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Voltage Noise Accompanying Chemically-Induced Depolarization of Insect Photoreceptors

R. Payne

Department of Neurobiology, Research School of Biological Sciences
Australian National University, P.O. Box 475, Canberra City, A.C.T. 2601, Australia

Abstract. Intracellular potentials are recorded from photoreceptors in a superfused preparation of the retina of a locust compound eye. Chloral hydrate and alkyl alcohols induce a rapid, superfusing reversible depolarization of these photoreceptors when dissolved in the saline. Analysis of voltage noise accompanying depolarization by chloral hydrate suggests that depolarizing ionic pathways are opened briefly and randomly in time in the photoreceptor membranes. This conclusion is supported by measurements of the cell resistance and of voltage noise amplitude as a function of membrane potential. Replacement of superfusate sodium by choline reversibly reduces the effects of chloral hydrate, suggesting that the ionic pathways opened are permeable by sodium. The voltage noise induced by chloral hydrate is compared to that during depolarization by steady illumination of the same cell. As the illumination intensity is increased, the amplitude and the shape of the power spectrum of light-induced voltage noise approach those of drug-induced noise at the same depolarization level. The possibility that these phenomena represent alterations in the mechanism of phototransduction is discussed.

Key words: Alcohols – Induced photoreceptor noise – Phototransduction.

Introduction

The retinula cell of the locust compound eye has been the subject of several investigations into photoreceptor transduction mechanisms following Scholes' (1964) detection of the cell's response to single photons. This photoreceptor generates one discrete depolarizing pulse or "bump" of amplitude 1–7 mV for every photon effectively absorbed and little 'spontaneous activity' in darkness if it is fully dark-adapted (Lillywhite 1977). Each bump is thought to involve the opening of many sodium channels in the rhabdom membrane (Fulpius and

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Bauman 1969; Wong 1978) and the bumps fuse to form slow, graded receptor potentials when bright flashes of light are shone on the cell. Changes in receptor potential characteristics can therefore be attributed to changes in the efficiency, latency and amplitude of the underlying responses to single photons.

This paper describes a preparation of the locust retina in which the photoreceptors are in contact with a saline bathing medium so that changes can be made in the ion concentrations in the saline, and chemicals can be introduced. Measurements were made of changes in transduction induced by alkyl alcohols and chloral hydrate, which were dissolved in the saline bathing the retina at concentrations sufficient to cause anaesthetic effects when applied to a variety of other tissues (Armstrong and Binstock 1964; Seeman 1972; De Felice and Alkon 1977). The effects of alcohols reported in this paper are intended as control results to help recognize, in future experiments, anaesthetic side-effects of drugs that are usually specific to a particular biochemical pathway. These anaesthetic effects may be important when applying high concentrations of lipid-soluble drugs to the medium bathing the retina in an attempt to modify transduction.

Materials and Methods

1.1 Adults from a laboratory culture of *Locusta migratoria,* reared under a 16 h/8 h cycle of light and darkness, were dissected during the light period. The animals were decapitated, and one compound eye was removed by slicing through the cuticle around the eye. The eye was waxed, facing upwards, upon a perspex block. A cut was made across the eye, close to its base and parallel to it, and the top section removed to expose a ring of photoreceptors around the rim of the remaining eye-section, while the photoreceptor connections to the lamina remained intact. Before dissection the locust had been dark-adapted for 20 min and the dissection was carried out under weak red light to maintain the dark adaptation.

The perspex block upon which the eye had been mounted was placed in a perspex chamber of volume 1 ml. Oxygenated ringer of composition (mM): Na, 200; Cl, 205; K, 4; Ca, 0.5; glucose, 90; TRIS, 9; pH 7.0, was passed across the retina at a rate of 3 ml/min. A similar solution containing the drug under investigation was oxygenated in a separate bottle. A tap at the entrance to the perfusion chamber was used to switch between solutions.

1.2 Stimulation and Recording

Photoreceptors were stimulated along their exposed length with light from a green light-emitting diode (Siemens, LD57C), peak wavelength 560 nm. To saturate a cell's response to light, white light from a tungsten microscope lamp was focused on the preparation. Single micropipettes of resistance 50–100 MΩ and double barrelled micropipettes (Werblin 1975) were pulled from borosilicate glass and filled with 3M potassium acetate. An amplifier similar to that of

Gage and Eisenberg (1969), with output to the bath indifferent electrode, was used to record from micropipettes impaling the distal ends of photoreceptors on the cut surface of the retina. A voltage-to-current converter (Gage and Eisenberg 1969) enabled constant current to be passed down one barrel of double electrodes irrespective of electrode and cell resistance. The input voltage to the V-I converter was taken as a measure of the current passed. Direct measurement of currents of up to 10 nA passed through electrodes of the type used showed no distortion of the linearity of the V-I conversion. The indifferent electrode was a silver wire connected to the bath by a 3M potassium acetate/agar bridge.

1.3 Recording from Double Electrodes

While resistive coupling between electrode barrels was less than 500 KΩ when measured with an electrode in saline, it rose to several MΩ when the electrode first penetrated a cell. This extra coupling could be removed by withdrawing the electrode 2–3 μm. Pressure of the electrode on the cell membrane opposite the point of penetration of the cell would produce a resistance which might account for this coupling. To estimate coupling inside cells, a photoreceptor's response was saturated while measuring cell resistance by applying a 20 Hz square wave of current, amplitude 0.4 nA, down one electrode barrel. Shaw (1969) using two electrodes in the same cell, shows that the cell membrane is short circuited when the cell's response is saturated. Any resistance seen using double electrodes under the same conditions was therefore attributed to coupling. For data collected from them to be considered valid, cells had to show an input resistance under saturating illumination that was less than 10% of that in the dark.

