früher gefunden und konnte auch unter den Ver- 
suchsbedingungen dieser Arbeit (s. Tab. 3) bestä-
tigt werden. 
Überraschend ist der experimentelle Befund —
 wenn man den komplexen Aufbau der Membran-
barrieren bedenkt —, daß die hydraulische Leit-
fähigkeit unter exosmotischen und endosmotischen 
Bedingungen innerhalb der angegebenen Fehler-
grenzen gleich ist. Eine Polarität des Wasserflusses,
d. h. eine Abhängigkeit der Flußgröße bei gleicher 
chemischer Potentialdifferenz von der Richtung durch 
die Membran, liegt demnach anscheinend bei Valo-
nia utricularis nicht vor.
In der Literatur finden sich eine Reihe von ex-
perimentellen Nachweisen dieses Phänomens (s.
z. B. die Zusammenfassung bei DAINTY 12). DAINTY 
hat gezeigt, daß eine Polarität bei hintereinander-

geschalteten Membranen, die sich in ihrem Hydrata-
tionszustand, bzw. in ihrem Reflexionskoeffizienten 
unterscheiden, zu erwarten ist. Er hat aber auch 
gleichzeitig auf die Fehlermöglichkeiten, die vor 
als durch die bereits diskutierten ungerührten 
Schichten auftreten können, hingewiesen. Deshalb 
befindet es zur Zeit fraglich, wie weit dieses Phänomen 
tatsächlich in der Natur vorkommt. Für Valonia 
Utricularis läßt sich auf jeden Fall aufgrund der ab-
gehandelten Fehlerbetrachtung eine Polarität des 
Wasserflusses ausschließen.

Wir danken der Deutschen Forschungsgemeinschaft 
die Unterstützung dieser Arbeit durch Personal-
und Reisemittel sowie für die Bereitstellung von 
Arbeitsplätzen in der Biologischen Anstalt Helgoland 
auf Helgoland und in der Stazione Zoologica, Neapel.

The Significance of Metabolic Energy and the Ion Pump for the Receptor Potential of the Crayfish Photoreceptor Cell *
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Institut für Neurobiologie der KFA Jülich
(2. Naturforsch. 26 b, 1311—1321 (1971); received August 3, 1971)

Isolated crayfish retinas were poisoned by dinitrophenol (DNP) or ouabain (OU). The changes 
of the extracellularly recorded receptor potential (ReP) evoked by short or long stimuli were 
measured.

The changes of the shape of the ReP after poisoning by OU are the same, except the transient 
increase, as appear after depolarizing the retina by high external potassium concentration. DNP 
has different effects on the ReP. It causes a prolongation of the amplitude \( h_{\text{max}} \) and an increase 
of the shape quotient \( h_{\text{max}}/h_{e} \) (Fig. 9).

Especially the rate of decrease of the excitability under different stimulus programs was 
measured (Tab. 5). 
DNP experiments show that the inexcitability occurs the earlier the higher the incident light 
intensity is.
The time needed depends on the stimulating efficiency of the light rather than on the direct 
amount of energy.
The loss of excitability rather depends on the energy of the total light applied than on the stimulus pattern. 
Concerning the inexcitability OU shows the same effect as DNP: it occurs the earlier the more 
the retina is excited. 
These results contradict the hypothesis that the receptor potential is a consequence of a light 
induced change of the activity of an electrogenic pump mechanism. Fig. 10 shows the electrogenic 
pump mechanism (EPM) as opposed to the conductance increase mechanism (CIM).

Following former investigations about the effect 
of some metabolic inhibitors on the receptor potential 
of the retina of the hermit crab we wanted to 
study the influence of 2,4 dinitrophenol more 
thoroughly for short (< latency) and long stimuli. 
Our main interest was attached to the influence of 

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Postfach 365. 

* In remembrance of Prof. Dr. Heinz Steinitz (Jerusalem) 
with gratitude and friendship.
the light stimulus program on the rate of decrease of the excitability of the receptor cells poisoned by DNP.

Additionally we wanted to study the action of ouabain on the receptor potential of the visual cells under different light stimulus conditions.

**Material and Method**

The mass response of the photoreceptor cells of the isolated layer of the retina of the crayfish Astacus leptodactylus ESCHSCHOLZ was recorded with a method described elsewhere (Stieve). External electrodes were used. The vessel in which the retina was placed was continuously perfused with van Harreveld's solution (V. Harreveld). In certain periods of the experiments a solution containing 2·10⁻⁴ mol/l 2,4-DNP or 10⁻³ mol/l OU was added to the normal solution. In some experiments OU was used in lower concentrations (10⁻⁷ and 10⁻⁸ mol/l). D I and D II are two types of perfusion described elsewhere (Stieve).

The pH of the solution was always close to 7.7. The temperature of the retina throughout the experiment was always close to 15 °C, the maximum deviation being ±1 °C.

Two different sources of white light were used. In most of the experiments a mercury super pressure lamp Philips CS 100 W/2 (beam A) was used; in one set of experiments (series C) an additional background illumination by a halogen filament lamp, Philips 12 V Typ 7023 100 W, (beam B) was used. The maximum light intensity applied amounted to 6200 lux and was named \( I = 100 \). It can be diminished by neutral density filters (Bausch & Lomb).

