

to pinocytose or by taking part in a common response to stimuli initiated by other inducers.

Resumen. El transporte de albumina en el intestino delgado del cerdo esta asociado con un aumento en el transporte de fluido. Ambos transportes son inhibidos por altas concentraciones de calcio. Ausencia de calcio tambien inhibe el transporte de albumina. Condiciones optimas en el

transporte de albumina y fluido se obtienen usando Cl_2Ca 4 mM en el medio. Se deducen analogias al comparar estos resultados con resultados similares en ameba, obtenidos de la literatura.

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High Electrical Discharge Frequency During Aggressive Behaviour in a Mormyrid Fish, *Gnathonemus petersii*

In the mormyrid fish *Gnathonemus* the rate of the electric organ discharge is variable¹⁻⁵. As a large number of investigations indicate, following different kinds of stimulation⁶⁻⁸ and even during periods of aggressive behaviour^{4, 9, 10}, maximal frequency never exceeded 50–60 Hz. The only observation where mormyrids emitted higher frequencies was made by LISSMANN¹, who mentioned for a mechanically stimulated *Gnathonemus senegalensis* emission rates of 130 Hz. The present paper furnishes evidence that *Gnathonemus petersii* emits continuously 'high frequency' bursts of up to 140 Hz during aggressive behaviour. This frequency surpasses the frequency range observed in resting and swimming fish.

Eight *Gnathonemus petersii* (16–12.5 cm; peak to peak voltage, measured in water, about 1.4–2.5 V¹¹; duration of fish pulse 300 μsec) were held each for several days in 150 l glass aquarium at 26–27.5°C, into which *Mormyrus rume* (20.5 cm; 6.0 V¹¹; 750 μsec) was introduced each time for 3–5 min. The attacks were filmed on a video equipment (SONY). A system of 3 pairs of carbon electrodes, oriented vertically and horizontally, was used to pick up all electrical pulses of the fish¹². Each pair of the 3 pairs of carbon electrodes was connected to one preampli-

fier. Amplified pulses were rectified, the three signals summarized and fed into a tape recorder. To distinguish the discharges emitted by the 2 fish – characterized by different amplitude and duration – the tape was played back into an oscilloscope. The images on the screen were recorded on 35 mm film moving past the open shutter at 50 cm/sec. Tape recordings were also fed into a computer (Didac 800) which had been programmed to perform interval histograms and instantaneous frequency histograms. Each experiment was started with control recordings of each fish prior to the attacking behaviour. Results obtained in one fish (*G. petersii* No. 1) are described in detail.

During 10 min control *G. petersii* No. 1 discharged a mean frequency of 11 Hz. In Figure A a characteristic peak on the left side of the histogram of pulse intervals corresponds to spontaneous burst-like acceleration of the discharge frequency (mode: 26 ms \pm 38 Hz). The shortest interval observed during the entire control period was of 22 ms.

During the following 180 sec period of aggressive behaviour, the discharge pattern of *G. petersii* No. 1 changed characteristically. The mean frequency now increased to 41 Hz and *G. petersii* emitted long lasting 'high frequency' bursts (inset in Figure B). In each of the four 180 sec periods of experiment 38–47 'high frequency' bursts occurred. The bursts displayed 2 kinds of pattern: in one the low frequency interburst activity (a in inset of Figure B) was followed by a particular pattern, in which intervals of 8 and 15 msec regularly alternated; (b in inset of Figure B), the final part of these bursts was characterized by a constant frequency of 117 Hz; (c in inset of Figure B). The second kind of pattern was similar to the part b) of the first kind of pattern (see inset in Figure B, end of preceding burst before a). In both cases the bursts ended abruptly (arrows in inset of Figure B), each one being separated from the other by an interburst activity characterized by several long intervals up to 806 msec.

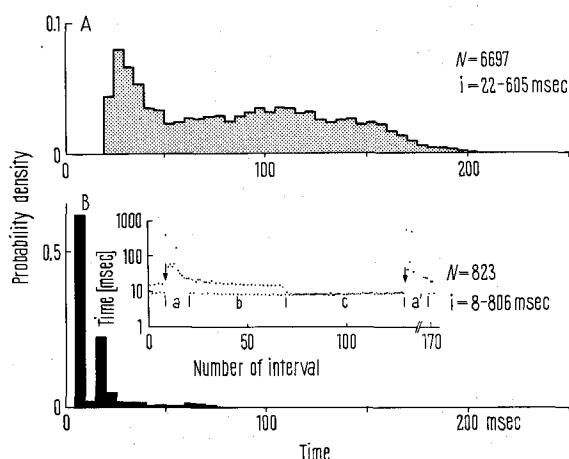


Fig. 1. Interval histograms of electrical pulses of *G. petersii* No. 1. A) control, $T = 10$ min. B) aggressive behaviour, $T = 20$ sec. N , number of intervals; i , shortest and longest interval. Probability density of intervals plotted against intervals duration. inset in B). 'High frequency' bursts of *G. petersii* No. 1 during aggressive behaviour. Interval (ms) plotted against number of interval. a) and a') Inter-burst activity. b) and c) Burst with 2 types of activity (see text). Duration of b) 535 msec, of c) 692 msec. Arrows, end of bursts.