1.4 Computations

Records of cell voltage noise were filtered with a high-pass RC filter (half-power frequency 0.5 H_z with a slope of −6 dB per octave) and a 4 stage active low-pass filter (half-power frequency 160 H_z and slope −48 dB per octave). The filtered record and an unfiltered one were digitized "on line" by a PDP-8 computer at a rate of 500 samples per second and stored on floppy disc. Mean membrane potential was calculated from the unfiltered record, while the filtered one was used for noise analysis. Power spectral density functions were calculated by a fast Fourier transform technique (Bendat and Piersol 1971). Between 100 and 200 Hz the spectra were corrected for the loss of power due to low-pass filtering of the signal.

1.5 Chemicals

Octan-1-ol, butan-1-ol and chloral hydrate were of analytic quality. When dissolved in saline none of these chemicals had any effect on electrode tip

potentials, indifferent electrode potential or on voltage noise. Drugs were generally applied by solution in the bathing media. In some experiments, drugs were applied directly onto the surface of the retina via a micro-syringe. A broken glass micropipette, diameter 50–100 μm was connected to a motor-driven syringe filled with 100 mM chloral hydrate dissolved in saline. The motor was stepped to force 10 μl pulses of liquid out of the electrode tip, which was positioned over the retina close to the site of the recording electrode.

Results

2.1 Recording from the Preparation

Retinula cells penetrated with single electrodes could be held impaled for up to two hours. Penetration of retinula cells was not always accompanied by a clear drop in recorded electrical potential, possibly due to electrode penetration through intervening glial or retinula cells. All potentials in this paper are therefore referred to a cell's potential in the dark.

After $^1/_2$ h of dark-adaptation following the dissection, single photon responses – "bumps" – of amplitude 1–4 mV were recorded from cells. Fully dark-adapted cells showed negligible spontaneous activity, as found by Lillywhite (1977). Maximum depolarizing potential changes during the peak transient of a cell's response to light (V_{max}) were in the range 40–75 mV. Cells exhibiting V_{max} less than 40 mV were rejected. Both the values of V_{max} and of bump height are similar to those recorded from intact animals (Shaw 1969; Lillywhite 1977). Experiments on the preparation generally lasted for less than 1 h. During some longer experiments satisfactory recordings were obtained 3–4 h after dissection.

2.2 Effects of Anaesthetics on Resting and Receptor Potentials

Upon addition of 60 mM chloral hydrate or 0.8 mM octanol to the perfusate photoreceptors rapidly depolarized towards V_{max}, the maximum response of the cell to light (Fig. 1). The "resting potential" of the cell (its potential in the dark) came close to or equalled, but never exceeded V_{max} even when using larger doses of anaesthetic. Receptor potentials elicited by 10 ms flashes of a green light-emitting diode (Methods; Sect. 1.2) decreased in height as the cell depolarized, being abolished as the cell's potential neared V_{max}, but were never reversed. Some cells underwent an initial peak depolarization followed by a fall away from V_{max}, the cell remaining insensitive to light throughout (Fig. 1). On removal of drug from the perfusate, cells repolarized and the resting potential returned to within ± 5 mV of its original level.

Receptor potential time course was not consistently altered by octanol or by doses of up to 60 mM chloral hydrate (Fig. 2). Larger doses of chloral hydrate (60–100 mM) induced a "tail" on the falling phase of receptor potentials elicited

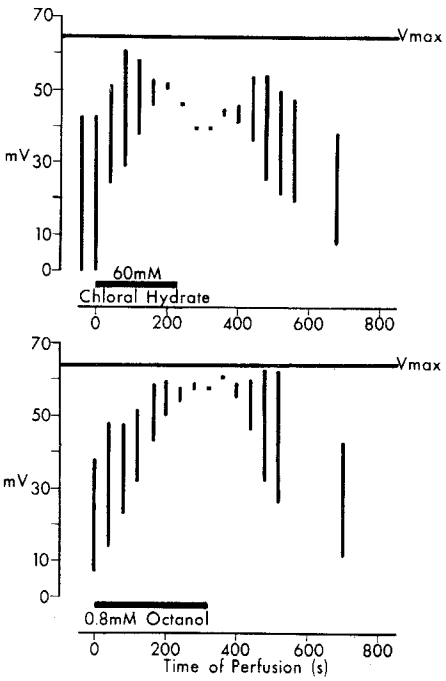

Fig. 1. Behaviour of a photoreceptor treated successively with chloral hydrate and octanol. Black bars represent the amplitude of receptor potentials in response to 10 ms light flashes. The bottom of each bar represents the cell's potential in the dark relative to its potential at the beginning of the experiment

