Miscellaneous features of electroreceptors in *Gnathonemus petersii* (Günther, 1862) (Pisces, Teleostei, Mormyriformes)

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ABSTRACT. The cutaneous electrical activity of ampullary and tuberous electroreceptor organs of *Gnathonemus petersii* (Teleostei, Mormyriformes) was studied in seven specimens. The spontaneous activity levels of 38 ampullary primary afferents showed significant differences between individual fishes rather than between different loci within a single specimen. Spontaneous activity of ampullary electroreceptor organs is not constant, but depends on some unknown factor, e.g. occlusion of the ampullary opening or local accumulation of ions. Both under Saffan and Hypnodil anesthesia it proved possible to record ampullary primary afferent activity from anesthetized specimens. Ampullary organ activity is modulated by Electric Organ Discharges and by respiratory potentials. Respiratory potentials also modulate the cutaneous electrical activity of mormyromasts. The ion composition of the water influences the cutaneous electrical oscillations of the Knollen organs.

KEY WORDS : Ampullary electroreceptor organ, conductivity, electroreception, *Gnathonemus petersii*, Knollenorgan, mormyromast, tuberous organ.

INTRODUCTION

Electroreceptor organs of electrosensitive fish are usually divided into several categories with different functions or properties : tuberous and ampullary organs (MOLLER, 1995; KRAMER 1996).Tuberous organs are used for the detection of electric high-frequency (300<f<10000 Hz) fields caused by Electric Organ Discharges (EOD's). Within the group of tuberous organs the Knollenorgans of the mormyriformes are used for the detection of electric fields caused by EOD's of other fish, whereas the mormyromasts are used for the detection of distortions of the electric fields caused by the fish's own EOD. Ampullary electrosensory organs, on the other hand, are used for the detection of low-frequency (0.1<f<100 Hz) electric fields not caused by EOD's. These fields emanate from a variety of sources including all aquatic organisms and bottom structures of the environment (KALMIJN, 1972). In the marine environment ampullary organs are centimetre-long jelly-filled canals, the so called ampullae of Lorenzini. In the freshwater environment ampullary organs are only small skin-deep invaginations called microampullae. The present paper deals with freshwater ampullary and tuberous organs.

Earlier studies on catfish ampullary electroreceptor organs in *Clarias gariepinus* revealed that the level of spontaneous activity is related to the topology of the receptor organs. Ventral ampullary organs have lower resting discharges than dorsal ones. Not much is known about the cause of this phenomenon (PETERS & MAST, 1983). Originally it was thought that the firing rate of ampullary organs was related to the number of receptor cells that converged to the same single primary afferent. However, this was not the case (PETERS & MAST, 1983; PETERS et al., 1997; TEUNIS et al., 1990). It might very well be that, for instance, the type of innervation somehow determines the resting discharge rate.

The aim of the present pilot study was to investigate if, in *Gnathonemus* ampullary organs, a similar place-related level of spontaneous activity could be found. In addition some cutaneous recordings of non-ampullary, tuberous organs were made.

MATERIAL AND METHODS

The experiments were performed on seven specimens of *Gnathonemus petersii*, varying in length from 11 to 18 cm (TL), individually kept in full glass containers of about 20 l in the aquarium of the LPN-CNRS, Gif-sur-Yvette France. The temperature was kept between 21 and 25 ºC. The composition of the (Paris tap) water was, in mM : Mg 0.132, Si 0.099, Na 0.826, Ca 1.62, K 0.084, P 0.0, S 0.274, Zn 0.015 (data provided by M. Veron). The conductivity of the tap water was 331 ± 12 µS/cm at 25 ºC and pH 6.3. That of the tank water was 422 µS/cm at 25 ºC and pH 5.8.

Fish were first anesthetized by immersion in Saffan (Serum- und Impfinstitut, Basel) 2 mg/l for induction, and 1.5 mg/l for maintenance of narcosis. Then they were transferred to a 31 x11.5 x 5 cm perspex tray for mechanical fixation and artificial respiration at 30 to 100 ml/min. Electrical conductivity of the circulating water (taken from the stock tank) including the anesthetic was 422 µS/ cm. On two occasions Hypnodil was used as an anesthetic

in a concentration of 30 mg/l for induction, and 5 mg/l for maintenance. For local application of water with a different ion composition to the skin, a ring of putty, inner diameter 1 cm, was placed on the skin.