The light intensity of beam B was adjusted to be 1/60 of that of beam A, \( I = 0 \). Since the two lamps had different spectral emissions, the adjustment was performed by using a beam A intensity diminished to 1/60 (by means of neutral density filters) as reference and adjusting the intensity of beam B so that stimulation by beam B evoked the same height of response of the retina. The desired light intensity of beam B could roughly be determined by use of a lux meter.

Different programs for the light stimulation were used. Among others they can be compared by measuring the average light intensity \( I \) which is a measure of the light energy which, per unit time, is applied to the retina and is averaged over long periods (\( \geq 10 \) min). \( I \) is measured in relative values [%].

**Procedure**

After a pre-period of 30 or 60 min during which the preparation, in normal \( v. \) Harreveld's solution, was allowed to adapt to temperature and a constant stimulus sequence of 1 stimulus per 10 min the vessel was perfused with solution containing the inhibitor. In various series different stimulus programs were applied as described later on.

The experiments differed as to the following parameters:

- stimulus duration \( \tau \),
- stimulus interval \( \Delta t \),
- stimulus intensity \( I \),
- average stimulus intensity \( I \).

The number of experiments in each series was 7. In series B to F frequent stimuli were given from \( t_{30} \) to \( t_{60} \); the poison was added at \( t_{61} \).

During the main period the receptor potential ReP gradually became smaller and finally disappeared.

The changes of size and shape of the recorded ReP's were evaluated quantitatively:

**A** For short stimuli (stimulus duration \( \tau \) about 6 ms)

- \( h_{\text{max}} \) the amplitude of the maximum [mV],
- \( t_{1} \) the latency — the period from stimulus begin to the first visible increase of the ReP [ms],
- \( t_{\text{max}} \) the peak-amplitude-time — the time from stimulus begin to the maximum amplitude of the ReP [ms],
- \( t_{2} \) the time in which the ReP decreases from \( h_{\text{max}} \) to \( h_{\text{max}}/2 \) [ms].

**B** For long stimuli (\( \tau \) about 1000 ms)

- \( h_{\text{max}} \) the amplitude of the maximum [mV],
- \( h_{0} \) the amplitude of the plateau of the ReP [mV] measured at the end of the stimulus (in order to determine the shape quotient \( h_{\text{max}}/h_{0} \)),
- \( t_{\text{max}} \) the peak-amplitude-time [ms],
- \( h_{a} \) the amplitude 500 ms after the end of the stimulus [mV].

If necessary the stimulus duration \( \tau \) will from now on be quoted as index of the measurement (e.g. \( h_{\text{max}} \), \( t_{\text{max}} \) evoked by a stimulus of 6 ms duration).

In the after-period, when the retina had become inexcitable or at least the ReP had decreased to less than 20% of its reference value (see below), the vessel was again perfused with inhibitor-free \( v. \) Harreveld's solution. This period lasted at least 60 min until a marked recovery of the ReP could be stated.

The changes of the ReP were expressed in per cent of the reference value i.e. the value of the ReP recorded immediately before the application of the inhibitor in each experiment.

As a measure for the rate of loss of the excitability the time was measured in each experiment after which \( h_{\text{max}} \) of the ReP amounted to 50% (50% time \( t_{50\%} \)), 30% (\( t_{30\%} \)) and 20% (\( t_{20\%} \)), respectively, of the reference value.

The intensity dependence of the height of the ReP was in some series (D and E) tested with stimuli of 6 ms duration and a stimulus interval of 2 min.
After a sequence of stimuli with falling light intensity a stimulus sequence with increasing intensity was applied. The tested intensity range was 3 log units. In the beginning, middle, and end of all series a stimulus with maximum light intensity ($I = 100$) was used. The average values of the responses to these three stimuli were taken as reference values, $h_{\text{max}} = 100$ per cent.

**Results**

**DNP**

6 series of experiments with retinas poisoned by DNP were performed which differed in respect of the stimulus pattern and will be described in detail below.

**Changes of the shape of the ReP**

The effect of DNP on the ReP evoked by short stimuli was studied in series A, while in series B long stimuli were used.

After poisoning the retina by DNP the ReP gradually decreases until the retina becomes inexcitable.

On 6 ms stimuli (series A) the shape changes: In the course of $h_{\text{max}}$ decreasing $t_1$ increases, $t_{\text{max}}$ increases less and $t_2$ decreases. Table 1, Figs. 1 and 2.

On 1000 ms stimuli (series B) $h_{\text{max}}$ becomes smaller more rapidly than $h_e$, i.e. the shape quotient $h_{\text{max}}/h_e$ decreases, $t_{\text{max}}$ becomes longer. The peak-amplitude-time $t_{\text{max}}$ for long stimuli is however not prolonged significantly more than for short stimuli. The interpolated $t_{\text{max}}$ value for the ReP's with an $h_{\text{max}}$ of 50% of the reference value is $114 \pm 9\%$ for $r$ about 6 ms and $122 \pm 6.5\%$ for $r$ about 1000 ms. Table 2, Figs. 3 and 4.

