

Correlation of the electrical and intrinsic optical signals in the chicken spreading depression phenomenon

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Abstract

This paper presents some results on the correlation between the electrophysiological and intrinsic optical signals (IOS) of spreading depression waves in chicken retinae. We first show that the peak of the time derivative of the electrophysiological wave occurs precisely when the optical signal reaches the electrode tip. Second, by comparing bath applications of propranolol and glycerol it can be shown that the slow potential shift is not directly correlated to the intrinsic optical signal. Propranolol depresses the amplitude of the electrical wave, although the intrinsic optical signal continues being visible. On the other hand, we observe total absence of the IOS under glycerol, while the electrical wave is always present. Correlations of this kind are relevant for a deeper understanding of the underlying mechanisms of the spreading depression phenomenon. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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The spreading depression (SD) phenomenon was discovered by A. Leão in 1944 [8]. Since then, SD waves have been investigated and successfully elicited in all regions of the gray matter, including the retina, which constitutes its most accessible part. Intrinsic optical signal (IOS) perceptibility of SD is made possible due to transient changes mainly in the extracellular osmolarity, which is a side effect of the massive motion of ions between extra and intracellular spaces. Translocation of ions also yields an electrical profile, which is present in all retinal layers during SD waves [2,3]. Despite the fact that they may share the triggering event, IOS and electrophysiological signals may be separately accessed and individually influenced, as we show here.

We have simultaneously measured optical (reflected light) and electrophysiological signals of SD waves, and their synchronism has been investigated in control experiments as well as under bath application of drugs. The beginning of the brightness profile obtained from IOS is concomitant with the peak of the time derivative of the slow potential shift in all cases.

Analysis of IOS and electrophysiological profiles was performed by treating retinae with propranolol and glycerol. These drugs are found to influence the behavior of SD waves in different ways [12,13], but their form of action and consequences to the retinal tissue are still subject of debate.

Experiments are carried out on 6–12-day-old chickens [5]. Immediately after the chick being decapitated, the eyeball is dissected, cut at the equatorial plane, and the vitreous humour is removed. The eyecup is immersed in a Petri dish perfused at a rate of 4 ml/min with Ringer solution (pH 7.4), containing 0.1 M NaCl, 6 mM KCl, 1 mM MgCl₂, 1 mM NaH₂PO₄, 1 mM CaCl₂, 30 mM NaHCO₃, 30 mM glucose and 5 mM Tris. In experiments with propranolol (0.5 mM) and glycerol (5%), drugs are added to the Ringer solution. Temperature is maintained at 29°C (±1°C). Waves are elicited chemically with 0.1 M KCl (approx. 50 µl applied by means of a micropipette) at the eyecup border every 15 min. Electrophysiological measurements are performed using glass electrodes filled with 1 M KCl (10 µm tip diameter) positioned in the inner plexiform layer. The reference electrode is a silver chloride pellet electrode, positioned in the bath surrounding the eyecup. Electrical signals are low-pass filtered at 10 Hz, and digitally recorded.

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Optical signals are recorded in video. Electrical and optical recording systems are synchronized by an audio signal (one pulse) common to both acquisition systems. The microelectrode becomes negative in respect to the reference electrode during SD wave propagation [11].

Concerning electrical signals, the derivative of the function with respect to time is computed. At particular instants of time, selected from the electrical wave, snapshots are taken from the optical images, and a 30–40 pixels (corresponding to 0.20–0.27 mm of retinal tissue) wide strip oriented normally to the wavefront is cut. Its brightness profile is then obtained by computing the densitometry in each column [1]. Optical wave onset is considered as a 10% change in brightness as compared to the maximum attained brightness of the wave. Electrical wave duration is measured from the onset (5% as compared to the maximum) of the potential shift until the returning of the signal to the baseline as considered after the wave has propagated [11].

Amplitude of the electrical signal was found to be directly correlated to the speed of the optical wave, as measured from the IOS (that is, wavefront speed). In control experiments ($n = 51$), while speed varied from 1 to 5 mm/min, amplitudes varied from 10 to 25 mV, showing a correlation coefficient of 0.8. Moreover, derivative and amplitude have shown a correlation coefficient of 0.85. The mean value of the amplitude was found to be 19 ± 1 mV ($P < 0.05$) and the mean speed was 3.4 ± 0.2 mm/min ($P < 0.05$). Both measures agree with already known values, namely, 20 mV and 3 mm/min [10]. We found the peak of the derivative to be 17 ± 8 mV/s ($P < 0.05$), occurring always before the electrical wave peak.

