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DENERVATION REVEALS TWO COMPONENTS OF NEUROTRANSMISSION IN ELECTRORECEPTOR SYNAPSES

R. C. PETERS,* E. L. GROEN, M. M. VAN DER SLUIS, P. F. M. TEUNIS and K. A. WILHELM
Laboratory of Comparative Physiology, University of Utrecht, Jan van Galenstraat 40, 3572 LA Utrecht, The Netherlands

Abstract—Denervation-induced changes in the synaptic efficacy of single electroreceptors in catfish (*Ictalurus nebulosus*, Teleostei) were studied *in vivo* under alfadolone anaesthesia. At 16°C the following effects were found 48 h post-operatively: (1) the average amplitude of the extra-dermally recorded spikes decreased from 100 to less than 20 μV; (2) the average resting discharge decreased from 40 to less than 25 spikes/s; (3) neither the sensitivity nor the frequency characteristic changed.

The results indicate that the resting discharge and the modulation mechanism of sensory synapses are controlled by different biochemical mechanisms. The resting discharge seems to be related to the trophic function of the afferent nerve and to its generator region, whereas the modulation mechanism is apparently associated with the receptor cell.

Stimulus transduction in teleost ampullary electroreceptors is thought to be mediated by the following processes. The stimulus, an electric potential difference over the receptor, causes the basal faces of the receptor cells to depolarize or hyperpolarize, which in turn modulates voltage-sensitive Ca-channels in the basal membranes. The Ca influx through these channels controls the release of a chemical transmitter, which depolarizes the generator region of the afferent nerve fibre. In the absence of a stimulus, a spontaneous flow of neurotransmitter supports an afferent "resting discharge" which enhances the sensitivity of the receptor. A "bias-current" keeps the basal face depolarized, thus sustaining spontaneous transmitter release. In addition a Ca-controlled transient potassium current is believed to contribute to the stimulus transduction.[2,17] According to this concept the spontaneous transmitter release and the modulation of transmitter release share a common physiological basis.

Not all experiments support the hypothesis that the spontaneous afferent discharges and modulation of the transmitter release are based on the same mechanism. Spontaneous afferent discharges and modulation of neurotransmitter do not follow the same course if subjected to temperature changes,[10] nor are they correlated.[3,12] If the spontaneous afferent discharge is affected, the stimulus transduction does not always change (Ref. 14 and Peters R. C., unpublished observations).

Earlier studies showed how the sensory synapse can be influenced by denervation.[1,15,16]

The aim of the present experiments is to study electrophysiologically the effects of denervation on the functioning of the ampullary electroreceptors. By following the denervation-induced changes in both the stimulus transduction and the spontaneous afferent discharges we have collected new facts that help us to reduce the uncertainty in the model of the electroreceptor synapse.

EXPERIMENTAL PROCEDURES

Animals

Seventeen catfish, *Ictalurus nebulosus*, weighing 150–230 g were obtained from a fish farm. Before the experiments they were kept in full glass containers, vol. 250 l, in Utrecht tap water at 16°C (for ionic constituents see Ref. 13). They were fed on minced beef, trout pellets, and occasionally earth worms. During the electrophysiological measurements and surgery the fish were put in a small perspex tray, vol. 1.8 l, so they could be given artificial respiration (100 ml/min). Between the experiments they were transferred into the buffer tank of the respiration system, vol. 4.5 l, which also served as recovery chamber. The water temperature varied from 16°C during the electrophysiological recordings to 18°C between the recordings.

Anaesthetics

Before denervation the fish were briefly anaesthetized with tricaine (MS222), 1:2000 (Sandoz, Basel); denervation took place under MS222, 1:4000.

During the electrophysiological recordings the fish were anaesthetized with alfadolone (Saffan; Glaxovet, Harefield), 24 μg/g i.m.

Denervation

Electrosensory afferents of the anterior lateral line nerve which are concurrent with the r. ophthalmicus superficialis VII and innervate a small area between the two nasal openings were cut 5–10 mm caudal of the dorsal barbel. At this site the ophthalmicus superficialis VII crosses the opthalmicus superficialis V (Fig. 1).[9] After exposure and cutting of the nerve the wound was sutured with Ethicon 6-0 surgical.