After poisoning the after-potential $h_a$ decreases in about the same degree as $h_{\text{max}}$, i.e. steeper than $h_e$. The changes are reversible: 90 min after washing out the poison the ReP amounts to about 14% of the reference height.

The changes are in good agreement with those in the experiments with *Eupagurus* (Stieve 3). The changes show some characteristic differences to the changes due to depolarization of the cell membrane caused by variation of the external potassium concentration (Stieve 1):

In case of increased external potassium concentration $h_{\text{max}}$ decreases, $t_1$ does not change significantly, $t_{\text{max}}$ and $t_2$ decrease, $t_2$ strongly; $h_{\text{max}}/h_e$ increases.

The influence of the stimulus program on the rate of decrease of excitability.

| stimulus-duration $r$ about 6 ms | time [min] | $h_{\text{max}}$ | $t_1$ | $t_{\text{max}}$ | $t_2$
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>a</td>
<td>60</td>
<td>1.3 ± 0.2 mV</td>
<td>23 ± 1 ms</td>
<td>91 ± 9 ms</td>
<td>330 ± 58 ms</td>
</tr>
<tr>
<td>b</td>
<td>90</td>
<td>86 ± 6%</td>
<td>108 ± 6%</td>
<td>102 ± 8%</td>
<td>78 ± 23%</td>
</tr>
<tr>
<td>c</td>
<td>120</td>
<td>48 ± 0%</td>
<td>128 ± 5%</td>
<td>118 ± 7%</td>
<td>71 ± 20%</td>
</tr>
<tr>
<td>d</td>
<td>150</td>
<td>24 ± 7%</td>
<td>160 ± 13%</td>
<td>128 ± 10%</td>
<td>56 ± 18%</td>
</tr>
<tr>
<td>e</td>
<td>180</td>
<td>10 ± 5%</td>
<td>176 ± 12%</td>
<td>122 ± 14%</td>
<td>61 ± 21%</td>
</tr>
<tr>
<td>f</td>
<td>300</td>
<td>7 ± 3%</td>
<td>182 ± 14%</td>
<td>148 ± 33%</td>
<td>122 ± 39%</td>
</tr>
</tbody>
</table>

Table 1. Measured quantities of the ReP during series A (DNP): The peak amplitude $h_{\text{max}}$; the latency $t_1$; the peak-amplitude-time $t_{\text{max}}$; and the time in which the ReP decreases from $h_{\text{max}}$ to $h_{\text{max}}/2$, $t_2$. a-value: pre-period; b-values: main period; c-value: after-period (B 12—B 72).

| stimulus-duration $r$ about 1000 ms | time [min] | $h_{\text{max}}$ | $h_e$ | $h_a$ | $h_{\text{max}}/h_e$ | $t_{\text{max}}$
<table>
<thead>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>60</td>
<td>2.5 ± 1.0 mV</td>
<td>1.2 ± 0.4 mV</td>
<td>0.4 ± 0.1 mV</td>
<td>2.12 ± 0.09 mV</td>
<td>108 ± 11 mV</td>
</tr>
<tr>
<td>b</td>
<td>70</td>
<td>86 ± 7%</td>
<td>80 ± 6%</td>
<td>50 ± 4%</td>
<td>107 ± 2%</td>
<td>87 ± 6%</td>
</tr>
<tr>
<td>c</td>
<td>80</td>
<td>67 ± 4%</td>
<td>66 ± 4%</td>
<td>34 ± 5%</td>
<td>102 ± 2%</td>
<td>94 ± 7%</td>
</tr>
<tr>
<td>d</td>
<td>100</td>
<td>40 ± 4%</td>
<td>51 ± 5%</td>
<td>80 ± 10%</td>
<td>78 ± 4%</td>
<td>129 ± 12%</td>
</tr>
</tbody>
</table>

Table 2. Measured quantities of the ReP during series B (DNP): $h_{\text{max}}$; $h_e$ (amplitude of the plateau of the ReP measured at the end of the stimulus); $h_a$ (amplitude 500 ms after the end of the stimulus); $h_{\text{max}}/h_e$ (shape quotient); and $t_{\text{max}}$. a-value: pre-period; b-values: main period; no c-value, as only short stimuli were applied in the after-period (B 12—B 72).
stimulus-duration \( \tau \) about 6 ms
\[
\begin{array}{cccccc}
\text{time} & h_{\text{max}} & t_i & t_{\text{max}} & t_2 \\
\text{[min]} & \text{[mV]} & \text{[ms]} & \text{[ms]} & \text{[ms]} \\
\hline
a & 55 & 1,1 \pm 0,2 & 20 \pm 1 & 87 \pm 6 & 144 \pm 6 \\
b_1 & 85 & 101 \pm 5 & 76 \pm 3 & 74 \pm 4 & 72 \pm 7 \\
b_2 & 115 & 47 \pm 10 & 90 \pm 6 & 67 \pm 3 & 44 \pm 6 \\
b_3 & 145 & 19 \pm 6 & 84 \pm 11 & 50 \pm 6 & 29 \pm 4 \\
b_4 & 175 & 3,6 \pm 0,3 & 115 \pm 27 & 56 \pm 8 & 27 \pm 5 \\
c & 260 & 21 \pm 4 & 138 \pm 8 & 102 \pm 5 & 59 \pm 4 \\
\end{array}
\]

