 and 1) and did not change systematically when modulation depth was varied, indicating that phase-locking to the CF was independent of modulation depth (MD). In contrast, coefficients with respect to the AMF increased, from 0 to 0.1 at 12% modulation depth to values between 0.1 and 0.5 at 96% modulation depth. Thus, phase-locking to the AMF improved with increasing MD (Mogdans and Bleckmann 1999).

Although many toral lateral line units of *Carassius* clearly encoded the AMF, they were not especially sensitive to amplitude modulations. In the range tested, a clear response to amplitude modulations was only obtained if the modulation depth was ≥ 36% (Plachta et al. 1999). Midbrain acoustic units of catfish (*Ictalurus*) respond to amplitude modulations with thresholds around 20- 30% (Plassmann 1985).

IV. Discussion

The electrosensory system of weakly electric fish is not only sensitive to amplitude, but also to phase modulations. For instance, while studying the electrosensory system, Kawasaki (1993) tested various depths of phase modulation, while maintaining the depth of amplitude modulation constant (at 10%). At 1.4 µs of phase modulation (peak-to peak), the African electric fish *Gymnarchus niloticus* showed small (0.03 Hz/min) but distinct JARs. The strongest JAR (0.9 Hz/min) occurred at a phase modulation of 90 µs. As the modulation depth increased further, progressively smaller JARs were observed. At 750 µs, no JAR occurred (Kawasaki 1993).

In the present study, MON units responded tonically when amplitude modulation depth was maximal (96%), i.e. MON units of goldfish responded to each modulation cycle with about the same number of spikes. When modulation depth was less than 96%, units tended to discharge with a decreasing number of spikes to increasing numbers of AM cycles (Fig. 15 A and B).

4.5 Level Response Functions

Behavioral studies have shown that the central lateral line system of fishes gets the information necessary to detect changes in the amplitude of water motions. This has also been shown in physiological studies. Although in many cases the highest stimulus amplitudes used were not sufficient to saturate the neural responses, dynamic amplitude ranges of

up to 90dB have been found in higher order lateral line units (Bleckmann 1994). In addition, the threshold of individual primary lateral line afferents vary by at least 20 dB (Coombs and Fay 1993; Bleckmann and Mohr 1998), thus stimulus amplitude might also be encoded by recruiting more and more of the higher threshold fibers. A third parameter which might be useful for amplitude discrimination is phase locking since phase locking increases with increasing stimulus amplitude until saturation was reached (e.g. Topp 1983; Bleckmann and Mohr 1998).

In posterior lateral line nerve fibres of goldfish both the degree of phase-locking and the discharge rates increased with increasing displacement amplitude of the vibrating sphere. At stimulus levels just above threshold, units respond to a stimulus with a modulation of ongoing discharge rate, i.e., they exhibit phase-locking without substantially increasing their discharge rate. With increasing displacement amplitude, the degree of phase-locking increased and reached a plateau at stimulus levels about 20 dB above threshold. In many units, the degree of phase-locking decreased at higher stimulus levels (Mogdans and Bleckmann 1999).

The results of the present study show that the discharge rates and the degree of phase-locking of MON units also may increase with increasing displacement amplitude of the sphere. Thus the information about the stimulus amplitude is preserved at the level of the MON. In many MON units the degree of phase-locking reached a plateau at stimulus levels

greater than 160 µm and even decreased at higher stimulus levels, due to the fact that these units started to discharge more than one spike per stimulus cycle. In terms of sphere vibration amplitudes, thresholds of the units varied between 25 µm and 250 µm. Most level response functions were steep, i.e., discharge rates increased over a narrow range of displacements (50 µm to 175 µm). In addition most MON units were saturating, i.e., discharge rates reached a plateau, but in the peripheral lateral line most units were non-saturating, i.e., discharge rates did not reach a plateau (maximum vibration amplitudes tested at a CF of 50 Hz were 1250 µm and 950 µm) (Mogdans and Bleckmann 1999).

4.6 Comparison with electrosensory units

The responses of type O units of *Eigenmannia* to an increase in stimulus intensity are phasic-tonic. To a stepwise increase of intensity, the units respond with a peak of activity that decays to a steady state with a time constant of about 3 sec. When the phasic part of the response reaches saturation (at about -23 db) the tonic firing level still increases with increasing intensity. The phasic peak now has a rise time of several seconds, behaving as though depressed. The time constant of decay is also much longer (about 15-20 sec) at high intensities (-20 to -10 db). Finally, a strong suppression of the response occurs, reaching zero activity at -10 db. This is also demonstrated by stimulating *Eigenmannia* with linearly increasing amplitudes up to -15 db (Thompson and Spencer 1966).