Recording the activity of an electroreceptor organ was done with tungsten needle electrodes, insulated apart from the tip, and a PAR 113B amplifier with a TEAC cassette recorder. Tuberous organs were recorded at a bandwidth of $300 < f < 10.000$ Hz; ampullary organs were recorded at $300 < f < 3000$ Hz. The ground electrode was placed near the tail of the fish.

In order to study the effect of ion composition of water on electroreceptor organ functioning, three solutions were made with equal conductivity but with different ion contents. We used either normal local tap water, or diluted KCl, or diluted fish Ringer's (WOLF, 1963). Conductivity of water was 331 ± 12 µS/cm at 24.8 °C and pH 6.2. The conductivity of the isoconductive KC-solution was 342 ± 11 at 21 °C and pH 6.3. The conductivity of the isoconductive Ringer's solution was 335 ± 14 µS/cm at 21 °C and pH 6.4.

In order to visualize electroreceptor organs, we incidentally applied a 1% neutral red solution in tap water in the ring on the skin for some minutes.

Differences between dorsally and ventrally situated ampullary organs in two specimens were tested with Kruskal-Wallis non-parametric test for independent samples.

RESULTS

Recognition of electroreceptor organs

It proved rather difficult to identify beyond doubt the three classes of electroreceptor organs by means of visual inspection and electrophysiological recording. It is true that there were small, large and intermediate organs, and that the large organs or Knollenorgans were pinkish as described by earlier investigators (BENNETT, 1965; BENNETT, 1967; SZABO, 1974; SZABO & FESSARD, 1974), but to the inexperienced eye there was an overlap between the classes. According to Jean-Pierre Dénizot (LPN-CNRS) application of the dye toluidine blue stains the ampullary organs blue and the tuberous organs reddish, but we refrained from applying this dye because we feared that the stain would affect functioning. It is also true that ampullary organs give 'regular' spontaneous activities with more bass components than do the tuberous organs when the recordings are made audible via a speaker. The sound from tuberous organs is of a higher frequency. Moreover they tend to oscillate after being touched by an electrode, or if the water height and water composition changes, or if the resistive load on the organs changes.

At the start of the experiment it was very difficult to find functioning ampullary organs. The candidates, the small organs, did not yield spikes. Not even after administering distilled water, which increases the amplitude of the recorded spikes, could activity be recorded (Fig. 1). Eventually we discovered that mechanically opening the ampulla pore with the electrode tip made recording possible.
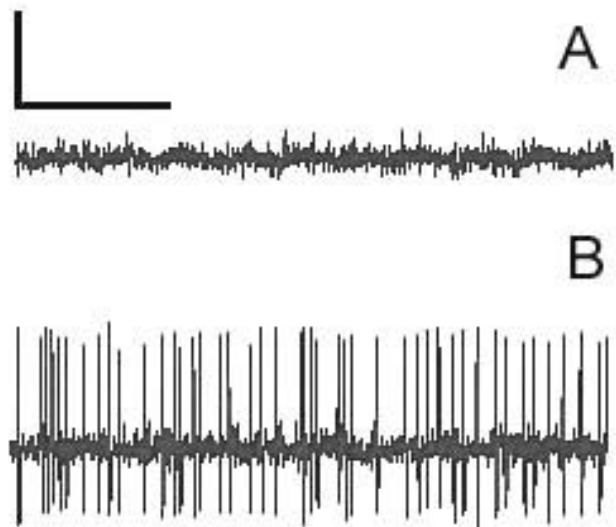