Table 3. Measured quantities of the ReP during series A' (OU, short stimuli): \( h_{\text{max}} \), \( t_i \), \( t_{\text{max}} \), and \( t_2 \). a-value: pre-period; b-values: main period; c-value: after-period (F11—F18).

stimulus-duration \( \tau \) about 1000 ms
\[
\begin{array}{cccccc}
\text{time} & h_{\text{max}} & h_{e} & h_{a} & h_{\text{max}}/h_{e} & t_{\text{max}} \\
\text{[min]} & \text{[mV]} & \text{[mV]} & \text{[mV]} & \text{[mV]} & \text{[ms]} \\
\hline
a & 60 & 1,3 \pm 0,2 & 0,6 \pm 0,1 & 0,3 \pm 0,1 & 2,0 \pm 0,1 & 110 \pm 9 \\
b_1 & 90 & 104 \pm 6 & 94 \pm 11 & 95 \pm 18 & 116 \pm 9 & 82 \pm 4 \\
b_2 & 120 & 53 \pm 9 & 43 \pm 10 & 30 \pm 10 & 132 \pm 10 & 82 \pm 3 \\
b_3 & 150 & 19 \pm 6 & 15 \pm 4 & 10 \pm 2 & 128 \pm 12 & 77 \pm 7 \\
b_4 & 180 & 6 \pm 1 & 4 \pm 1 & 2,4 \pm 0,7 & 145 \pm 14 & 84 \pm 12 \\
c & 270 & 37 \pm 4 & 32 \pm 3 & 13 \pm 3 & 119 \pm 8 & 129 \pm 6 \\
\end{array}
\]

Table 4. Measured quantities of the ReP during series A' (OU, long stimuli): \( h_{\text{max}} \), \( h_{e} \), \( h_{a} \), \( h_{\text{max}}/h_{e} \), and \( t_{\text{max}} \). a-value: pre-period; b-values: main period; c-value: after-period (F11—F18).

Series A

In this series the lowest average light intensity of all experiments is used: short stimuli (\( \tau \) about 6 ms) and long stimulus interval (\( \Delta \tau \) 10 min).

Pre-period: \( t = 0 \) to \( t = 60 \) min, \( \tau \) about 6 ms, \( \Delta \tau \) 10 min. Main period: DNP from \( t = 61 \) min, \( \tau \) about 6 ms, \( \Delta \tau \) 10 min, \( I = 100 \); \( I = 0,75 \) per cent. After-period: \( \tau \) about 6 ms, \( \Delta \tau \) 10 min, \( I = 100 \), \( n = 7 \); experiment numbers B 01—B 71, Table 1, Figs. 1 and 2.
Table 5. All series of experiments compared concerning the decrease-times \( t_{50\%}, t_{30\%}, \) and \( t_{20\%} \) of the amplitude of the ReP, \( \text{h}_{\text{max}} \), after addition of either DNP or OU. A statistical comparison of the decrease-times was made. In the following some examples of the \( t_{90\%} \)-values are given; the other values compare accordingly. In each case two series of experiments were compared and \( p \) was determined.

<table>
<thead>
<tr>
<th>Series ( \rightarrow )</th>
<th>( p )</th>
<th>Series ( \rightarrow )</th>
<th>( p )</th>
<th>Series ( \rightarrow )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{A}<em>{\text{DNP}} ) and ( \text{D}</em>{\text{DNP}} )</td>
<td>0,0001</td>
<td>( \text{D}<em>{\text{DNP}} ) and ( \text{E}</em>{\text{DNP}} )</td>
<td>&gt; 0,25</td>
<td>( \text{D}<em>{\text{OU}} ) and ( \text{D}</em>{\text{DNP}} )</td>
<td>&gt; 0,25</td>
</tr>
<tr>
<td>( \text{B}<em>{\text{DNP}} ) and ( \text{D}</em>{\text{DNP}} )</td>
<td>0,0025</td>
<td>( \text{D}<em>{\text{DNP}} ) and ( \text{F}</em>{\text{DNP}} )</td>
<td>0,1</td>
<td>( \text{A}<em>{\text{OU}} ) and ( \text{D}</em>{\text{OU}} )</td>
<td>0,0001</td>
</tr>
</tbody>
</table>

**Series B**

A much higher average light intensity is applied in this series.

![Graph](image)

Fig. 3. ReP's under the influence of DNP (Series B). \( r \) about 1000 ms, \( I = 100 \), \( A \) after 60 min in v. H a r r e l d’s solution; DNP added at \( t = 61 \); \( b_1 \) at \( t = 90 \); \( b_2 \) at \( t = 120 \); \( b_3 \) at \( t = 150 \); \( b_4 \) at \( t = 210 \); DNP washed out at \( t = 211 \); \( c \) at \( t = 330 \) (B 42).

Pre-period: \( t = 0 \) to \( t = 30 \) min, \( r \) about 1000 ms, \( \Delta r \) 10 min, \( t_{60} \) to \( t_{40} \) min, \( r \) about 1000 ms, \( \Delta r \) 10 min, \( I = 100 \). Main period: DNP from \( t = 61 \) min, \( r \) about 1000 ms, \( \Delta r \) 10 min, \( I = 100 \); \( I = 125 \) per cent. After-period: \( r \) about 6 ms, \( \Delta r \) 10 min, \( I = 100 \), \( n = 7 \); experiment numbers B 12—B 72, Table 5, Figs. 3 and 4.