Electrophysiology and signal processing

Single unit afferent activity was recorded extracellularly from the dorsal head region (cf. Fig. 1) by means of tungsten

*To whom correspondence should be addressed.
Abbreviation: FWHM, full width at half maximum.

microelectrodes.[1,2,4,6] The electroreceptors were stimulated with sinusoidal currents applied via a fork-shaped silver wire that was placed around the recording electrode, about 2 mm from the skin. In this way a local, uniform stimulus field was created in which currents of 1 nA delivered by the stimulus electrode corresponded with a current density of 0.5–1 nA/cm^2 at the body surface.[3,12] The stimulus frequencies varied between 1 and 3 Hz depending on the purpose of a particular experiment (see results). The recorded spike trains were processed into post-trigger time histograms (averaging) and interspike interval histograms by means of a desk top computer (Bimex, Laboratory of Comparative Physiology Software).

The sensitivity of the electroreceptors was expressed as the average modulation depth of the spike trains, as inferred from the post-trigger time histogram, divided by the stimulus strength (spikes per s/nA per cm^2). The average spike frequency was taken as a measure for the resting discharge. For the description of the interspike interval histograms we used the peak value (modus) and the full width at half maximum (FWHM).

Effects of denervation

To describe the effects of denervation we measured the sensitivity, frequency characteristic, spontaneous afferent discharge rate, and extracellularly recorded spike amplitude of the electroreceptors both before and after denervation. The non-denervated (contralateral) electroreceptors were used as controls. In addition the effects of sham denervation were tested. The effects of denervation were measured 1 day (18–24 h), 1.5 days (26–30 h), and 2 days (42–49 h) post-operatively.

Differences between the various parts of the experiments were tested statistically with the rank sum test or with the *t*-test.[5]

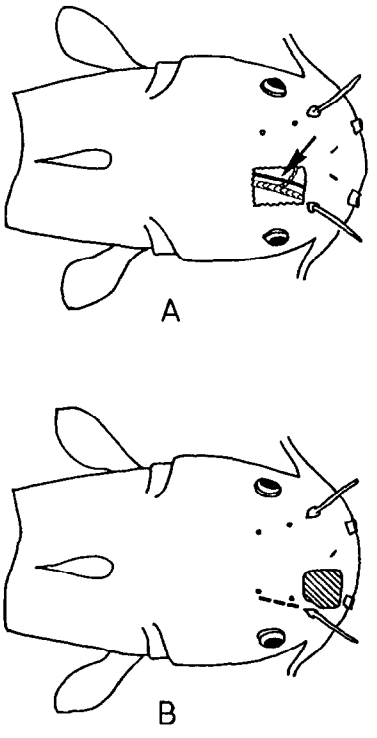

Fig. 1. *Ictalurus nebulosus* dorsal view. (A) Denervation site with n. ophth. sup. VII (black) and V exposed. The arrow points to the transection. (B) Denervated skin area (hatched) with microampullae.

RESULTS

Spike amplitude

Afferent nerve single unit signals recorded from the opening of the electroreceptor were usually of the order of 100 μV. The amplitude strongly depends on the position of the electrode with respect to the ampulla lumen (Fig. 2). To find out whether denervation affects the recorded spike amplitude, we always positioned the electrode in such a way as to maximize the recorded signal. The spike amplitude had not changed significantly 1 day post-operatively; 1.5 days post-operatively a significant difference was found. Two days post-operatively no spikes could be recorded (Fig. 3). These data were collected from 50 electroreceptors in three catfish.

Resting discharge

In the same 50 electroreceptors the average spontaneous activity calculated from the post-trigger time histogram was found to decrease post-operatively. In the controls, the resting discharge was 40 spikes/s whereas after denervation the spike rate decreased to 30 and 22 spikes/s on days 1 and 1.5, respectively. The latter change was statistically significant. After 2 days no spikes could be recorded (Fig. 4).

