Spreading Depression: Investigating This Complex System

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Abstract

We present here two manipulations done on the perfusion solution during the propagation of the spreading depression waves in the in-vitro chicken retina. For the first time, the electro-physiological and optical signals were acquired simultaneously. Comparing both of them, we show the relationship of the characteristic slow potential shift to the brightness profile and to the propagation velocity. By applying barium and barbiturate to the perfusing solution it was possible to demonstrate that the electrical and optical waves are not directly correlated.

1: Introduction

The phenomenon of the spreading depression is a biological experimental model for waves occurring in excitable media. Other commonly investigated systems are the Belousov-Zhabotinsky waves, in Chemistry, and the Bénard instability, in Physics. In all of them it is possible to devise three phases, namely, quiescent, excited and refractory. Besides that, the presence of a positive feedback or auto-catalysis is also a relevant feature, which takes place in at least one process in the system.

The SD waves have been successfully elicited in all parts of the central nervous tissue, including the retina, which constitutes the most accessible part of the central grey matter in vertebrates. The retina is particularly suitable to devise the intrinsic optical signal (IOS) with naked eye, because of the natural transparency of the tissue and the presence of a black background (the pigment epithelium). Moreover, it is naturally separated from the cerebral cortex and divided in layers. The chick retina has a further advantage, it is avascular, what helps a lot with its maintenance *in vitro* for several hours.

The IOS perceptibility of SDs, to wit, the milky wave visibility, is made possible due to changes in the osmolarity and to the massive motion of ions between the extra and intracellular media. The wave is the outcome of several concomitant phenomena taking place at the cellular level, which, if we could get to know each one, would be extremely easily interpretable events. Although governed by (presumably simple) natural laws, the SD is considered to be a complex system, because of the strong dynamic and non-linear interactions between its inumerable elements. The previsibility of the waves helps one to disentangle its most relevant features, whereas the infeasibility to separately analyze each concomitant phenomenon plays against it.

One way out of this dilemma is to maneuver some processes which occur locally, that is, at the cellular level, and scrutinize the global result. We propose here two different manipulations performed in chicken retinas, namely, the treatment of the *in vitro* retinas with barium chloride and with barbiturate. We simultaneously measured the optical and electro-physiological signals, aiming a greater comprehension of the synchronism between both of them. We were able to compare the slow shift potential, velocity and brightness profiles of the waves.

The depression of the amplitude of the slow potential shift and of the velocity during the retinal SD under the effect of barium have been shown in [1]. Although the majority of glial potassium channels are blocked with the application of barium, we hypothetize that, yet in this casi, the massive movement of ions and the shrinkage of the extracellular space happen, producing an optical amplitude as clear as the control waves.

The mechanisms through which barbiturates act on any neural tissue are not unambiguous [2]. An increased electrical excitability with low concentrations of this substance has been reported, and also a depression on the excitability for longer periods or higher doses. Albeit frequently employed as a sedative and antiepileptic drug, concerning the SD the barbiturate seems to increase excitability and the velocity of propagation of the waves, not interfering with the optical profile, though.

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2: Material and Methods

The experiments were carried out on 6 to 12 days old chicken. The procedures used here have been described elsewhere [3]. Briefly, the chick is decapitated, the eye is cut at the equatorial plane and the vitreous humour removed. The posterior chamber is immersed in a petri dish perfused (at a rate of 4ml/min) with Ringer solution containing 0.1M NaCl, 6mM KCl, 1mM $MgCl_2$, 1mM NaH_2PO_4 , 1mM $CaCl_2$, 30 mM $NaHCO_3$, 30mM glucose and 5mM Tris; pH is adjusted to 7,4. In the experiments with barium, $BaCl_2$ (to a final concentration of 0.5mM) is added to the perfusing solution, and the same is carried out with barbiturate (natrium-barbital), at a concentration of 0.5mM. The temperature is mantained at 29°. The waves are elicited chemically (with 0.1M KCl) each 15 minutes.

The electro-physiological measurements are performed in the IPL (inner plexiform layer), using glass micro-electrodes to extracellular recording (tip of 10 to 20μM). The electrical wave is recorded with a 12-bit A/D converter, and stored at a PC-486 computer. The optical signal is recorded in video, for later digitization and analysis. At least two control waves are acquired while the retina is perfused with standard Ringer solution, and then it is treated with either barium chloride or barbiturate. After the solution has been changed twice, more waves are obtained. At last, the retina is treated with standard Ringer one more time, again at least a control wave is acquired, and the recovery of the tissue verified. The synchronism between the two waves is accomplished through the recording, with a microphone plugged in the video, of the sound of the key pressed to initialize the A/D convertion.

The electrical signal is examined and the following data obtained: duration, amplitude and peak of the derivative (time and value). At the instants taken from the electrical wave, the respective frames are acquired from the optical images, and digitized to 256 grey levels. The brightness profile of a strip 30 pixels wide is evaluated. Then, to enhance contrast, mapping of the strip to the whole range of gray levels (256) is usually applied.