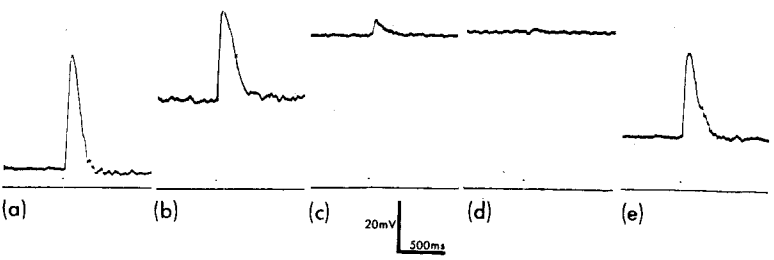

Fig. 2. Receptor potentials recorded during treatment with 60 mM chloral hydrate. **a** Before; **b** 60 s; **c** 150 s; **d** 210 s after introducing the drug. **e** shows a response recorded 7 min after perfusion with chloral hydrate had ceased. The bottom traces show the time of stimulation by a flash of intensity 10^{14} photons/cm^2/s

as the cell depolarized, approximately doubling their decay time. Doses of less than 60 mM chloral hydrate produced depolarizations roughly proportional to the concentration of the drug used. The lowest concentration of chloral hydrate to depolarize photoreceptors consistently was 10 mM.

100 mM butanol reversibly depolarized photoreceptors by 20–40 mV without change in the time course of their receptor potentials. Larger doses of butanol could not be applied because of its insolubility in saline.

The measured time course of anaesthetic effects, as illustrated in Figure 1, was limited by the time constant of filling of the perfusion chamber (20 s). A crude measure of the true latency of a cell's response to chloral hydrate was

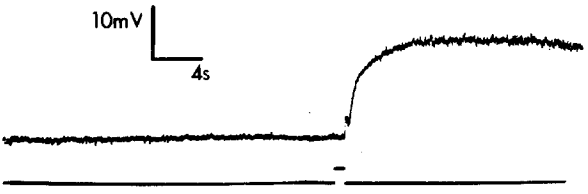

Fig. 3. Initial response of a photoreceptor to a pulse of 100 mM chloral hydrate applied locally via a micro-syringe (see text)

obtained using a microsyringe positioned close to the recording site (Methods, Sect. 1.5). The latency of depolarization following a 10 µl pulse of 100 mM chloral hydrate in saline was less than one second (Fig. 3). The depolarization was accompanied by a characteristic increase of noise. Recovery of a cell's potential was usually complete within 90 s of drug application. Keeping the preparation in darkness had no effect on the magnitude or approximate time course of anaesthetic-induced depolarization of photoreceptors.

2.3 Anaesthetic-Induced Voltage Noise

Voltage noise recorded from photoreceptors in the dark had a variance, $\overline{E^2}$, of 0.006 mV^2 (mean of 12 cells; SE = 0.002). A dark-adapted photoreceptor depolarizing in the dark during perfusion with salines containing chloral hydrate, octanol, or butanol exhibited increased low frequency voltage noise.

As a cell depolarized, the amplitude of the membrane noise initially rose, reaching a maximum at a depolarization (V_{peak}) of 10–20 mV. With further depolarization the membrane noise amplitude declined, disappearing if the cell was allowed to approach V_{max} before removal of anaesthetic. On removal of drug from the perfusate this process was reversed (Fig. 4). In no experiment could individual voltage events be resolved unequivocably in the final stages of recovery from drug.

Samples of noise recorded during slow wash-out of chloral hydrate from the bath were digitized (Methods, Sect. 1.4) and the signal variance and mean depolarization calculated. In Figure 5 the difference between noise variance, $\overline{E^2}$, during and after the action of chloral hydrate is plotted against the mean depolarization, V, of a photoreceptor during gradual removal of anaesthetic. The shape of the curve and the position of its maximum suggested comparison with the study by Katz and Miledi (1972) of acetylcholine-activated ionic channel noise.

If the depolarization is driven by the random opening of channels in the membrane then Katz and Miledi's simple model (which ignores cell cable properties and membrane electrical nonlinearities) predicts that voltage noise variance $\overline{E^2}$, and mean depolarization, V, will be related by the following equation:

$$\overline{E^2} = Vka\left[\frac{V_D - V}{V_D}\right]^3, \tag{1}$$

where V_D is the difference between a cell's natural resting potential and the equilibrium potential for ion movements in the channels, a is the amplitude of a

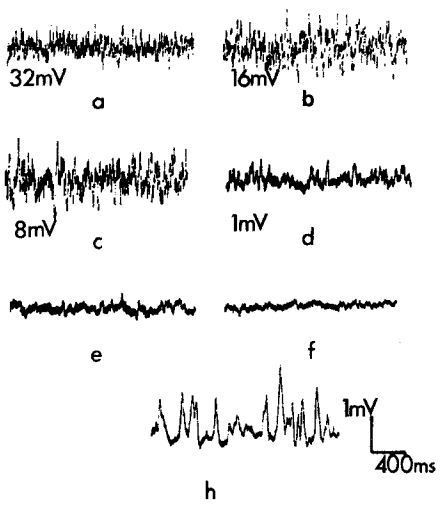

Fig. 4. Voltage noise, at the depolarizations shown, during gradual removal of 40 mM chloral hydrate (**a**–**d**). The final states of drug action (**e** and **f**) and the response to subsequent weak illumination **h** are also shown