The inter-spike interval measurements of the same 50 receptors gave similar results. The modus shifted significantly from 22 to 40 ms 1.5 days post-operatively; the FWHM increased from 18 ms in the

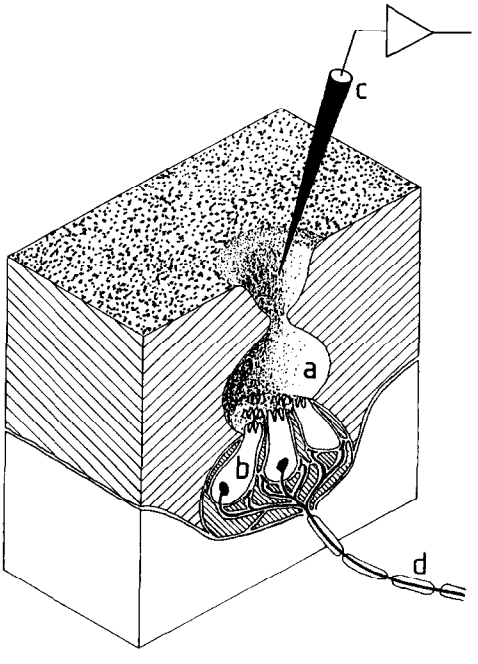

Fig. 2. Schematic drawing of a piece of skin with an electroreceptor showing the position of the recording electrode. The drawing was made after a scanning electron micrograph made by C. Eigenhuis and J. van der Linden. a, ampulla lumen; b, receptor cells; c, electrode; d, afferent fibre. Overall dimensions of the ampulla 150 μm.

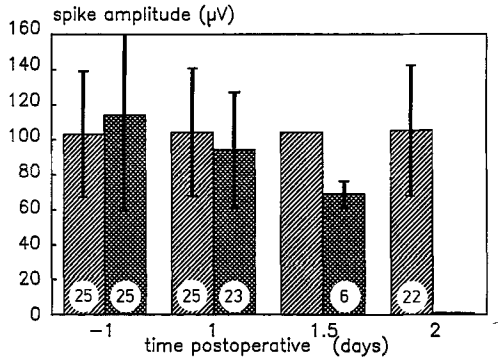

Fig. 3. Extradermal spike amplitude after denervation. The figures in the columns refer to the numbers of tested receptors. Hatched columns, controls; cross-hatched columns, post-operatives. No control measurements were performed at day 1.5; instead we took the average of the controls of day 1 and day 2. Bars are standard deviations.

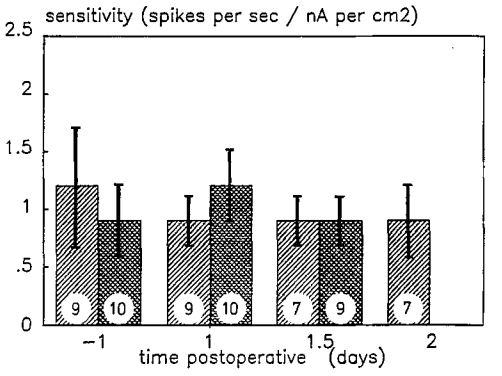

Fig. 5. Sensitivity at 10 Hz after denervation. The figures in the columns refer to the numbers of tested receptors. Hatched columns, controls; cross-hatched columns, post-operatives. Bars are standard deviations.

controls to 25 and 26 ms after 1 and 1.5 days post-operatively.

Sensitivity

The input–output curves were measured with a 10 Hz stimulus; the slope of the *I–O* curve was taken as "the sensitivity" of the receptor. For this part of the experiment 19 electroreceptors were measured in two fish; 10 of these were test receptors and nine were controls (Fig. 5). Particular attention was paid to possible changes in sensitivity during the decrease of the resting discharge. Hereto the behaviour of individual electroreceptors was followed. No significant changes in sensitivity were found after denervation.

Frequency characteristic

The frequency characteristic was determined in the linear part of the dynamic range. Because the measuring of a frequency characteristic took a relatively long time the results for 1 and 1.5 days post-operatively were pooled. Data of 15 receptors were collected in four fish; six of these receptors were controls. No significant changes could be found post-operatively (Fig. 6).

Sham experiments

In three fish sham operations were performed. Spike amplitude, spontaneous afferent discharge, sensitivities and frequency responses were determined

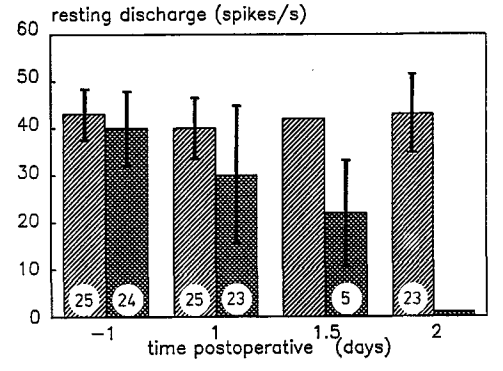

Fig. 4. Resting discharge after denervation. The figures in the columns refer to the numbers of tested receptors. Hatched columns, controls; cross-hatched columns, post-operatives. No control measurements were performed at day 1.5; instead we took the average of the controls of day 1 and day 2. Bars are standard deviations.