Figs. 1 and 2 show electrical and optical concomitants from a wave with bath application of propranolol. Time 0 is arbitrarily chosen, before the wave onset. The optical wave spreads in the direction of the electrode tip, which is reached at 15.7 s. This is exactly the instant of time of the derivative peak, as taken from the electrical wave. This fact is noticed in all analyzed waves, either in controls or under the effect

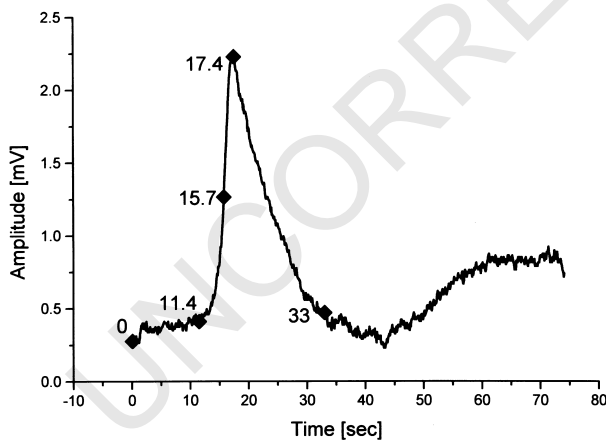


Fig. 1. Slow potential shift of a wave under the effect of propranolol (amplitude 2 mV). Numbers in seconds (see Fig. 2). Polarity: negative up.

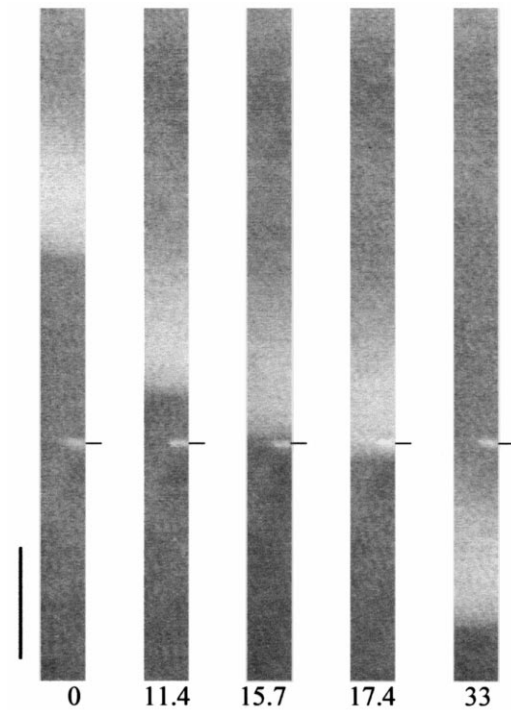


Fig. 2. Strips taken from the optical wave from Fig. 1, moving downward. Scale bar: 0.5 mm (75 pixels). Numbers in seconds.

of drugs. Fig. 3 shows brightness profiles of two waves (one of them is the wave from Fig. 2), both evaluated at the instant of time where the derivative reaches its maximum value. In addition, brightness profiles taken at 0 s are presented in order to exhibit the electrode tip position relative to the waves. Note that background brightness is distorted by the pipette. It is clear from this figure that the

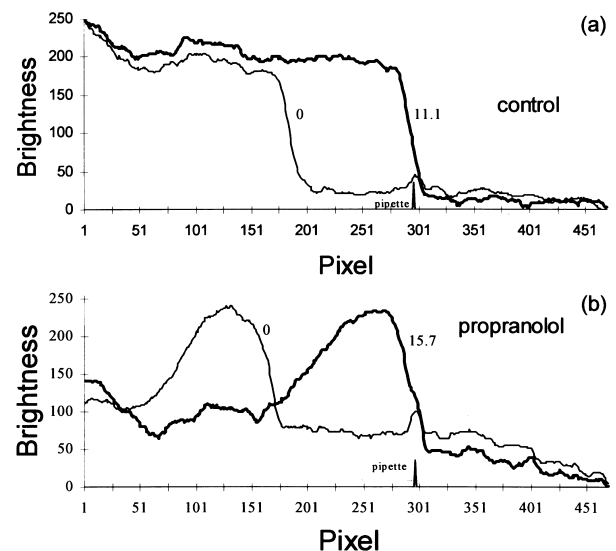


Fig. 3. IOS brightness profiles (a) of a control spreading depression wave and (b) of a wave with bath perfusion of propranolol. Pipette position is indicated on the picture. Both waves are moving to the right. Numbers near the curves in seconds.

beginning of brightness change coincides with the pipette position. This analysis has been performed in 51 control waves and 30 waves under the effect of drugs. With a precision of 0.05 s and 0.02 mm as set by the experimental apparatus, IOS onset happens simultaneously to the electrical wave derivative peak.

The optical wave recovers faster under the effect of propranolol. In Fig. 3a, a profile taken from a control wave at 11.1 s occupies most of the retinal area (in this case, approx. 300 pixels, or 2 mm). On the other hand, under perfusion with propranolol, even during the same experiment, the complete optical profile can be visualized inside the screen area, as shown by Fig. 3b at 0 s and at 15.7 s. Moreover, the end of the electrical wave is, in this case, concomitant with the restoration of the background brightness (data not shown).