These values show that the time needed until the retina loses its excitability is shorter when the retina is stimulated with longer and more frequent stimuli.

![Graph](image)

Fig. 4. Values of \( m_{\text{max}} \), \( h_{\text{max1000}} \), \( h_{\text{max}}/h_e \) (shape quotient), and \( t_{\text{max1000}} \) in series B (B 42).

**Series C**

In this series the same average light intensity is used as in series B but the light is applied mainly by a steady background illumination the intensity of which is called \( I_b \), whereas the stimulus intensity is referred to as \( I_s \). Without DNP the background illumination produces a decrease of the height of the
ReP to a steady value of about 20–30% of the height of a response without background illumination.

Pre-period: \( t = 0 \) to \( t = 30 \text{ min} \), \( \tau \approx 6 \text{ ms} \), \( \Delta \tau = 10 \text{ min} \), \( I_b = 100 \), from \( t = 0 \) on an additional permanent background illumination, \( I_b = 1.7 \). Main period: DNP from \( t = 61 \text{ min} \), \( \tau \approx 6 \text{ ms} \), \( \Delta \tau = 10 \text{ min} \), \( I_b = 100 \); \( I_b = 1.7 \); \( I = 125 \text{ per cent} \). After-period: \( \tau \approx 6 \text{ ms} \), \( \Delta \tau = 10 \text{ min} \), \( I = 100 \), \( n = 7 \), experiment numbers B03 – B73, Table 5, Figs. 5 and 6.

The \( t_{50\%} \) value as well as the other two values of rate of loss of excitability in this series are smaller than in the B series. The decrease of the height of the ReP following stimuli of different length (1000 ms in the B series and 6 ms in the C series) was measured.

![Fig. 5. ReP's under the influence of DNP (series C). \( \tau \approx 6 \text{ ms}, I = 100 \). a₁ after 30 min in v. Harrer evel's solution; at \( t = 45 \text{ min} \) additional background illumination with \( I_b = 1.7 \); a₂ at \( t = 70 \); DNP added at \( t = 71 \); b₁ at \( t = 100 \); b₂ at \( t = 130 \); b₃ at \( t = 160 \); b₄ at \( t = 220 \); DNP washed out at \( t = 221 \); c at \( t = 340 \) (B 73).](image)

**Series D**

Pre-period: \( t = 0 \) to \( t = 30 \text{ min} \), \( \tau \approx 1000 \text{ ms} \), \( \Delta \tau = 10 \text{ min} \), from \( t = 31 \text{ min} \), \( \tau \approx 1000 \text{ ms} \), \( \Delta \tau = 1 \text{ min} \), every 5th stimulus = test stimulus, \( \tau \approx 6 \text{ ms} \), \( I = 100 \), \( n = 7 \), experiment numbers A61 – A69, Table 5.

In this series the average light intensity is 25% smaller than in series C. The \( t_{50\%} \) times etc. are slightly but not significantly shorter in D than in C.

Compared to series B these results show that the \( t_{50\%} \) times etc. depend on the stimulus duration; the values are smaller for short stimuli than for long stimuli. C compared to B and to D shows that the decrease does not depend on how often the stimulus is changed but on the total amount of light energy, which could not be taken for granted.

**Series E**

The stimulus program is almost similar to series D, the average stimulus intensity half as much.

Pre-period: \( t = 0 \) to \( t = 30 \text{ min} \), \( \tau \approx 500 \text{ ms} \), \( \Delta \tau = 1 \text{ min} \), from \( t = 31 \text{ min} \), \( \tau \approx 500 \text{ ms} \), \( \Delta \tau = 1 \text{ min} \), every 5th stimulus = test stimulus, \( \tau \approx 6 \text{ ms} \), \( I = 100 \). Main period: DNP from \( t = 61 \text{ min} \), \( \tau \approx 500 \text{ ms} \), \( \Delta \tau = 1 \text{ min} \), from \( t = 61 \text{ min} \), every 5th stimulus = test stimulus, \( \tau \approx 6 \text{ ms} \), \( I = 100 \); \( I = 50 \text{ per cent} \). After-period: \( \tau \approx 6 \text{ ms} \), \( \Delta \tau = 1 \text{ min} \), \( I = 100 \); \( n = 7 \); experiment numbers A72 – A80.

The decrease-times of the excitability, \( t_{50\%} \) etc., except \( t_{50\%} \), are somewhat, but not significantly, smaller than in series D. In this series the dependence of the height of the ReP on the stimulus intensity was tested in each experiment to determine the relative height of the ReP evoked by a stimulus intensity corresponding to the average stimulus intensity \( I \). On the average a stimulus intensity of 50% results in a \( h_{\text{max}} = 85 \pm 1\% \), \( \tau \approx 6 \text{ ms} \). If \( t_{50\%} \) depends on the energy of the stimulating light \( t_{50\%} \), should be prolonged to 200% of the value.
of series D; if $t_{50\%}$ depends on the stimulating efficiency of the stimulating light $t_{50\%}$, should be prolonged to 118% of series D. The measured values are closer to the value predicted by the latter assumption.