Fig. 1. – Demonstration of the lack of response in ampullary electroreceptor organs, compared to the cutaneous activity recorded from a Knollenorgan. A : Cutaneous electrical activity of an ampullary organ submersed in distilled water. B : Cutaneous electrical activity of a Knollenorgan submersed in distilled water. Scale markers : 100 µV, 50 ms.
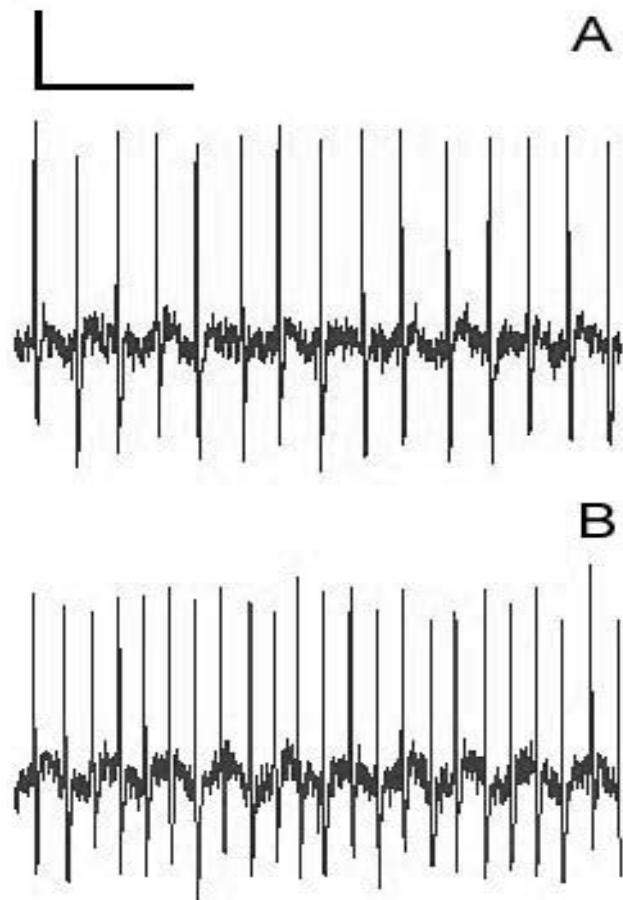

Fig. 2. – Cutaneous activity recorded from ampullary electroreceptor organs in specimens of *Gnathonemus* anesthetized with Saffan (A) 1.5 mg/l, and Hypnodil (B) 5 mg/l. Scale markers : 100 µV, 50 ms.

Anesthetics : Saffan and Hypnodil

Both under Hypnodil- and Saffan anesthesia it proved possible to record activity from the pores of the ampullary organs (Fig. 2). The effect of both anesthetics on sensory function was not rigorously investigated. Both under Hypnodil and Saffan anesthesia the Electric Organ Discharges (EOD) continued to some extent. Most likely the depth of anesthesia plays a part here.

Recording of ampullary organs

Probing with the electrode into the ampulla pore resulted in a high frequency series of spikes, Once 'opened', the ampulla continued to fire until, after some minutes, it sometimes stopped firing, but the recorded spikes did not change in amplitude. An example of such a recording is given in Fig. 3. The spontaneous activity of 38 ampullae was recorded at six spots, in two fishes. The sites were dorsally and ventrally between the dorsal fin and snout, and on the snout. The Kruskal-Wallis test revealed no differences between dorsally- and ventrally-situated ampullary organs (p=0.92), but a significant difference between the two specimens (p=0.003). Average interspike interval lengths of more than 800 intervals for each ampulla are given in table 1. Further, the ampullary organs responded to ventilatory movements of the operculum, if the fish recovered from anesthesia (Fig. 4). Also the EODs caused strong modulations of the spontaneous activity (cf. BELL & RUSSELL, 1978)(Fig. 5).