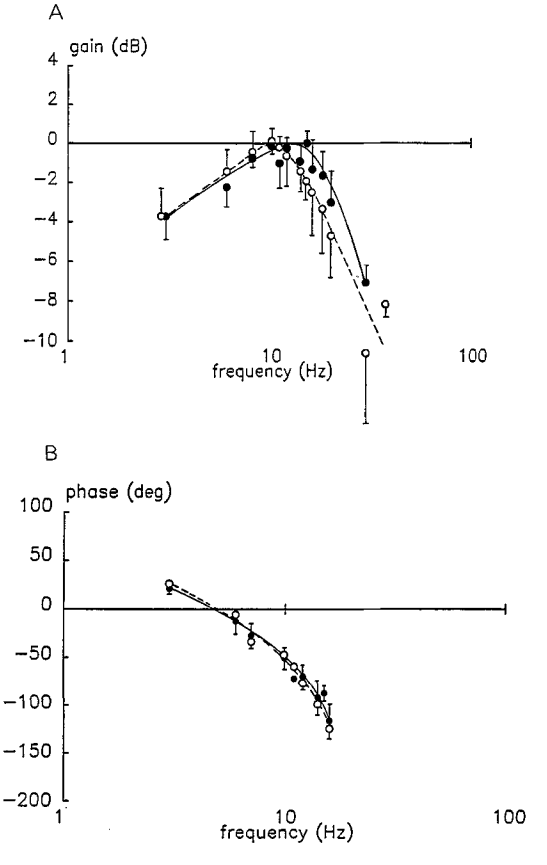

Fig. 6. Frequency response. (A) Gain and (B) phase versus frequency. Open circles, controls; filled circles, post-operatives (1 day and 1.5 day pooled). Bars are standard errors of the means (A), or standard deviations (B).

as in the denervation test. No significant differences could be found between test and control receptors with regard to spike amplitude, spontaneous afferent discharge rate, and variability of the spontaneous discharges. Statistically significant differences between unpaired controls were sometimes found. Neither the frequency responses nor the sensitivity was affected by sham operations.

DISCUSSION

Morphology

The physiological effects of denervation on transduction in electroreceptor synapses can be discussed in the light of earlier morphological work. In *Kryptopterus* electroreceptors at 22–24°C, the nerve shows signs of degeneration first: swollen mitochondriae with disrupted cristae, and multivesiculate bodies 6–12 h post-operatively. The cleft between nerve and receptor cells becomes irregular and the synaptic depressions in the nerve terminal seem to be reduced. The presynaptic bodies lose their shape, whereas the remainder of the organelles in the receptor cells are unchanged. At 18–24 h post-operatively the nerve terminals withdraw completely from the receptor cells. Thereafter degeneration proceeds in the receptor cells until finally, at 72 h, the secretory cells too degenerate.[16] Further it was found that the afferent nerve of the tuberous organs of *Gnathonemus* degenerates within 48 h post-operatively, whereas degeneration of the secondary receptor cells takes 2–12 days.[15] A more or less similar time course for the degeneration of ampullary electroreceptors would seem to be possible in *Ictalurus*.[8]

Temperature

The experiments with *Kryptopterus*[16] were performed at 23°C, whereas the experiments reported here were performed at 16°C. According to a rough estimate at 16°C the metabolic processes and degeneration are slowed down by a factor of two with respect to 23°C. So at 16°C the first effects of denervation can be expected 12–24 h post-operatively. Our study shows the first noticeable effects, viz. smaller spikes and a lower discharge rate, 24–36 h post-operatively, which is in good agreement with the expectation. Hereafter we shall consider that the morphological changes in our experiments follow the pattern described for *Kryptopterus* ampullary receptors[16] and *Gnathonemus* tuberous receptors.[15]

Spike amplitude

The decrease of spike amplitude 24 h post-operatively can be explained as follows. After denervation the afferent terminals withdraw from the receptor cells, thereby reducing their membrane contact. Whatever mechanism, capacitive or resistive, underlies the spread of afferent action potentials to the extradermal space, withdrawal of the afferents will reduce the electric coupling between afferent terminal and extradermal space. This can be one of the causes of the reduced extradermally recorded action potentials. We did not find any indications of clogging of the lumen.