3: Results

3.1: Barium

During the perfusion with barium, seventeen waves were acquired. Seven of them presented a complete depression of the extracellular slow shift potential (Fig. 1), while the others exhibited a partial depression (Fig. 2), with mean amplitude of 6.6 mV. In order to evaluate the depression in amplitude, we compared each set of experiments separately, to wit, each barium wave was compared uniquely to the mean values obtained for the control waves of the same retina. We found that the amplitude of the electrical signal was depressed to 39 ± 11% ($p<0.05$).

The peak of the derivative was also strongly depressed, showing a mean decline to 31% (n=10) of the control values. On the other hand, the duration of the waves exhibited even a slight increase, when compared to their own control values (102 ± 20%). These results suggest a lower synchronism for potassium to get out of the extracellular medium, admitting the hypothesis that the potential shift is due to the clearance of potassium, promoted by glial cells.

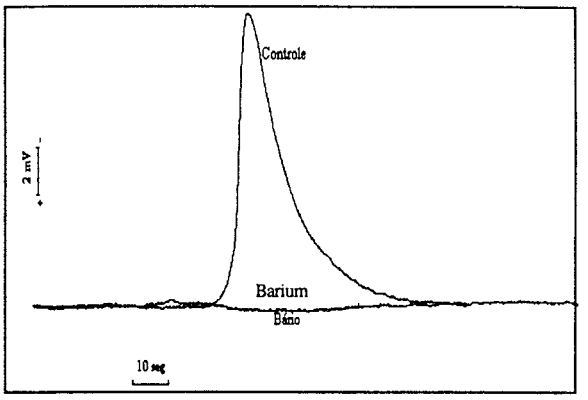

Figure 1. Electrical signal - control wave and under the effect of barium chloride (0.5mM). Amplitudes are indicated.

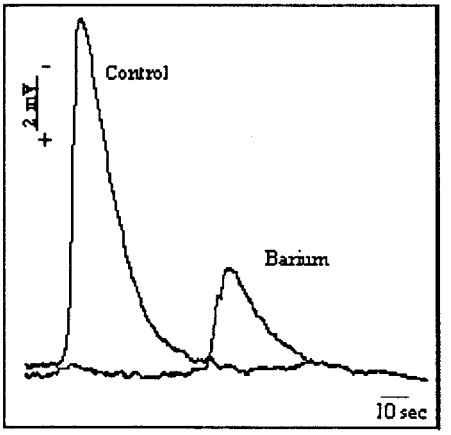

Figure 2. Electrical signal - control wave and under the effect of barium chloride (0.5mM). Amplitudes are indicated. Numbers are in seconds (see text)

Fig. 3 shows the brightness profiles of the waves from Fig. 2. The numbers are the instants of time when the profiles were evaluated. They correspond to the relevant points in the slow shift potential, that is, to the beginning of the signal acquisition (marked 0), the starting of the electrical wave, the peak of the derivative, the peak of the electrical signal, and finally the return to the base line. The IOS has never been depressed completely during the perfusion with barium, even when there was no slow potential shift. In the majority of the waves, it presented an amplitude comparable to the control waves, as exhibited in Fig. 3. The most affected parameter was the wave velocity, determined from the recordings in video. The control waves presented an average of 2.8 ± 0.9 mm/min (n=40), and with the perfusion of barium, 0.9 ± 0.2 mm/min (n=10). Because of the low variation in the optical profiles we can conclude that the characteristic potential drop in the extracellular space and the massive ion exchange between extra and intracellular space are not linearly related, probably the ionic current responsible for it represents a small part of the ionc shift. There was apparent correlation between the propagation velocity and potential drop, more particularly with its time derivative.