Fig. 4. – Cutaneous activity recorded from an ampullary electroreceptor organ, modulated by ventilatory potentials. The whole recording lasts about .9 s, and corresponds roughly to 2/3 of a respiratory cycle. *Gnathonemus* recovering from affan anesthesia. Scale marker : 100μ.
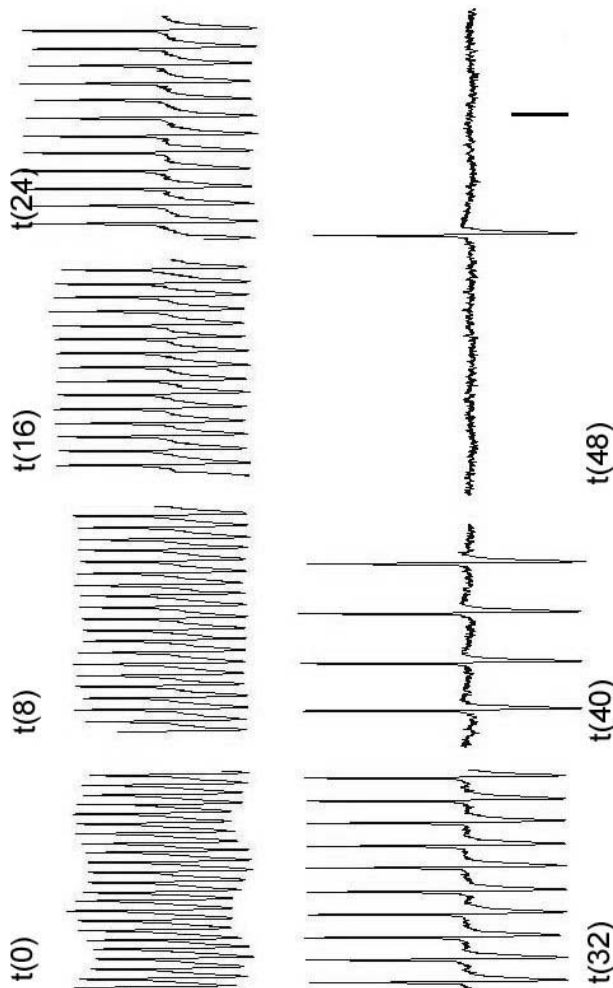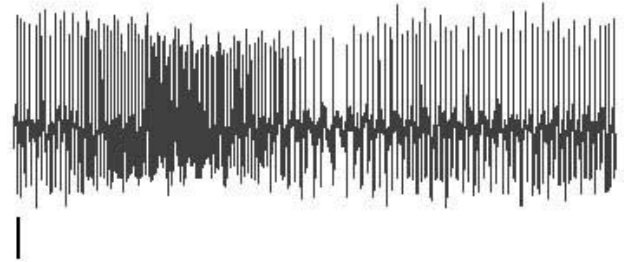

Fig. 3. – Cutaneous activity recorded from an ampullary electroreceptor organ after opening its pore with a tungsten needle electrode. The blocks each represent 50 ms recordings, made every 8 s. Before opening the pore the ampullary organ is 'silent'. After opening it starts with a high frequency spontaneous activity that slows down and eventually stops. The spikes remain large, indicating that the ampullary pore is still open. Scale marker : 200 μV.
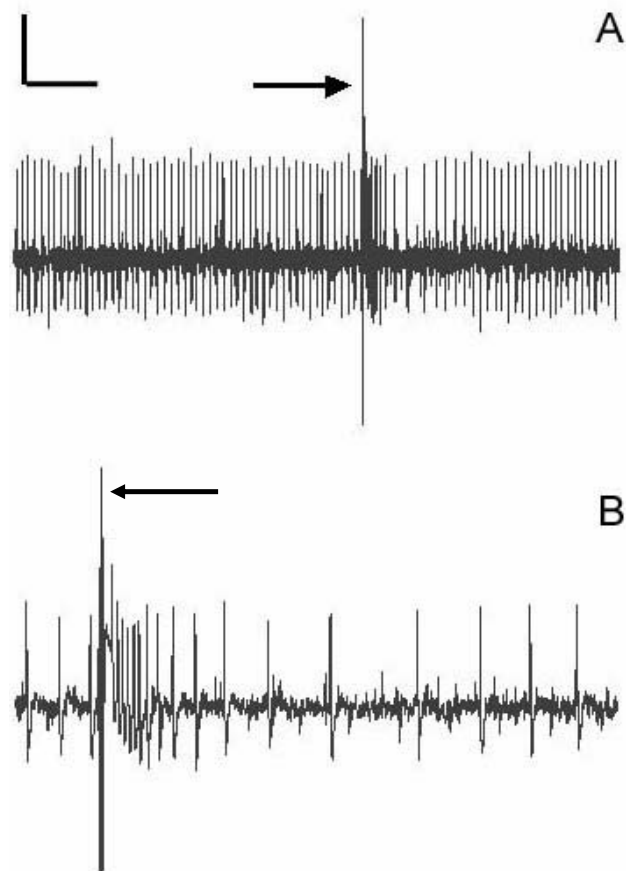

Fig. 5. – Cutaneous activity recorded from an ampullary electroreceptor organ, modulated by an Electric Organ Discharge, while *Gnathonemus* is recovering from Hypnodil anesthesia. Scale markers : 200 μV, 150 ms (A), and 30 ms (B). Arrow : Electric Organ Discharge.