Resting discharge

The decrease of the spontaneous discharge rate might also be caused by the withdrawal of nerve afferents from the receptor cells. A decreased synaptic area would lead to a decreased synaptic efficacy: a smaller portion of the postsynaptic membrane, or less transmitter receptors, would be exposed to the depolarizing action of the transmitter. Such a loss of synaptic efficacy, however, should be reflected equally well in the sensitivity; this was not found. On the contrary, throughout the denervation experiment the sensitivity remains unchanged as does the frequency characteristic. Another explanation for the reduced resting discharge could be a change in the threshold of the spike generator. An increased threshold would result in a decrease of spontaneous activity but would not affect the sensitivity. An increase in threshold, however, would imply a hyperpolarized postsynaptic membrane, but hyperpolarization is unlikely because denervation means disintegration of the afferent fibre and consequently depolarization. Moreover a threshold would cause non-linearities in the *I–O* curve for weak stimuli, but this was not found. A third cue is given by the morphology. Fields and Ellisman[7] have demonstrated that there is a relation between the resting discharge of the ampullae of Lorenzini and the synaptic morphology. Deep synaptic invaginations correspond to a high level of resting activity, whereas the absence of invaginations corresponds to a low resting activity. A similar morphological succession can be seen in the denervated synapses of the electroreceptors of *Kryptopterus*:[16] undamaged receptors have deep synaptic invaginations, degenerated synaptic complexes show no invaginations at all. These findings seem consistent with our electrophysiological results, although we disagree with the implicit statement of Fields and Ellisman[7] that the resting discharge rate is positively correlated with the sensitivity. Unpublished observations (Peters R. C.) have revealed that the sensitivity of *Scyliorhinus* ampullae remains constant when the resting discharge is manipulated. If the receptor cells of the ampullary organs follow a degeneration pattern similar to those of tuberous organs after denervation[15] our conclusion is that the resting discharge rate is apparently related to the integrity of the afferent nerve and not to the sensory cells. It might be that the "trophic" function of the afferent nerve is somehow involved in sustaining the resting discharge and that cutting the nerve abolishes the trophic properties. This conclusion would challenge the conception that the resting discharge is caused by a steady flow of transmitter, unless of course it is the trophic function of the nerve that controls the resting level of transmitter release.

Sensitivity and frequency characteristic

The sensitivity and the frequency response of the electroreceptors do not change as long as afferent activity can be recorded. Although the synaptic organization must have been seriously affected within the recording period this is not reflected in the stimulus transduction, i.e. sensitivity. Apparently the transmitter releasing mechanism is still intact. This is consistent with the data found for *Gnathonemus* tuberous receptors.[15] The fact that a probably already damaged synapse does not affect the frequency response means that the limitations of the stimulus transduction are not dictated by the synapse alone. It is very likely that properties like "sensitivity" and "frequency response" have to be sought in other parts of the receptor cell as well, as was suggested for the ampullae of Lorenzini in *Scyliorhinus*.[11] In that case they could reflect elementary kinetics of presynaptic components like Ca-channels, or other stimulus transduction-related cellular systems. Additional experiments (Teunis P. F. M. *et al.*, in preparation), focusing on smaller time windows and with modified denervation techniques, support the data presented above. These experiments which follow the electrophysiological behaviour of individual electroreceptors demonstrate again unequivocally the differences between "sensitivity" and "resting discharge".

CONCLUSION

Comparison of our data with existing experimental evidence leads to the postulation of two "systems" involved in stimulus transduction: (1) a "resting discharge system" which is ultimately a property of the afferent fibre and which might somehow be related to its "trophic function"; (2) a "modulation system" which is a property of the receptor cell, and which could be the voltage-controlled transmitter release system. On the other hand, the many inconsistencies mentioned in the introduction lead us to look for another explanation.

We propose the hypothesis that both components are part of a feedback loop in which the firing frequency of the afferent fibre reflects the metabolic energy needed to compensate for the disturbances in the electrochemical homeostasis of the receptor cell.

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