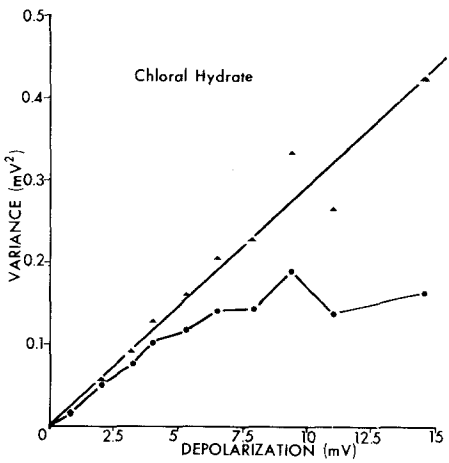

Fig. 5. Voltage noise variance (increase from the final value) plotted against photoreceptor depolarization during the removal of 50 mM chloral hydrate. Circles show raw data, triangles are data corrected for non-linear summation (see text). V_D was set at 55 mV. The cell's potential after recovery from drug was 1.5 mV below that before drug treatment, while voltage noise variance was unchanged at 0.006 mV^2. Variance was computed from samples of duration 2 s

single voltage event due to channel opening and k is a factor dependent on the shape of that event. By differentiating Eq. (1), Katz and Miledi showed that the voltage noise variance would be maximal at a depolarization, V_{peak}, equal to $V_D/4$.

Using the 'correction factor', $[V_D/(V_D-V)]^3$, a linear plot of the noise variance against depolarization (< 15 mV) was obtained for the data of Figure 5 and for nine other cells, in agreement with Eq. (1). V_D was set in each case to be four times the depolarization (V_{peak}) at which noise was maximal. At depolarizations above 15 mV, the fit with theory deteriorates, noise variance falling below that predicted.

The value of V_{peak} for 9 cells, 10.5 mV ± 1 mV (SE), means that the value of V_D predicted by Eq. (1), 42 mV, lies in the range of maximum depolarizations obtainable using large amounts of anaesthetic (Sect. 2.2). For the cell of Figure 5

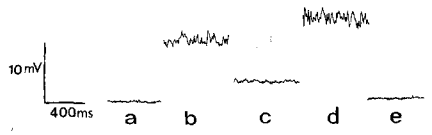

Fig. 6. Reduction in amplitude of depolarization and voltage noise following complete replacement of perfusate sodium by choline. The recordings shown were made: **a** before drug treatment; **b** after 3 min treatment with 30 mM chloral hydrate; **c** 2 min after switching to sodium-free ringer; **d** 2 min after reverting to sodium ringer; **e** after subsequent removal of chloral hydrate from the perfusate

the amplitude of the voltage event underlying the noise was predicted to be 42 μV, assuming a double-exponential event shape ($k = e/4$). Nine cells had a mean event size of 36 μV ± 8 μV (SE). Assuming a driving force of 60 mV and a cell input resistance of 15 MΩ (Sect. 2.5), a voltage event of 36 μV would arise from a conductance event of amplitude 40 pS. This value is in the upper range of those expected of single ionic channels, 3–200 pS (Neher and Stevens 1977).

2.4 Effects of Replacement of Perfusate Sodium

Observations made on similar preparations of compound eyes (Stieve 1964; Fulpius and Bauman 1969; Wulff 1973), were confirmed using the perfused locust retina. Complete replacement of perfusate sodium by choline reversibly reduced receptor potentials of amplitude 14.2 ± 1.1 mV (SE) to 2.7 ± 0.4 mV ($n = 6$) within 120 s of changing ringers but the receptor potential could not be further reduced over a longer period in sodium-free ringer. No significant changes in resting potential occurred. Receptors were also depolarized to a steady level by continuous illumination. 120 s after the introduction of sodium-free ringer, the depolarization of these cells dropped from 9.1 mV ± 0.7 mV (SE) to 3.8 mV ± 1 mV ($n = 6$) while the standard deviation of voltage noise (photon shot noise) accompanying the depolarization dropped to 24% ± 9% of the original value.

The latter effect of sodium replacement was compared with that upon the voltage noise induced by chloral hydrate. After 120 s perfusion with sodium-free ringer, voltage noise observed from six cells during steady depolarization induced by 30 mM chloral hydrate was reversibly reduced in amplitude (SD) to 29.1% ± 7.1% of its original value, while the depolarization dropped from 8.5 mV ± 1 mV to 4.7 mV ± 1 mV (Fig. 6). Replacement of sodium in the perfusate therefore reduces the voltage noise and the depolarization induced both by chloral hydrate and illumination but cannot abolish either.

2.5 Experiments Using Double-Barrelled Electrodes

These experiments were performed to obtain three results:

(*i*) measurement of the cell input resistance and its dependence on membrane potential;

(*ii*) a demonstration of the effect on input resistance when anaesthetics are applied; and

(*iii*) the effect of membrane potential on voltage noise.

Preparation of double-barrelled electrodes and precautions against coupling resistances occurring between barrels are outlined in Methods, Sect. 1.3.

(i) Cell Input Resistance. The input resistance of cells with maximum responses greater than 40 mV and the dependence of cell resistance on imposed membrane potential varied from cell to cell. Cell input resistance ranged from 7 MΩ to 35 MΩ, similar to values recorded by Shaw (1969). Cells with low input resistance at their natural potential in the dark (less than 15 MΩ) showed little variation of resistance as membrane potential was changed by current injection, behaving like linear resistors as the cell's potential was changed by ± 40 mV. Cells with input resistances of more than 15 MΩ exhibited unchanged resistance to increasing hyperpolarizing currents but resistance fell with depolarization. It is possible that the differences are due to shunting of the higher resistance to hyperpolarizing currents by a leakage resistance at the seal between the electrode and the membrane of some cells. Figure 7 shows the averaged voltage-current curve for 6 cells.