TABLE 1

Average interspike intervals of spontaneous activity of ampullary electroreceptor organs in *Gnathonemus petersii*

Ampullae, Spontaneous activity	Average interspike intervals of n ampullary organs in fish 1 (ms)	Average interspike intervals of n ampullary organs in fish 2 (ms)
Dorsal	15 ± 7 S.D. (n = 8)	9 ± 6 S.D. (n = 6)
Ventral	13 ± 4 S.D. (n = 9)	9 ± 5 S.D. (n = 15)

Recording of tuberous organs, i.c. Knollenorgans

The resistive load, i.e. the conductivity of the water, determines the amplitude of the recorded cutaneous signals. After administration of a Ringer's solution in a putty ring, no spikes could be recorded, whereas after administration of distilled water the amplitude of the spikes increased about tenfold (Fig. 6).
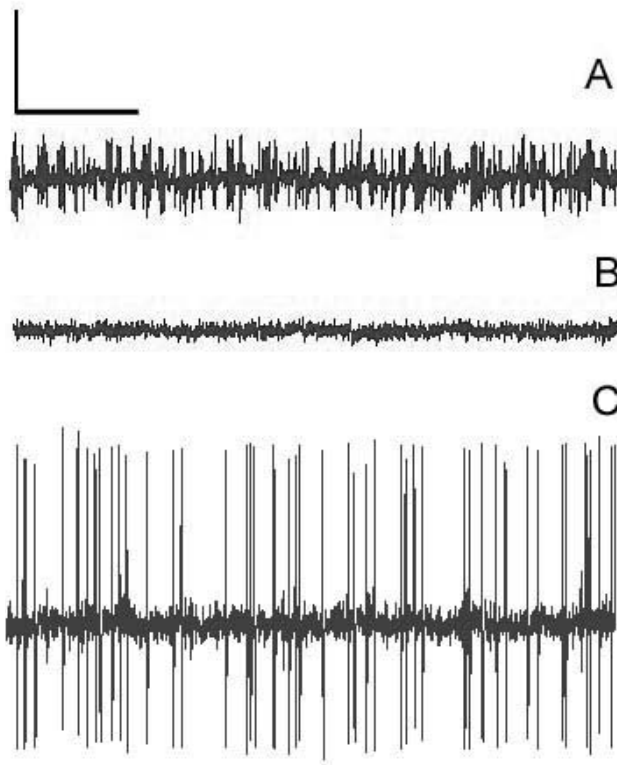

Fig. 6. – Cutaneous activity recorded from a Knollenorgan in three media with different electrical conductivities. A : tap water; B : Ringer's solution; C : Distilled water. Scale markers : 200 µV, 200 ms.

Touching the Knollenorgans with an electrode can evoke ringing i.e. oscillations. We found it was not necessary to dry the skin as mentioned by previous authors (BENNETT, 1965 ; BENNETT, 1967 ; SZABO, 1962 ; SZABO, 1963 ; SZABO & FESSARD, 1965). Even with 3 cm of water over the skin, ringing could be recorded at 1 cm distance from the skin. Administration of 1 % neutral red on the skin and subsequent rinsing resulted in patches of brown precipitate more or less over the tuberous organs (Fig. 7).

Since earlier studies in catfish ampullary electroreceptors revealed that they are very sensitive to changes in the water composition (ROTH, 1971), we also tested how the ion composition of the water influenced Knollerorgan functioning. We therefore changed the water within a circular ring placed on the skin around an organ. We took care to match the electrical conductivities. Surprisingly the water composition changed the oscillations of the Knollenorgans (Fig. 8). When normal tap water was administered the 'ringing' activity disappeared, whereas it reappeared when Ringer's or KCl solutions were applied. Further respiratory movements proved to modulate the firing or oscillation frequency of tuberous organs, i.e. mormyromasts (Fig. 9).

DISCUSSION

Anesthesia

Both Saffan and Hypnodil were satisfactory anesthetics for recording the cutaneous activity of the electroreceptor organs, apparently because electroreceptor organs do not contain GABA membrane receptors, which are the target of both anesthetics (PETERS et al., 2001 ; GRANT et al., 1999).

Ampullary organs

The recordability of the ampullary activity seems to depend on the 'openness' or 'closedness' of the pores. In freshwater catfish this phenomenon is not seen to this extent. The course of the frequency change (Fig. 3) demonstrates that it is not the openness of the ampulla per se that influences recordability. The end of the recording in Fig. 3 shows that the amplitude of the spike remains large, whereas the frequency drops. So the electrical recording condition did not change. It is not clear how this phenomenon should be interpreted. Perhaps the spontaneous activity of the ampullary organs depends somehow on the water composition. The stock tank water must have been rather polluted since its resistivity amounted to 442 µS/cm versus Paris tapwater 332 µS/cm. Earlier it was demonstrated that the water composition influences both spontaneous activity and evoked activity in catfish ampullary organs (ROTH, 1971 ; PETERS & WESTERINK, 1999). The spatially related differences of spontaneous activity found in catfish ampullary organs were not seen in *Gnathonemus*.