**Series F**

In this series the stimulus program is quite similar to series D and E, only the stimuli are shorter and the average stimulus intensity is much smaller.

Pre-period: $t=0$ to $t=30$ min, $\tau$ about 50 ms, $\Delta t$ about 10 min, from $t=31$ min on $\tau$ about 50 ms, $\Delta t$ 1 min; every 5th stimulus = test-stimulus, $\tau$ about 6 ms, $I=100$. Main period: DNP from $t=61$ min, $\tau$ about 50 ms, $\Delta t$ 1 min, every 5th stimulus = test-stimulus, $\tau$ about 6 ms, $I=100$; $t$ lasting about 5 per cent. After-period: $t$ about 6 ms, $\Delta t$ 10 min, $I=100$, $n=7$; experiment numbers A 129–A 135.

In this series the $t_{50\%}$ times are significantly longer than in the D series.

The intensity-dependence of the height of the ReP on 6 ms stimuli was also tested in this series. In the mean a stimulus intensity of 5% results in a $h_{max}$ of $47 \pm 3$ per cent. If $t_{50\%}$ depends on the energy of the stimulating light, $t_{50\%}$ in this series should be prolonged by a factor of 20 compared to series D, if, however, it depends on the stimulus efficiency only by a factor of 2.1. The factor found is 1.65 which is much closer to the value predicted by the second assumption.

**Ouabain**

In pre-experiments OU in concentrations of $10^{-7}$ mol/l and $10^{-5}$ mol/l showed so little effect that all the later experiments were carried out with OU of a concentration of $10^{-5}$ mol/l.

In 2 series of experiments corresponding to series A and D of the DNP — set the influence of OU on the ReP was tested. The effect of OU on the ReP's evoked by short and long stimuli was studied in series A', where short and long stimuli were applied in the same experiment.

Poisoning the retina by OU results, after a transitorial increase in height of the ReP, in a gradual decrease, until the retina finally becomes inexcitable.

On 6 ms stimuli $h_{max}$ increases and afterwards decreases again. $t_1$ decreases significantly as long as $h_{max}$ increases and stays short thereafter. $t_2$ decreases strongly. Tab. 3 and Figs. 7 and 8.

On long stimuli (1000 ms) $h_{max}$ increases and decreases later. $h_e$ shows almost the same behaviour but it decreases a bit steeper than $h_{max}$, i.e. the shape quotient $h_{max}/h_e$ increases, $t_{max1000}$ decreases a bit less than $t_{max6}$. Tab. 4 and Figs. 8 and 9.

The after potential ($h_a$) decreases closely parallel to the changes of $h_e$. All these changes are reversible. 90 min after washing out the OU $h_{max}$ amounts to 20% and $h_{max1000}$ to 40% of the reference value.

There are some obvious differences between the changes in shape after treating the retina with OU as compared to DNP in the course of the decrease of the ReP.

In OU there is a transient increase of the height $h_{max}$ and a transient decrease in the latency $t_1$ which both are absent in DNP. Additionally $t_{max}$ decreases in OU and increases in DNP. The diminution of $t_2$ is more pronounced in OU than in DNP.

On long stimuli the differences are corresponding and in OU $h_e$ falls steeper than $h_{max}$ ($h_{max}/h_e$ increases) which is inverse in DNP ($h_{max}/h_{max}$ decreases).

It is remarkable that the changes in shape of the ReP, in respect of all measured values ($h_{max}$, $t_{max}$ etc), due to poisoning of the retina by OU are similar to the changes due to an increase of the external potassium concentration (that means the same changes as occur when the retinula cell is depolarized or hyperpolarized). The initial phase of OU action is similar to a hyperpolarization of the cell, the second similar to depolarization.

**The influence of the stimulus program on the rate of decrease of excitability**

**Series A' (OU)**

The average light intensity of the stimulating light is not quite as low as in series A (DNP).

Pre-period: $t=0$ to $t=60$ min, $\tau$ about 6 ms, $\Delta t$ 10 min, every 30 min a stimulus, $\tau$ about 1000 ms, $I=100$. Main period: OU from $t=61$ min, $\tau$ about 6 ms, $\Delta t$ 10 min, every 30 min a stimulus, $\tau$ about 1000 ms, $I=100$; $I$ about 4 per cent. After-period: $t$ about 6 ms; $\Delta t$ 10 min, every 30 min a stimulus, $\tau$ about 1000 ms, $I=100$, $n=7$; experiment numbers F11–F18, Tab. 5, Figs. 7, 8, 9.

**Series D (OU)**

In this series a much stronger average stimulus light intensity is applied.
Fig. 7. ReP's under the influence of OU (Series A', short stimuli). $\tau$ about 6 ms, $I=100$. a after 55 min in v. Harreveld's solution; OU added at $t=61$; $b_1$ at $t=80$; $b_2$ at $t=100$; $b_3$ at $t=200$; (during the main period from $t=61$ to $t=211$ every 30 min an additional stimulus of $\tau=1000$ ms was applied); OU washed out at $t=211$; $c$ at $t=295$ (F 13).

