STUDIORUM PROGRESSUS

Response of the Ampullae of Lorenzini to Static Combined Electric and Thermal Stimuli in *Scyliorhinus canicula*[1]

In recent years the role of the ampullae of Lorenzini, especially in Elasmobranchs, for electroreception became more and more evident (MURRAY[2-4]; DIJKGRAAF and KALMIJN[5,6]; OBARA and BENNETT[7]). Behavioural experiments, for example, by KALMIJN[8] revealed that sharks and rays actually seem to employ this quality of stimulus in their natural life. Most attention has been paid to the study of the dynamic component of electrosensitivity of the Lorenzinian ampullae (LA). The present paper deals with the influence of constant electric stimuli upon the static neural impulse patterns in single ampullary afferents. Stimulus conditions of this kind would appear, for example, when the fish is swimming with constant velocity in the earth's magnetic field; in other words, when the fish uses electroreception as an aid for navigation.

Methods. The experiments were performed with single mandibular ampullae of Lorenzini which were dissected from the dogfish (*Scyliorhinus canicula*) adapted to 6 °C for half a year (for detail see HENSEL and NIER[9]). Because of the well-known thermosensitivity of the LA (SAND[10]; HENSEL[11]), the temperature was held constant during all measurements by means of electrically isolated and thermostatically controlled thermodes just above and below the ampullary capsule at 7, 13, 19, or 25 °C. Isotonic solutions, with components according to FÜHNER[12], were used in the experiments.

The electric currents were applied by inserting thin glass-coated platinum electrodes (tip diameter 2 µm) into the ampullary canals; the input of the stimulating current in the orifice of the ampullary canal was isolated from ground by a broad air gap (ca. 1 cm). The indifferent electrode (platinum) was placed near the nerve emerging from the isolated capsule. The generator (Grass stimulator S 44) supplied dc and square wave impulses of variable duration, intensity and polarity. Electrode potentials were compensated by a bucking voltage between indifferent electrode and ground. In order to achieve constant current conditions, a 100 MΩ resistance was installed in series to the stimulated LA, limiting the stimulating current between 0 and ± 100 nA. The input impedance of the preparation was in the range of 180 to 250 kΩ (in agreement with findings of WALTMAN[13]). Since a considerable part of the applied current was short circuited by interstitial fluids and ground connections, all current values were only relative ones; they corresponded approximately to those values given by MURRAY[4].

For recording, single units were used whenever possible (microdissection method). The neural impulse patterns were recorded by means of platinum electrodes, preamplifiers (Tektronix RM 122) with a frequency range of 80 to 1000 cps, a double beam oscilloscope (Tektronix RM 565) and a Grass camera. In later experiments, the stimulus parameters and impulse patterns were stored by a magnetic tape recorder (Hewlett Packard 3960D) and evaluated by a digital computer (IBM 1130).

Results. In Figure 1 original recordings of neural impulse patterns in the ampullary afferents are shown for

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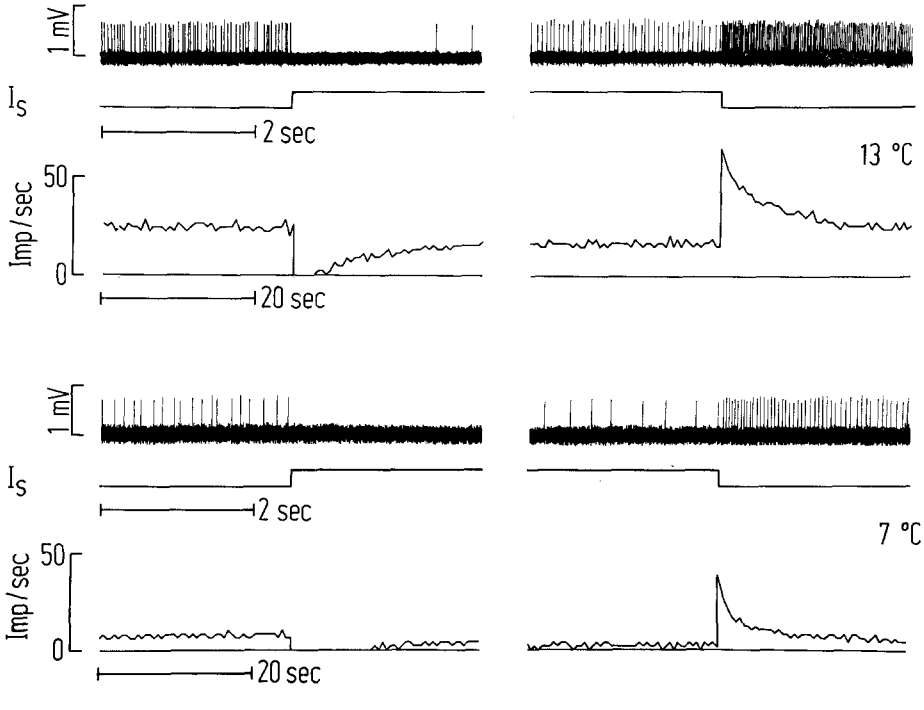