IV. Discussion

The JAR of *Eigenmannia* is continuously graded, not only with stimulus frequency but also with intensity, i.e. voltage gradient of the stimulus, over a wide dynamic range. It is non-habituating. After a long rest period the first response may be up to 50% larger than subsequent (Thompson and Spencer 1966). Due to the suppression of activity at high intensities the number of peaks at multiples of the stimulus interval increases. The dynamic range of intensity between threshold and maximal stimulus amplitude is about 1:40 in gymnarchids and 1:100 in gymnotoids (Bullock et al. 1975).

4.7 Histology

The MON is the principal first-order lateral line nucleus found in the majority of anamniotic vertebrates (New et al. 1996). This study confirms that lateral line information is processed in the MON. In addition the study shows that many MON units are especially sensitive to amplitude modulations, i.e. the sensitivity to amplitude modulations already emerges at the level of the medulla. Further studies are needed to uncover the biological significance of amplitude modulation sensitivity.

5. Summary

This study investigated the responses of MON units to hydrodynamic stimuli. In 41 experiments sinusoidal water motion was applied by a stationary vibrating sphere. In all experimental trials, the evoked spike time profile produced by constant frequency stimuli (33, 50, 100, 200Hz) was compared with the profile produced when the same stimuli were amplitude modulated. A total of 116 units were recorded using indium electrodes or glass pipettes.

If stimulated with single frequency water motions, two types of units could be distinguished. Type 1 units showed phasic responses and fired only a few action potentials at the beginning of the stimulus. Type 2 units showed a sustained discharge, and in some units responses were especially pronounced if high- frequency stimuli were applied.

The majority of MON units had a best-frequency (highest number of spikes per stimulus) at 100 Hz (45%), followed by 200 Hz (42%), 50 Hz (13%), and 33 Hz (11%). Units showed heterogeneous tuning characteristics including low-pass, band-pass and high-pass qualities. However, in most MON units tuning was weak or absent.

The responses of MON units to amplitude modulated sine wave stimuli were characterized by a burst of discharge to each modulation cycle. In most units the response profile was phasic to constant amplitude pure

sine wave stimuli, but changed to tonic for amplitude modulated pure tone stimuli.

When modulation depth was maximal (96%), units responded to each modulation cycle with about the same number of spikes. When modulation depth was less than maximal, units tended to discharge with a decreasing number of spikes to increasing numbers of AM cycles. When modulation depth was 24% or smaller, discharge patterns resembled the responses to unmodulated CFs, i.e. units responded with an on-response to the first AM cycle but responded only weakly to successive AM cycles.

Level-response functions obtained with pure sine wave stimuli exhibited saturation in most units (Figs. 16-19), i.e. discharge rates reached a plateau at higher stimulus amplitude. In some units level response functions were steep, i.e., in these units discharge rates increased over a narrow range of displacements (50 μm to 160 μm).

Level-response functions obtained with amplitude modulated stimuli exhibited saturating level-response functions in all 42 units (e.g., Figs. 20-23), i.e. discharge rates reached a plateau at higher stimulus amplitudes. At stimulus levels just above threshold, units responded to the stimulus with a modulation of ongoing discharge rate, i.e., they exhibited phase-locking without substantially increasing discharge rate. With increasing

displacement amplitude, the degree of phase-locking increased and reached a plateau at stimulus levels of about 200-250µm.

Lateral line units in the MON of goldfish exhibited phasic or tonic responses to constant-amplitude stimuli. When stimulated with amplitude-modulated stimuli, most MON units exhibited phasic-tonic or tonic responses that were phase-locked to the modulation frequency. On average, phase-locking to the modulation frequency was greater than phase-locking to the carrier frequency. Thus, MON responses to amplitude-modulated sine wave stimuli differed from those of afferent fibres in the posterior lateral line nerve (strong phase-coupling to both carrier and modulation frequency) and were similar to those of midbrain lateral line units (strong phase-coupling only to the modulation frequency). Our data suggest that at least some of the response characteristics of midbrain units can be explained by the properties of units in the lateral line brainstem.

Lateral line units in the MON of goldfish increased their discharge rates and the degree of phase-locking with increasing displacement amplitudes of the vibrating sphere.

In many units the degree of phase-locking reached a plateau at stimulus

levels greater than 160 µm and decreased at higher stimulus levels due to the fact that units started to discharge more than one spike per stimulus cycle.

Most level response functions were steep, i.e., discharge rates increased over a narrow range of displacements (50 µm to 175 µm).

Goldfish medullary lateral line units encode a wide range of stimulus displacements.

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Education

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Professional experience

- 1990-1992 Secreteriat of Committee for Marine Wealth, Elbeida, Libya
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Patents and publications

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Conferences

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