Fig. 8. Values of $h_{\text{max}}$, $h_{\text{max}1000}$, $h_{\text{max}}/h_0$, and $t_{\text{max}}$ in series A' (F 13).

Pre-period: $t=0$ to $t=30$ min, $\tau$ about 6 ms, $A\tau$ 10 min, from $t=31$ min $\tau$ about 1000 ms, $A\tau$ 1 min, every 5th stimulus = test-stimulus, $\tau$ about 6 ms; $I=100$. Main period: OU from $t=61$, $\tau$ about 1000 ms; $A\tau$ 1 min, every 5th stimulus = test-stimulus, $\tau$ about 6 ms, $I=100$; $I=100$ per cent. After-period: $\tau$ about 6 ms, $A\tau$ 10 min, $I=100$, $n=7$; experiment numbers A 89 - A 95 (Tab. 5).


The values of this series are significantly smaller than the values of the series A' (OU) which clearly shows that also in the experiments, where ouabain is used, the time needed until the retina becomes inexcitable strongly depends on the light energy used for stimulation.

Discussion

Changes in shape of the ReP caused by DNP and OU

The action of DNP consists in decoupling the oxidative phosphorylation and in turn blocking the active transport which results in a decrease of the resting potential.

If the changes in shape of the ReP were due only to the decrease of the resting potential the changes should be the same as after a potassium depolarization. Since this is clearly not the case, DNP must have also other effects besides causing a decrease of the resting potential. It has been established (HOPFER et al. 4, LIBERMAN and TOPALY 5, see also

5 E. A. LIBERMAN and V. P. TOPALY, Biochim. biophysica Acta [Amsterdam] 163, 125 [1968].
DELBRÜCK that DNP is a good proton carrier in lipid bilayers. It seems probable that DNP also changes the conductivity of the membrane of the visual cells. This could account for the differences between the experiments with high external potassium concentration and DNP.

The main differences between the effect of OU and DNP on the ReP concern $t_{\text{max}}$ and $t_{\text{max1000}}$ and $t_2$ and $h_{\text{max}}/h_s$. OU however causes the same changes in all the measured values of the ReP as would have a potassium depolarization of the photoreceptor cells.

It is possible that the late OU effect is only caused by the increasing depolarization of the cell, whereas the DNP-effect consists in retarding the decrease of conductivity of the cell-membrane (falling phase of the ReP) in the same way as would have been the case for a low external calcium-concentration.

The time needed for the decrease of excitability

As a basis for comparison in Tab. 5 the decrease-times $t_{90\%}$, $t_{50\%}$, and $t_{10\%}$, are recorded for all series of experiments where DNP and OU were applied. On principle these values show the same type of dependences and are all suited for comparison. Therefore in the following only the $t_{90\%}$ times are considered since the others show similar results.

As a comparison of the B and D values shows, the $t_{90\%}$ time depends on the stimulus duration of the test stimulus: For short duration (6 ms) of the test stimulus one finds short $t_{90\%}$ times while for long test stimuli (1000 ms) longer decrease-times of excitability are measured.

Comparison of the DNP experiments series A and D and E and F shows that the inexcitability occurs the earlier the higher the stimulating light intensity is. The time needed depends on the stimulating efficiency of the light rather than on the amount of energy applied.

A steady background illumination has the same effect on $t_{90\%}$ as more intense periodic light stimuli when the average light intensity is the same (series C and D). The ratio of intensities of stimulus versus background illumination was 100 : 1,7.

That means: The loss of excitability depends — within the accuracy of the measurements — only on the incident light energy and not on the stimulus pattern. It might also have been possible that the receptor cells became more rapidly exhausted after frequent changes between light and darkness.

These results are in good correspondence with the data published by HAMDORF and KASCHEF who measured the oxygen consumption of the fly's eye.

The results are in contrast to the findings of HAGINS et al. about the vertebrate photoreceptor which has a higher oxygen consumption in the dark than during illumination. This shows that vertebrate photoreceptors and at least the compound eyes of arthropodes are working with basically different excitatory mechanisms.

Mechanism of the ReP

An important portion of the metabolic energy of the photoreceptor is used for an active ion transport across the cell membrane.

A comparison of the change in shape of the ReP during poisoning with DNP and OU with the depolarization of the photoreceptor cell caused by high external potassium concentration (STIEVE) shows that, due to the action of the two drugs, the resting potential of the photoreceptor cells decreases (see also SMITH et al. 9).

Under the term “ion pump” one summarizes the mechanism which causes an active ion transport across the cell membrane.

Two different mechanisms are postulated for the receptor potential of the photoreceptor cell in which the ion pump plays a different role (Fig. 10).

I Conductance increase mechanism (CIM)

The light stimulus causes an increase of the conductivity of the membrane of the photoreceptor cell. This in turn causes ionic currents to flow through the cell membrane, thus producing the ReP (FOURTES). The function of the ion pump is to reestablish the concentration gradients of the ions which are diminished mainly by the excitation.