Fig. 1. Original recordings from a single afferent unit for different temperatures and currents. Upper series: 13 °C; lower series: 7 °C, same ampulla. Upper line in each series: neural impulse patterns, middle line: strength of stimulating current, hyperpolarizing current upwards; lower line: computer evaluation of impulse rate (averaged for every half sec).

2 static temperatures (13 and 7 °C) and different electric currents. If a positive current of some nA was suddenly applied at the orifice of the ampullary canal, the receptor responded by a decrease of the impulse rate; the silent period was lengthened with higher current strength. Accordingly, a negative current step led to an increase of the impulse rate in the afferent nerve, the maximum of which sensitively depended on stimulus strength. The threshold current strength amounted to values of 0.2 to 1 nA for a 10% change in the neural impulse patterns in correlation to the applied current step. This electrosensitivity of the ampulla for quick changes in current strength, as shown by MURRAY[3,4] mainly at room temperatures, could be qualitatively verified within the total range of temperatures investigated here.

In 3 out of 28 preparations we found an inverse characteristic in electroreception of the ampulla: Negative currents diminished the impulse rate in the neuron, positive currents led to a rise in frequency. In one two-fibre preparation, there were both types of electroreception: one fibre displaying the normal behaviour as described above, and the second one, with lower spike amplitude but nearly the same threshold strength, responding in the opposite way. However, for these fibres, the electro- and thermosensitivity was less, the static impulse rates were very irregular, and the survival time was less than 1 h (in contrast to preparations in a good condition with 4 and more hours of normal behaviour).

However, we also found a static component of electroreception in the LA investigated here, when the neural impulse patterns were checked 3 or more min after the onset of a constant current flow. Figure 2 shows shifts in steady spike discharges of single afferent nerve units under long lasting dc. Mean values and form of the distribution were approximately the same when calculated 3 or 5 min after the onset of the dc stimulus, but the mean values