(ii) The Effects of Chloral Hydrate on Cell Input Resistance. Membrane resistance fell during sustained depolarization initiated by 40 mM chloral hydrate (Fig. 8). Cell resistance during this noisy depolarization is reduced by two factors, a dependence of the input resistance upon the potential observed in some cells, and a shunting parallel resistance which can be estimated by comparing the resistance of the cell before and during drug treatment in the linear region of the voltage-current curve, when the cell is hyperpolarized by current injection. This parallel resistance reduces the cell input resistance by 20–40% and it may reflect the channels that the analysis of voltage noise suggests are opening and closing during drug treatment (Sect. 2.3). The magnitude of the shunting pathway adequately explains the depolarization ($\sim 20\%$ V_{max}) seen if we assume a driving potential behind current flow in the shunting pathway equal to V_{max}, the maximum response of the cell to light.

Preliminary experiments were performed to investigate the dependence of the drug-induced drop in cell resistance upon the ion concentrations in the bath. Replacement of the sodium ions in the perfusate by choline was attempted before addition of chloral hydrate while the resistance was monitored from the potential drop caused by −1 nA current pulses. The anaesthetic-induced fall in cell resistance is prevented in the absence of sodium (Fig. 9c), occurring only when sodium is replaced (Fig. 9d). The drug therefore induces an increase in the membrane's sodium conductance. Note that replacement of perfusate sodium did not completely abolish the response to light (Fig. 9b), as found in similar preparations of other compound eyes (Stieve 1964; Fulpius and Bauman 1969; Wulff 1973).

(iii) Double-Electrode Experiments on Voltage Noise. Experiments were undertaken to investigate the relationship between the voltage noise amplitude

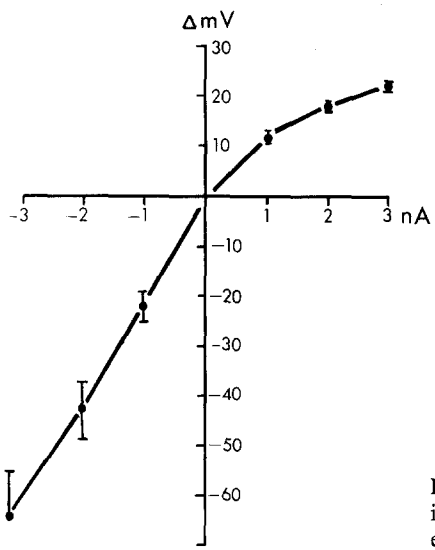

Fig. 7. Mean potential change produced by current injected into 6 cells through double barrelled electrodes. Bars indicate standard errors

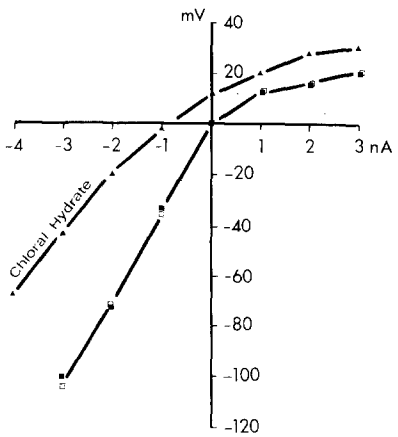

Fig. 8. Potential change produced by current injection into a cell before treatment with 40 mM chloral hydrate (filled squares) when depolarized 11 mV by chloral hydrate (triangles) and after recovery from chloral hydrate (open squares)

and the membrane potential during the application of anaesthetic and illumination. The results were checked to ensure that nonlinearities of the cell input resistance did not affect the results over the range of potential change observed. If the noise is caused by irregularities in the current, due to fluctuations in membrane conductance (l. c., channels opening), then increasing the electrical driving force behind ion movements in the channels opened should increase the current flowing and the voltage fluctuations seen. The amplitude of the voltage noise, its r.m.s. value, will depend on membrane potential, V_m according to the following relation, which neglects cell cable properties and resistance non-linearities.

$$E_{\text{r.m.s.}} \alpha |(V_{\text{Eq}} - V_m)|, \tag{2}$$

where V_{Eq} is the equilibrium potential for ion movements in the channels.

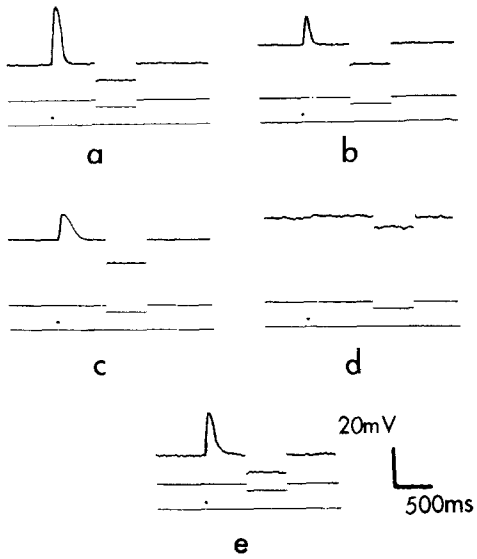

Fig. 9. Potentials recorded from a photoreceptor (top traces) while −1 nA current pulses (middle traces) and 10 ms light flashes (bottom traces) were applied during sequential perfusion with salines containing: **a** 200 mM Na, no chloral hydrate, **b** ONa (200 mM choline chloride) no chloral hydrate, **c** ONa, 60 mM chloral hydrate, **d** 200 mM Na, 60 mM chloral hydrate, **e** 200 mM Na, no chloral hydrate