Knollenorgans and mormyromasts

The Knollen organs and mormyromasts behaved as described by the early pioneers (BENNETT, 1965 ; BENNETT, 1967 ; SZABO, 1962 ; SZABO, 1963 ; SZABO & FESSARD, 1965 ; SZABO, 1967 ; SZABO, 1974 ; SZABO & FESSARD, 1974). Conspicuous is perhaps that respiratory movements, and consequently respiratory potentials (cf. KALMIJN, 1972), modulate the activity of mormyromasts. Further the composition of the surrounding water also has a strong effect on the cutaneous activity of Knollenorgans. It is possible that the diffusion potentials (liquid junction potentials) of the different fluids modulate the activity. On the other hand it is likely that the ionic composition somehow influences the electrochemical homeostasis of the receptor cells and thus causes ringing. It is generally thought that the path from receptor cells to the
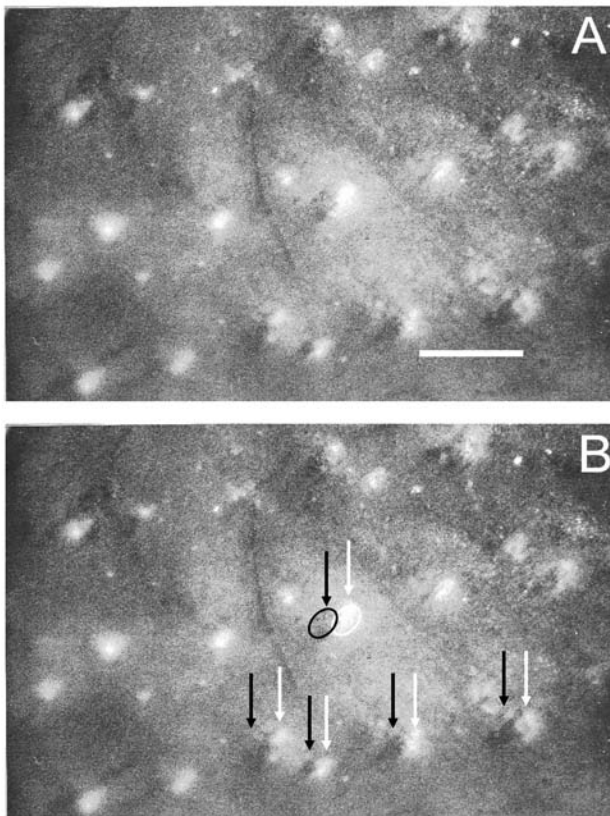
Fig. 7. – Photograph of tuberous electroreceptor organs after exposure to 1% neutral red solution. A : Plain photograph. B : Photograph with markers. Note the dark spots representing pre-cipitated neutral red (black oval, black arrow) covering half of the unpigmented electroreceptor organs (white oval, white arrow). Scale marker : 1mm.
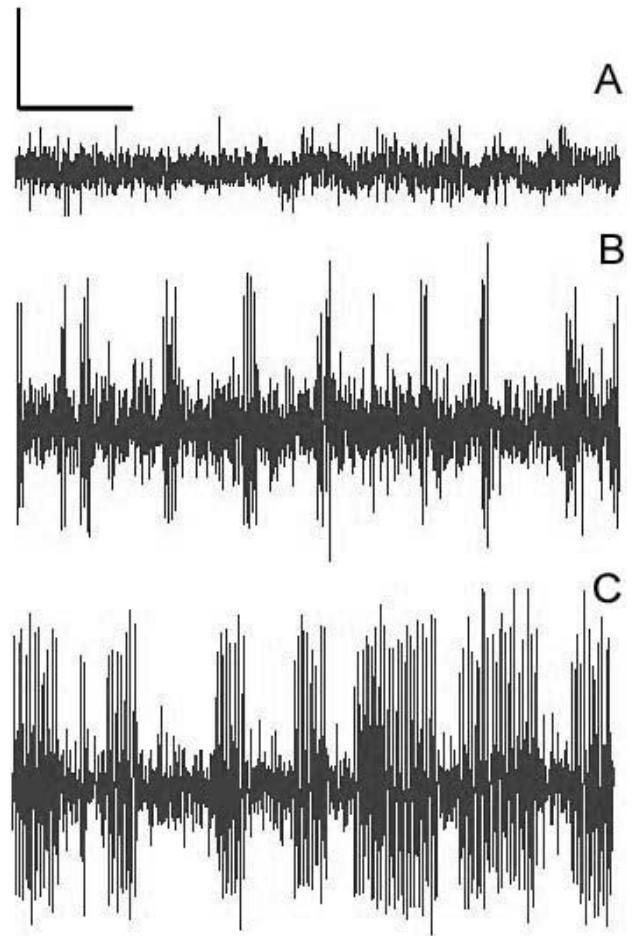