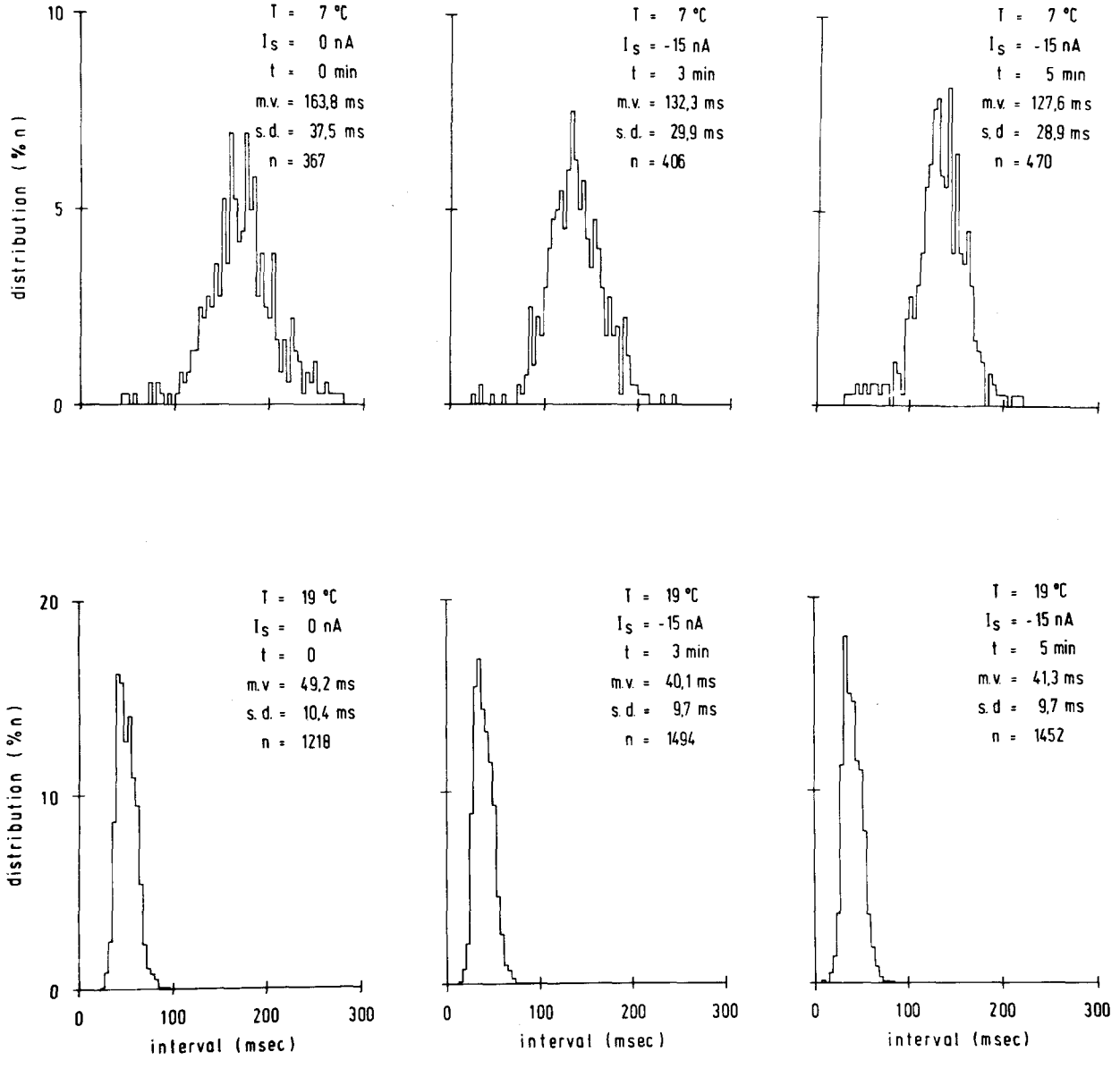

Fig. 2. Spike interval histograms for steady state impulse discharges. Ordinates: distributions of interval length; the sum of all analyzed intervals (n) for time periods of exactly 1 min was set as 100%. Abscissae: length of spike intervals (msec). Time (t) indicates the end of the analyzed period. Temperature (T), current (I_S), mean values (m.v.) and standard deviations (s.d.) are typed in the plots.

differed from the first one where no current was applied. There were no significant changes of the standard deviations when the experiment was repeated for higher current strength or for inverse polarity. The form of the distribution depended on temperature: at higher temperatures the standard deviation was smaller (lower series in Figure 2). However, when the standard deviations (s.d.) were related to the mean values (m.v.), s.d./m.v. was almost constant for all the conditions investigated here (between 20 and 30%). In the following, only the mean values (averaged for a given time period) of the impulse rate are reported, the significances of which are given by s.d./n.

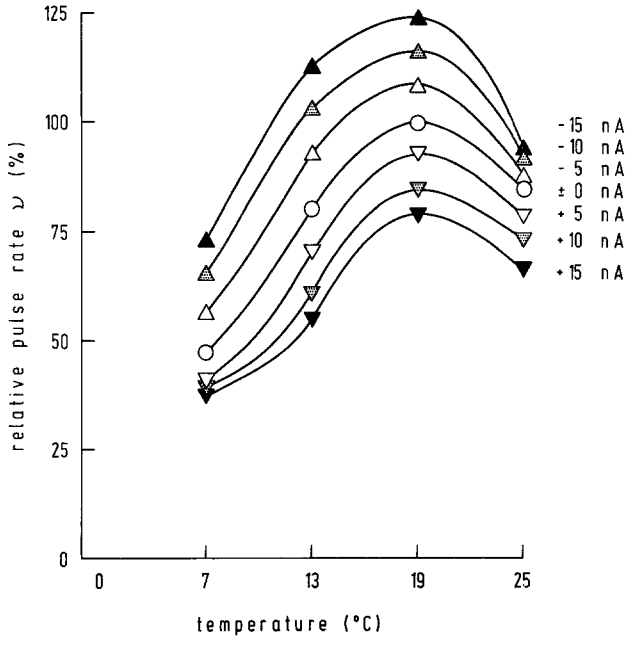

Fig. 3. Steady state temperature characteristics for different currents. Ordinate: averaged relative steady state impulse rate; the rate of every unpolarized ampulla was set 100%; 18 preparations. Abscissa: constant temperature. Applied currents are indicated for every plot. Depolarizing currents at the orifice of the canal are negative.