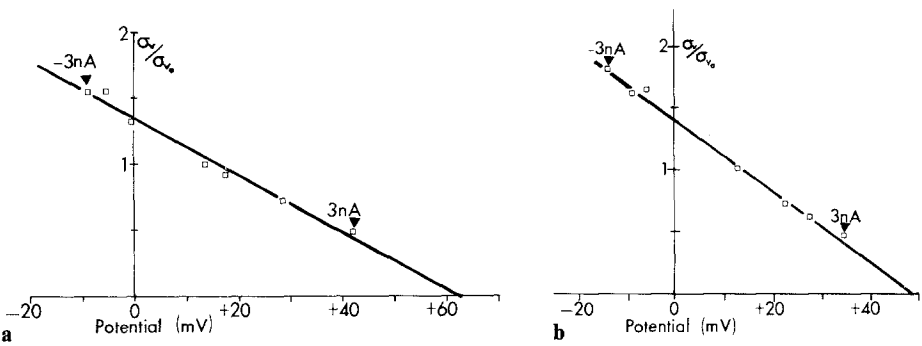

Fig. 10. Relative standard deviations of voltage noise, σ_v/σ_{v0} (4 s samples) plotted against photoreceptor potential during 5 s injections of current. **a** cell depolarized 14 mV by 40 mM chloral hydrate, **b** the same cell depolarized 13 mV by steady illumination after recovery from drug. Cell potentials are relative to those at the beginning of the experiment

Figure 10 summarizes the results. The r.m.s. values ($\sqrt{E^2}$) of voltage noise during steady illumination and during the application of chloral hydrate are linearly dependent on imposed membrane potential during 5 s injections of current, whereas the voltage noise in the absence of light or anaesthetic was not affected by current injection. By extrapolating plots such as Figure 10 to zero noise variance, the equilibrium potential for ion movements in the channels creating chloral hydrate-induced noise in four cells was predicted to be 64 mV ± 6 mV (SE) above the cells' natural resting potential and that behind the steady response to light (as opposed to the initial transient peak response to a brief flash) was 46 mV ± 2 mV (SE). Because electrode coupling was only controlled to within 10% of a cell's input resistance, these values must be regarded as upper limits.

Return of a photoreceptor's potential to its value before drug treatment for several seconds thus increased rather than reduced membrane voltage noise. Conversely, an experiment in which a cell's potential was held constant by applying increasing hyperpolarizing current during chloral hydrate treatment showed the usual increase in membrane noise. This increase in membrane noise is not, therefore, a result of the depolarization by the anaesthetic.

2.6 Power Spectral Analysis of Voltage Noise

Power spectral density functions (power spectra) were computed from sets of five records of voltage noise, each record being of duration two seconds (Methods, Sect. 1.4). Power spectra showing the effects of light and drugs on voltage noise were calculated by taking the difference between spectra before and during the treatment. Quantitative analysis of the spectra requires a model of the cable properties of the photoreceptor, a cell of complex structure. Cable analysis of the photoreceptor has not been attempted in this paper and comments are therefore, for the present, limited to the general properties of the power spectra and to comparisons between spectra recorded under different conditions.

The power spectrum of voltage noise recorded from a dark-adapted photoreceptor in darkness (which includes amplifier and electrode noise) falls as ^{1}/frequency, f, between 4 H_z and 180 H_z (Fig. 11a). This $^1/f$ relationship is typical of voltage noise recorded from high impedance glass micro-electrodes (De Felice and Firth 1970) and from 'resting' cell membranes (Review: Neher and Stevens 1977). During a depolarization of 12 mV induced by treatment with saline containing 40 mM chloral hydrate, low frequency voltage noise increased, and the power spectrum changed shape (Fig. 11a). The difference between spectra recorded before and during drug treatment, the power spectrum attributed to drug-induced noise, is flat between 1 and 20 H_z. Above 40 H_z it declines as $^1/f^2$ to $^1/f^3$, the steepness of roll-off varying from cell to cell. When all of the chloral hydrate has been removed from the perfusion chamber, and the potential and voltage noise have recovered, the power spectrum reverts to that before drug treatment (Fig. 11a).

While the membrane potential recovered during the gradual removal of chloral hydrate, the power spectrum of the drug-induced noise did not change in the steepness of roll-off, but the frequency at which the power dropped to half of its value at 1 H_z shifted to lower frequencies (Fig. 12 dotted lines). This is to be expected if current flow during one drug-induced voltage event is brief compared to the electrical time constant of the photoreceptor. The shape of the power spectrum then approximates to that of the power transfer function of the electrical filter that represents the cell membrane. At smaller depolarization less of the drug-activated channels are open in the membrane, which consequently has a higher resistance (seen as an increased input resistance in the experiments of Sect. 2.5). The resulting increase in the membranes electrical time constant accounts for the shift of the power spectrum to lower frequencies without change in shape.