Under the effect of propranolol, amplitude of the electrophysiological signal is depressed to 48% and the derivative peak to 38% ($n = 10$) of the control waves, whereas the speed is dampened to 83%. Inside the same group of waves (i.e., those under the effect of the drug) the correlation coefficient between amplitude and derivative is 0.96 ($n = 10$). There is no alteration on the electrophysiological waveform by comparing waves in the same experiment.

Bath application of glycerol completely depresses the IOS. Although the electrical signal remains, speed cannot be measured by the previously described method. In roughly half of the 22 waves analyzed in our experiments, the electrical profile showed an alteration in form, which is the case in Fig. 4 (notice the plateau and the second peak of the wave). In these profiles we consider the first peak with the purpose of evaluating amplitude. Amplitude is lowered to 45% ($n = 11$) of the control values, while the derivative peak drops to 67%. Duration is lowered to 88% ($n = 11$) of the control waves, despite the presence of the two peaks.

Retinae are very sensitive to the application of propranolol, which is not the case with some other drugs [12]. This implies that in the same experiment it is very difficult to

obtain two waves under the effect of propranolol. Nevertheless, the recovery of the retinal tissue is usually obtained by perfusing them with standard Ringer. Status of the retina is then judged from the optical composition of its background.

Propranolol is commonly considered to be an anti-migraine drug, besides many other possible uses. It is also known as a non-selective beta-adrenergic receptor-blocking agent [7]. Although its mechanisms of action are not well established [4], due to the fact that its effect on the electrical profile is much more pronounced than on the IOS, we conjecture that the main influence of propranolol occurs at the early phase of ionic translocation, that is, during intense neural excitation.

Changes of IOS associated with neural stimulation have usually been attributed to cell volume and extracellular space alterations [9]. Because of its high viscosity, glycerol obstructs the water movement between the intra and extracellular media, and we conjecture that during the occurrence of the SD this is the main cause for the depression of the IOS. Also the higher osmolality of the perfusion solution can be an explanation for that, and for the form alterations observed on the electrical profiles. We would like to point out that other optical waves can be devised [6]. Because the electrical concomitant is still present, one can show the existence of ionic reorganization during wave propagation.

Our results show that both propranolol and glycerol decreased the excitability of the retinal tissue. Amplitudes of the electrophysiological concomitant were lowered to 45 and 48% in the case of glycerol and propranolol, respectively. Derivative peak dropped to 67 and 38%, respectively.

Based on the electrical waveforms in control waves and under drug application we hypothesize that the processes taking place at the cellular level (ionic changes and depolarization) do happen on a much faster time scale for the spreading of the excitation than for the reorganization of the ionic composition of the extra and intracellular spaces.

Concomitant recording of optical and electrophysiological signals during retinal SD waves suggests the existence of separated physical processes underlying macroscopic variables. Field potential appears to be an important modulator of propagation velocity. By contrast, the IOS seems to be the result of volume alterations at the cellular level.

Concerning form alteration in the case of the electrophysiological signal with glycerol, such an alteration has not been observed in any of the control waves, or in the waves under the effect of propranolol. It remains to be investigated whether it can be a function of concentration, of the relative alteration in osmolality or of other experimental parameters such as temperature, perfusion rate or composition of the Ringer solution.

Simultaneous recording of electrophysiological and optical parameters of retinal SD waves permits a demonstration of the spatio-temporal relationship between the part of the ionic flux responsible for the electrical concomitant of SD waves and the intrinsic optical changes associated with

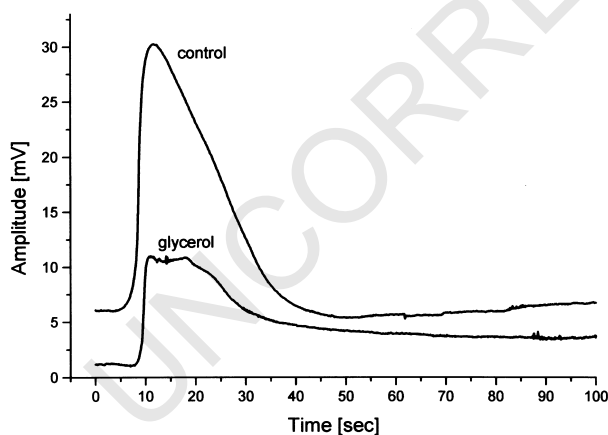


Fig. 4. Electrophysiological profiles of a control wave (amplitude 24 mV) and of a wave under the effect of glycerol (amplitude 10 mV). Polarity: negative up.

them. The electrode becomes sensitive to the electrical field 3–4 s before the region is invaded by the optical changes. At the derivative peak of the electrical wave the IOS onset is at the pipette tip. This is a confirmation of the customarily accepted assumption that the sensitivity of the electrode occurs in a spherical volume. It also suggests that the strength of the derivative peak, that is, the coherent ionic flux, is a relevant component of propagation mechanisms: blockage of the field depresses speed of propagation and lowering of the peak derivative is compatible with smaller amplitudes. As we have shown, the three above-mentioned parameters are not independent, but the functional relationship among them remains an open question.

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