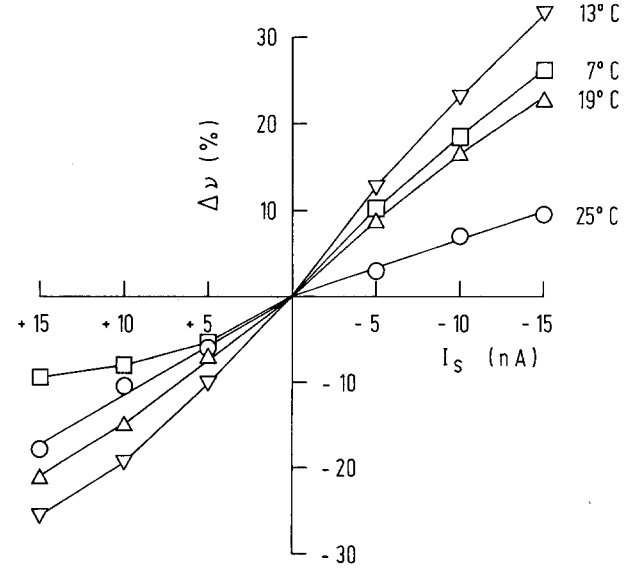

Fig. 4. Steady state current characteristics for different temperatures. Ordinate: averaged relative steady state impulse rate; the rate for the unpolarized ampulla was set 0% for every temperature. 18 preparations. Abscissa: current strength. Applied temperatures are indicated for every plot.

The impulse rate of isolated single nerve units was between 5 and 30 sec^{-1} depending on temperature. In order to standardize the evaluations, the impulse rate of the non-polarized ampulla at 19 °C was defined as being 100%. All other rates were normalized by a conversion factor depending on the preparations. The mean values obtained for all preparations under comparable conditions were averaged. Figure 3 shows the averaged relative values of steady state impulse patterns of the ampullae of Lorenzini in relation to different temperatures (18 preparations). In agreement with the investigations of HENSEL and NIER[9], the steady state temperature characteristics for unpolarized ampullae passed rather low values at 7 °C and the maximum rate at about 19 °C then to reach the falling section of the curve (25 °C). Single preparations, however, showed considerable deviations from this average steady state characteristic (cf. HENSEL and NIER[9], Figure 1).

Prepolarization of the ampulla yielded a marked shifting of these characteristics. Long-lasting negative currents at the orifice of the ampullary canal led to an increase in steady state impulse rate all over the temperature range, positive currents caused a decrease of the impulse rate; i.e. the dynamic and the static component of electrosensitivity of the ampulla were unidirectional over the whole temperature and current range investigated here. The shape of the steady state characteristics was not altered; especially the rate maximum remained at 19 °C. Higher currents did not make the shift in steady spike frequency more apparent. Currents of more than 20 to 30 nA even diminished again the effects given in the Figure (see below). If, as postulated by MURRAY[22], thermal and electrical stimuli were equivalent, we should have expected a distinct horizontal shifting of the maximum to lower temperatures for negative currents with the intensities used here. However, this equivalent suggested by MURRAY was based on investigations of the dynamic response of the ampulla, whereas our results are related to static responses.

In Figure 4 the static sensitivity of the ampulla to electric dc is plotted for different temperatures. The steady state impulse rate of the unpolarized ampulla was set zero for each of the tested temperatures; then the increase and decrease of impulse rates produced by additional electric currents were drwan in the ordinate. The Lorenzinian ampullae were most sensitive to electric stimuli at temperatures around 13 °C, the slope being maximal for hyperpolarizing as well as for depolarizing currents. At higher and lower temperatures, the slopes decreased; this effect was asymmetrical, depending on temperature and on current direction. In particular at 25 °C, the additional depolarizing electric current yielded a smaller increase of steady state impulse frequency, and at 7 °C the effect of hyperpolarizing currents which reduced the impulse rate decreased.

With higher current strength, the effects became more complex; currents of 50 nA and more did not, as a rule, produce a significant shift of the mean static neural impulse rate; i.e. the curves in Figure 3 tended back to the abscissa with increasing current strength. For the dynamic component of electroreception, this fact is described by MURRAY[4] as 'electrical overstimulation'. However, it was not convenient to extrapolate the average impulse rate versus current plots for greater current ranges because of too high a degree of dispersion.