II Electrogenic pump mechanism (EPM)

The receptor potential is caused by a change in the activity of an electrogenic ion pump which is controlled by light (SMITH et al. 9, 11).
Fig. 10. Two hypotheses regarding the cause of the ReP of the photoreceptor cell: CIM = Conductance Increase Mechanism, EPM = Electrogenic Pump Mechanism.

An ion pump is electrogenic if, for instance, it pumps sodium ions from inside to outside and potassium ions in the opposite direction in a ratio which is different from one. This example of only two ion species is very simple, but the following would also be true if other ion species were involved.

An electrogenic pump can also be a part of the resting potential of a photoreceptor cell. This, however, will be discussed below whereas now the question is dealt with whether the ReP is caused by an electrogenic pump mechanism.

In the case of the CIM the function of the ionic pump is to maintain the excitability. In this case the pump needs not to be electrogenic. In the case of the EPM causing the ReP the function of the pump is necessary for every single excitation and the pump must be electrogenic.

The most probable assumption is that the electrogenic pump is based on a transport-ATPase which is activated by sodium, potassium and by magnesium ions and inhibited by calcium ions. The fact that calcium and magnesium ions act synergetically rather than antagonistically (Stieve \textsuperscript{1,12}) is more in favour of the CIM than of the EPM.

In the here described experiments the ion pump was stopped in two different ways. OU is believed to block the pump directly whereas DNP blocks the active transport by means of stopping the production of energy-rich phosphate. The evaluation of the experiments with the two different drugs and the different stimulus programs leads to the rejection of one of the two hypotheses.

The experiments where the retina was poisoned by DNP show that the loss of excitability occurs the earlier the more the retina was stimulated by light. These results can be explained both by the CIM as well as by the EPM. As the energy-rich phosphate reserve becomes exhausted either the ion gradients decrease (CIM) or the pump activity in the retina as a whole gradually stops.

The experiments in which the retina was poisoned by OU show that also in these experiments the loss of excitability occurs the earlier the more stimulus energy is applied. This fact can only be explained satisfactorily by the CIM and not by the EPM.

OU should block the pump as soon as it reaches it. It is extremely improbable that it reaches the pump earlier when the retina is stimulated more intensely. It is also extremely improbable that OU stops the ReP by a different mechanism from that of blocking the ion pump which influences the membrane potential of the receptor cell membrane.

As a consequence these experiments rule out the EPM and make it highly probable that the ion pump restitutes and maintains the ionic gradients across the cell membrane which are necessary for the excitation caused by means of a CIM.

We do not see any simple explanation for the findings of Smith et al.\textsuperscript{9} who report that after poisoning the ventral eye of Limulus the excitability is lost already when the resting potential is decreased to about 50\% of its original value.

It seems quite probable that OU acts at the outside of the cell membrane whereas DNP probably has to penetrate the cell membrane to act intracellularly. One would therefore expect that, if both drugs act with the same rate, shorter $t_{90\%}$ times should occur in series A′ (OU) than in series F (DNP) since in both cases the average light intensity is almost equal. The opposite is the case: the $t_{90\%}$ time in the DNP-F-series is not significantly ($p=0,07$) shorter than in the OU-A′-series, whereas the two D values are almost equal. Since the standard deviation of the mean is extremely great in series F one can not discuss this result further. There are good reasons to assume that the $t_{90\%}$ time of series F is relatively too small in our experiments. It can also have something to do with the phase of increased sensitivity proceeding the loss of excitability after OU treatment. This obviously prolongs the time needed for the decrease in sensitivity, an effect which is stronger with lower light intensity. Therefore the relative difference is much greater for the $t_{90\%}$ time than for the $t_{90\%}$ time and even more for the $t_{90\%}$ time, as absolutely the $t_{90\%}$ time is the longest.

\textsuperscript{12} H. Stieve, Z. vergleich. Physiol. 47, 457 [1964].
Regarding all the DNP- and OU-experiments described the results make probable:

1. The reserves of the photoreceptor cell of energy-rich phosphate for the maintenance of the ion pump are small.
2. The excitability loss after poisoning the retina by OU occurs so slowly because the ionic gradients across the cell membrane and therefore the resting potential decrease gradually when the pump is blocked.

Does an electrogenic pump contribute to the resting potential of the photoreceptor cell?

The experiments described in this paper do not rule out the existence of an electrogenic pump in the photoreceptor cell. They only exclude that an electrogenic pump is the cause of the ReP. But there is also the possibility that an electrogenic pump under certain conditions contributes to the resting potential of the photoreceptor cell.

In the lateral eye of Limulus there are good reasons to assume that an electrogenic pump is existing which increases the resting potential under certain conditions and which seems to be initiated by light (compare Stieve\textsuperscript{13}, Fig. 6) if the stimulus is long and bright enough.

Millecchia\textsuperscript{14} describes the same type of phenomenon as "after-hyperpolarization" in the ventral eye of Limulus and Brown et al.\textsuperscript{15} in the photoreceptor of the barnacle as "post-illumination hyperpolarization". Poisoning this eye by OU makes the hyperpolarization quickly disappear, which indicates that this effect is a result of an electrogenic ion pump.

Our experiments concerning the contribution of the electrogenic pump to the membrane potential of the photoreceptor cells will be continued.

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\textsuperscript{14} R. Millecchia, Thesis, Rockefeller University, New York 1969.