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Figure B indicates that the attacking fish displayed intervals (modes: 9 and 15 msec = 111 and 67 Hz) shorter than the shortest interval observed during control.

The remaining seven *G. petersii* displayed a similar pattern of 'high frequency' burst activity during aggressive behaviour: the burst frequency (75 to 140 Hz) always surpassed the highest value of instantaneous frequency observed in the resting fish (50 to 66 Hz).

These 'high frequency' bursts appeared only when *Gnathonemus petersii* attacked the intruder. If a parallel or anti-parallel lateral display followed this attack, bursting of discharges continued and the bursts, now, were of longer duration. Bursting ended when *G. petersii* retreated.

In the literature the only 'high frequency' bursts (130 Hz) in mormyrids were indicated by LISSMANN¹ for a mechanically stimulated *Gnathonemus senegalensis*. In contrast, in our experiments continuous 'high frequency' bursts (up to 140 Hz) of fighting *G. petersii* were recorded during long periods of attack, whereas in 14 h of control the same resting fish never emitted instantaneous frequencies higher than 71 Hz. Also, swimming *G. petersii* never discharged frequencies exceeding 30 Hz⁴.

The significance of this particular discharge pattern, described for the first time for *G. petersii*, will be the subject of further observations.

Zusammenfassung. Der elektrische Fisch *Gnathonemus petersii* sendet beim Angriff auf einen anderen Mormyriden, *Mormyrus rume*, und während des Breitseitimpotenzierens «burst»-artige elektrische Entladungen von Frequenzen bis zu 140 Hz aus.

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A Method for Distinguishing Tetrodotoxin from Saxitoxin, by Comparing Their Relative Stabilities when Heated in Acid Solution

Tetrodotoxin (TTX) occurs naturally in puffer fish of the order Tetraodontidae and in newts within the genus *Taricha*. Saxitoxin (STX) is produced by marine dinoflagellates of the genus *Gonyaulax*, and is also found in many species of bivalve molluscs after they have been feeding on these dinoflagellates¹⁻³. The newts and puffer fish are unaffected by TTX; bivalves are not poisoned by TTX or STX. Most vertebrates, however, are paralysed by either toxin in doses of 5–20 µg/kg i.v. or i.p. Since these toxins have virtually the same mode of action they are very difficult to distinguish by means of their pharmacological effects^{2,3}. As no specific chemical tests have been discovered for either poison, identification of an unknown poison having these pharmacological effects is not easy⁴. The nerves of *Taricha* newts resist TTX but are blocked by STX⁵. TTX is not found in European newts and their nerves are not resistant^{6,7}.

Several remarks in the literature imply that STX is stable in strong acids, whereas TTX is most stable near pH 4 to 5^{2,8-14}. The poisons were therefore tested for stability at various pH values, diluting solutions of pure TTX and STX to concentrations equal to 8 mouse units/ml. The mouse LD₅₀ (1 mouse unit, MU) for TTX was found to be 0.24 µg/20 g body weight, so the TTX solution was made up to 1.92 µg/ml. The corresponding figures for STX were 0.206 µg/20 g and 1.65 µg/ml. The diluent was either HCl or 0.025 M sodium acetate/HCl buffer, depending upon the desired pH. The ability of the poisons to withstand heating to 100°C, for various times between 2.5 and 60 min, was tested at pH 0.64, 1.0, 1.28, 2.0, 3.0, 4.0 and 5.0. Portions of 4 to 5 ml were pipetted into conical centrifuge tubes. One tube was left unheated as a control and the others were placed in a boiling water bath for 2.5, 5, 7.5, 10, 20, 40 or 60 min, then removed and quickly cooled. The samples were brought to pH 2.5–3.5 with HCl or NaOH before bio-assay on mice, and loss of water by evaporation was corrected.

The amount of poison remaining in each tube after heating was estimated by a mouse death time technique similar to that described by SCHANTZ, McFARREN and their colleagues¹⁵⁻¹⁸. The samples were assayed on groups of 3–14 mice. Almost all the points shown in the Figure were estimated using groups of 12–14 mice. The volume of solution injected i.p. was 0.5 ml/20 g body weight. Therefore, if no destruction of poison had occurred in the sample, each mouse received 4 MU of poison. This amount of TTX would be expected to kill mice after a median time to death of 4 min; 4 MU of STX would produce a median time of about 3 min¹⁷. The unheated controls gave median times to death that did not differ significantly from these figures, when corrected for the presence of sodium

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