Fig. 8. – Cutaneous activity recorded from a Knollenorgan-gan, in 3 solutions with equal conductivities but with different ionic compositions. A : normal water. B : Ringer's solution. D : Diluted KCl. Conductivity of all solutions 335 ± 14 µS/cm. Scale markers : 50 µV, 50 ms.
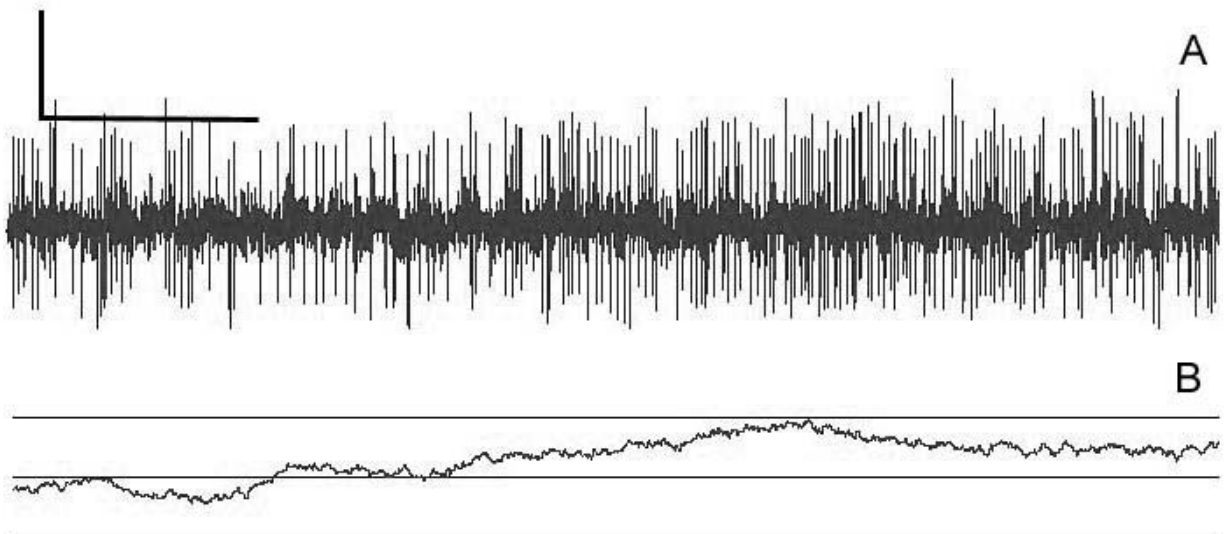

Fig. 9. – Cutaneous activity recorded from a mormyromast, modulated by ventilatory potentials. The right half of the recording shows an increased activity with respect to the left half. A : cutaneous recording. B : 'integrated signal' as running average (n=3000) of the absolute values of A. The whole recording lasts about .6 s, and corresponds to less than a respiratory cycle. Scale markers 50 µV/cm, 100 ms.

outer world is blocked by a capacitor, such as cell membranes, but there are also indications for a resistive path. External application of a 1% neutral red solution to the skin causes brown precipitates near the plug of the tuberous organ (Fig. 7). Such precipitates are, among other things, caused by high concentrations of ions (unpublished tests). This could mean that the plug of the tuberous organ is much more leaky than thought hitherto. In that case the ionic composition could influence the functioning of the tuberous organs either by liquid junction potentials or by the ion-mix.

Conclusion

We conclude from the data mentioned above that there is no position-related difference in the spontaneous afferent rate of the ampullary organs, and that the water composition plays an important part in the functioning of both ampullary and tuberous receptor organs.

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