The electric overstimulation obviously depended on the conditions of the preparation under investigation; it should be discussed with respect to the single preparation only.

Discussion. The present investigations demonstrate that there is a static component of electrosensitivity in the Lorenzinian ampullae, which is positioned at least 3 min after a change in the current strength. This static component was in the same direction as the dynamic one over the whole range of temperatures investigated here (7 to 25 °C); as a rule, a negative current applied to the orifice of the ampullary canal led to a rise of neural activity; a positive current yielded a fall. The amplitude of the static electrosensitivity, however, is very small compared to that of the dynamic component. The dynamic response in single afferent units to currents of sufficient strength could reach impulse rates of 160 sec^{-1} (MURRAY[4]; in our own experiments computer evaluation of single spike intervals even gave values up to 260 sec^{-1}). Relative to steady state rates, the dynamic component reached values of up to several 1000%, depending on temperatures. In contrast, the static shift in steady discharge frequency due to current was in every case less than 100%.

AKOEV and ILYINSKY[14] divided the ampulla of rays (*Raja clavata* and *Trygon pastinaca*) in phasic and tonic ones. In the dogfish, we found no basis for such a distinction. At 17 °C, all fibres in a good condition showed a steady impulse rate greater than zero. Certainly the fibres were silent at extreme temperatures (< 7 °C, > 25 °C); in this case, as well as during the silent period following a quick drop in temperature, negative currents could induce neural activity.

It is obvious that under static conditions of temperatures and electric currents, no equivalent of both can be established. If such an equivalent existed, the static frequency versus temperature characteristic of the ampulla would have to be shifted along the temperature axis (abscissa in Fig. 3) by an additional constant current, whereas, in fact, the curves are shifted along the frequency axis (ordinate in Figure 3). In the case of an equivalent, for example, a depolarizing current would increase the discharge frequency at low temperatures and decrease it at high temperatures, which is in contradiction to our finding that depolarization led to a frequency increase over the whole temperature range.

Finally the question remains whether the static component of electroreception has any meaning for the fish. While this paper was being prepared, ANDRIANOV et al.[15] described responses of central neurons of the electrosensory system in skates to linearly rising magnetic fields (of more than 2 Gs/sec). This rate of change of magnetic field strength corresponds to that which a fish should feel when swimming in the earth's magnetic field with a constant velocity of 50 cm/sec. However, the magnetic field was applied for 0.5 sec only, so that the dynamic responses to the induced electric fields were recorded. The static component of electroreception is two orders of magnitude smaller; therefore we should expect that the fish will record mostly quick changes of direction in the magnetic field[16].

Summary. The effect of long-lasting electric currents on the Lorenzinian ampullae at constant temperatures between 7 and 25 °C was investigated in the dogfish (*Scyliorhinus canicula*). Steady state neural impulse patterns in single afferent units were analyzed by plotting interval length histograms and computing mean values and standard deviations for currents between -100 and $+100$ nA. The mean values depended on temperature and on current strength; the relative standard deviations remained almost constant (ca. 20–30%). Negative currents, inserted at the orifice of the ampullary canal, led to higher, and positive currents to lower, steady impulse rates in the whole temperature range investigated here. This static component of electrosensitivity again disappeared at higher currents (of 50 nA and more; electric overstimulation). The maximum static response was two orders of magnitude less than the maximum dynamic component of electroreception. The electrosensitivity depended on temperature: the ampullae were most sensitive to electric currents between 13 and 19 °C. The maximal neural activity at 19 °C was not shifted to higher or lower temperatures by electric stimulation. A constant equivalent of electric and thermal stimulation throughout the tested temperature and current range could not be found.

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[18] The experiments were performed at the Biologische Anstalt Helgoland. We wish to thank Prof. Dr. O. KINNE for his hospitality and support and Mr. J.-K. HOLTMANN for his help during the adaption procedure. For the digital analysis of spike frequency on the IBM 1130 we wish to thank Dipl. Ing. A. TAGMAT, Bochum.

PRO EXPERIMENTIS

A Section Stretching Apparatus for Ultracryotomy[1]

Several procedures for stretching and handling semithin and ultrathin frozen sections have been described[2–7]. None of these methods, however, seems to be satisfactory, especially with respect to section stretching. Therefore a section stretching apparatus will be described, the main feature of which is that it is inherently adapted to the cutting edge of the glass knife. According to this principle,

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