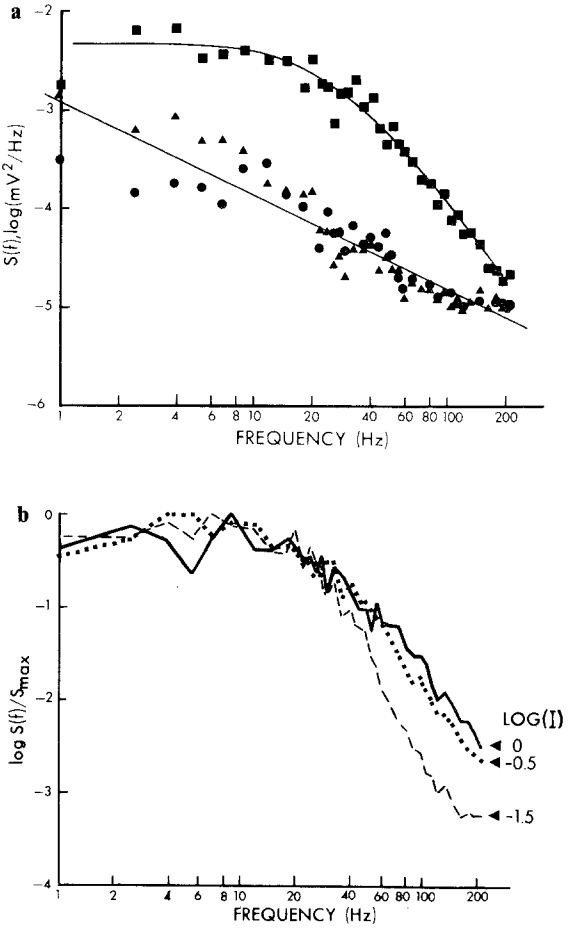

Fig. 11. a Power spectrum (squares) of voltage noise increase recorded during treatment with 40 mM chloral hydrate at a depolarization of 12 mV. The other spectra were recorded before (triangles) and after (circles) anaesthetic treatment. 22° C, **b** Power spectra of voltage noise increase on illumination of the same cell to a steady depolarization. Light intensities: 7×10^{11} photons/cm^2/s (dashed line), 9×10^{12} photons/cm^2/s (dotted line) and 3×10^{13} photons/cm^2/s (continuous line); wavelength 560 nm. The cell was depolarized 5, 12 and 16 mV respectively and the noise variance in each case was 0.7 mV^2, 0.44 mV^2 and 0.20 mV^2. 22° C; 10 s samples

Power spectra of voltage noise created by steady illumination after recovery from chloral hydrate treatment varied in shape, depending on the intensity of light used and hence on the depolarization reached (Fig. 11b). At low light intensities (less than 5×10^{11} photons/cm^2/s) the power spectrum declines at high frequencies as $1/f^3$ to $1/f^4$, while spectra recorded at higher light intensities approach a high frequency roll-off of $1/f^2$ to $1/f^3$. Once this final shape was attained, further increase in light intensity shifted the curve to higher frequencies without change in shape.

The change in spectral shape may be attributed to a shortening of the duration of the current event underlying a bump relative to the photoreceptor membrane's time constant (Dodge et al. 1968; Wong 1978). Shorter, light-adapted photon events have faster rise times than dark-adapted ones and so exhibit a more gradual decline of power at high frequencies. Single photon events therefore get shorter in duration as light intensity increases. The ratio of noise variance to depolarization (and so the photon event amplitude) also decreases at higher light intensities, as reported for other preparations by Dodge

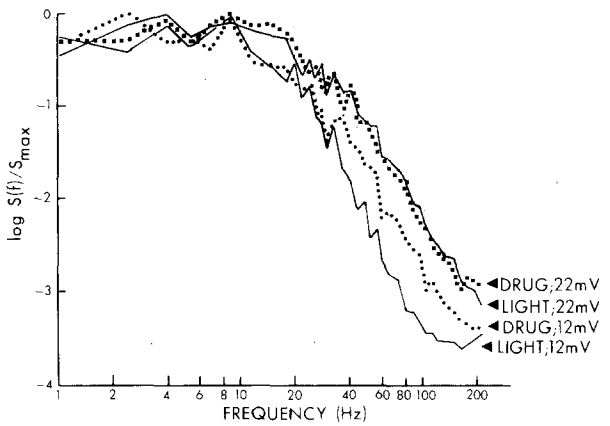

Fig. 12. Power spectra of voltage noise created by illumination (continuous lines) or by chloral hydrate (dotted lines). Spectra were recorded when the cell was depolarized 12 mV and 22 mV by drug or by subsequent illumination. Light intensities used were 7×10^{11} photons/cm^2/s and 3×10^{13} photons/cm^2/s; wavelength 560 nm. Variances of noise were: 0.7 mV^2 (light, 22 mV), 0.4 mV^2 (drug, 22 mV); 1.9 mV^2 (light, 12 mV), 0.5 mV^2 (drug, 12 mV). 22° C; 10 s samples

et al. (1968), and Wu and Pak (1978). The shortening of event timescale and the reduction in size may be attributed to two factors; shunting of the cell input resistance by the light-induced conductance increase, and adaptation of the underlying current events (Wong 1978). Light adaptation shortens and diminishes isolated single photon events of locust photoreceptors (Tsukahara and Horridge 1977), possibly through the latter mechanism.

Figure 12 compares noise produced by illumination of a photoreceptor with that recorded during prior treatment with chloral hydrate. Voltage noise recorded from cells depolarized to a steady level by a light of intensity less than 10^{12} photons/cm^2/s exhibit a power spectral shape that declines more steeply at high frequencies than that of noise from the same cell depolarized to the same extent by chloral hydrate. However, as light intensity is increased to 10^{13} photons/cm^2/s the power spectral shape of light- and drug-induced noise become very similar.

Over the same light intensity range the variance of photon noise decreases to approach that of chloral hydrate noise recorded in the same cell at the same depolarization levels. At a mean depolarization of 14 mV seven cells exhibited a ratio between the standard deviation of photon and drug noise of 1.58 ± 0.38. As the depolarization at which the two types of noise are compared is reduced, the amplitude of photon noise increases with reference to drug noise until, in the fully dark-adapted state, photon bumps of amplitude 500 μV−5 mV can be recorded compared with a predicted drug event size of about 35 μV (Sect. 2.3).

In summary, drug noise is similar to photon noise recorded from a light-adapted cell, *but as the photoreceptor dark-adapts under less intense illumination, photon current events broaden and increase in amplitude while drug*

current events are the same size at all depolarizations. Whether the matching spectra of Figure 12 are a product of the cell membrane time constant or the current event shape has not been determined.

Discussion

(i) Summary of Results

The simple anaesthetic molecules investigated have a complex effect on the electrophysiology of retinula cells. Analysis of the voltage noise accompanying rapid depolarization of receptors induced by these compounds suggests that the depolarization is caused by summed events of amplitude 35 μV. Converted to a conductance increase of 40 pS this event size is in the upper range expected of single ionic channels, $\sim 10^{-11}$ S (Neher and Stevens, 1977). The peak depolarization induced by large doses of anaesthetic, the effects of change in membrane potential by injection of current into the cell, and the relationship of the noise variance to depolarization all indicate an equilibrium potential for ion movements in these channels of 40–65 mV above a cell's natural resting potential. This range is close to that typical of the initial peak transient response to a light flash that saturates a cell's response, V_{max}, and the predicted driving force of the response to steady illumination. Ion replacement experiments suggest that the channels opened are permeable to sodium.

(ii) The Nature of the Elementary Events

Neutral anaesthetics inhibit or facilitate cation movements across a variety of artificial and natural membranes (Armstrong and Binstock 1964; Gage et al. 1975; Gutknecht and Tosteson 1970; Knutson 1961). Such a rapid, large and reversible depolarization, possibly because of an increased sodium conductance, has not, however, been described by these papers. Rather than cause a general leakiness to cations, the voltage noise recorded suggests that pathways to current flow through the membrane are being rapidly opened and closed. Does the voltage noise originate from the light-sensitive rhabdom or from the terminal of the photoreceptors?

The locust retinula cell is about 600 μm in length. The photoreceptive rhabdom extends 400 μm, from the cornea towards the basement membrane of the retina and an axon of length 200 μm continues through the basement membrane to synapse with a cell in the lamina (Shaw 1969). Signals initiated by light are thought to originate in the rhabdomal region and to travel the length of the cell by decremental electronic spread. As signals travel down the cable formed by the receptor cell, the high frequency components of the signal are lost (Review: Shaw 1978). Because the voltage noise induced by alcohols has, at all depolarizations, a faster or similar time course to that induced by light, there is no indication that the drug-induced noise originates further from the micro-electrode impaling the distal end of the rhabdom than light induced noise.

The large drops in input resistance and the rough agreement of the values of the driving force behind drug-induced depolarization, estimated from the effects of current injection and from the depolarization at which noise is maximal, suggest that the electrode was electrically close to the source of voltage noise. Whether such arguments rule out the possibility of the noise originating from synapses on the photoreceptor axon terminal depends on the value taken for the cell's cable length constant. Estimates by Shaw (1969) certainly rule out this possibility. No synaptic connections exist between retinula cells (Meinertzhagen 1976) and no suggestion of powerful synaptic feedback onto retinula cells has been made during previous electrophysiological studies.

Wong (1978) has proposed a model of light adaptation which describes a bump as the slow concerted opening of many ionic channels when a photon hits a dark-adapted cell. In the extreme light-adapted state, however, only one channel is opened by a photon. If the channels opened by light and anaesthetic are identical, then random opening of the channels by anaesthetic would be expected, as reported in Sect. 2.6, to mimic the response to steady illumination of a light-adapted cell. Photon events in a dark-adapted cell would be expected to be slower and larger than the events caused by random opening of single channels by anaesthetic. It is possible that the noise phenomena described in this paper are signs of alterations in the final stage of transduction – the opening of individual channels. Anaesthetics may cause channels normally opened by light to be activated by a rearrangement of molecules in the membrane or by the release of an internal transmitter.

Shaw et al. (1978) have also reported modifications of transduction in barnacle photoreceptors following treatment with alcohols. A sustained, reversible depolarization follows light flashes delivered to a photoreceptor in the presence of alcohols.

While speculation that the same channels are opened by light and anaesthetic is justified by the results described, clearer experiments are needed. It is necessary to localize the site of action of drugs and the source of current noise, and to establish the pharmacological identity of the channels by specifically blocking them. However, no specific blocker (like TTX for voltage dependent sodium channels) has yet been found for the channels opened by light in insect photoreceptor membranes.

Whatever the cause of the phenomena presented here, further investigation of activators and inhibitors of transduction on this preparation will have to account for drug effects not only in terms of the specific chemical reactions of a drug but also in terms of its possible anaesthetic activity. This may be of importance when applying high concentrations of lipid-soluble drugs outside cells in an attempt to modify processes inside.

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