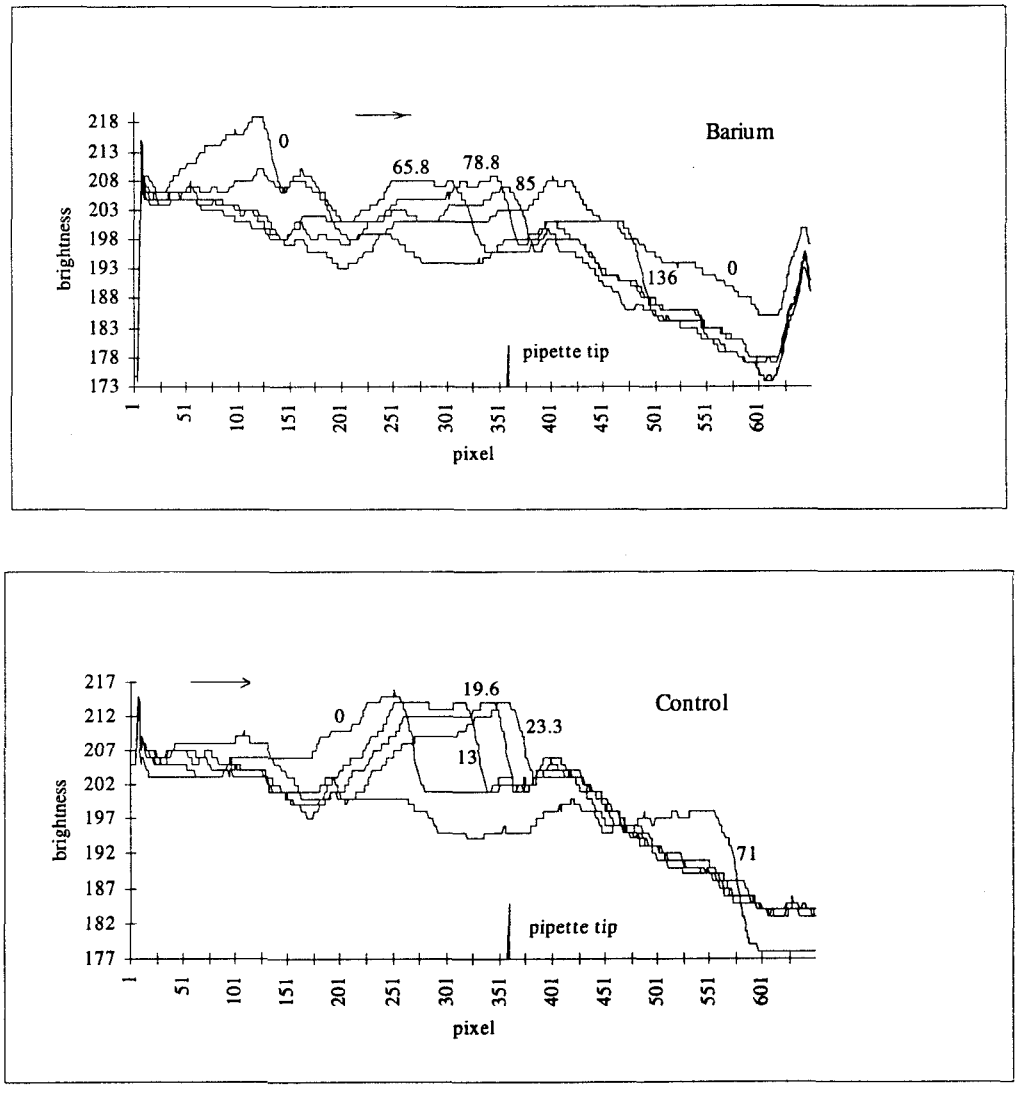

Figure 3. Brightness profiles of the waves from Fig. 2. Arrow denotes the direction of the wave.

3.1: Barbiturate

In [4], the influence of natrium-barbital on the retinal SD is analyzed for several concentrations of that substance, using the same methods described here. The most effective concentration was 0.5mM, and with it an increase of 20% on the wave velocity was verified.

The waves acquired during the perfusion with barbiturate showed an average amplitude of 111 ± 28% ($p < 0.05$), when compared to their control waves. Figs. 4 and 5 present two examples of such waves. Notice the different forms of the two waves with barbiturate. The peak of the derivative exhibited the same behaviour, always related to the amplitude of the potential shift. Furthermore, a marked augment has been observed in the velocity of spread, evaluated to 3.6 ± 0.8mm/min (n = 7). Comparing seven waves acquired under the effect of barbiturate to the control waves, what, in this case, is reasonable, given the relative low deviation, there is a mean increase of 18% in the velocity.

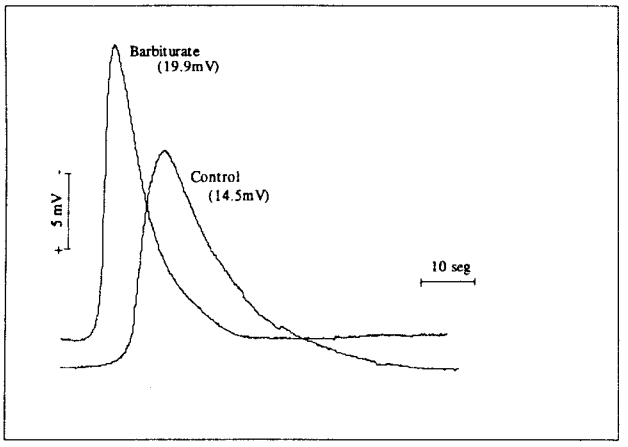

Figure 4. Slow potential shift of a control wave and during perfusion with natrium-barbital. Amplitudes are indicated near the waves.

In Fig. 6 we show the brightness profiles of the slow potential shifts from Fig. 5. The profiles are very similar, and the same behaviour has been observed on the other waves.

In two out of three trials, when the drug was applied we could identify an increase in amplitude followed by a decrease. This fits well to the mechanism of action of the barbiturate on neural tissues, as mentioned above, but it remains to be investigated in the future.

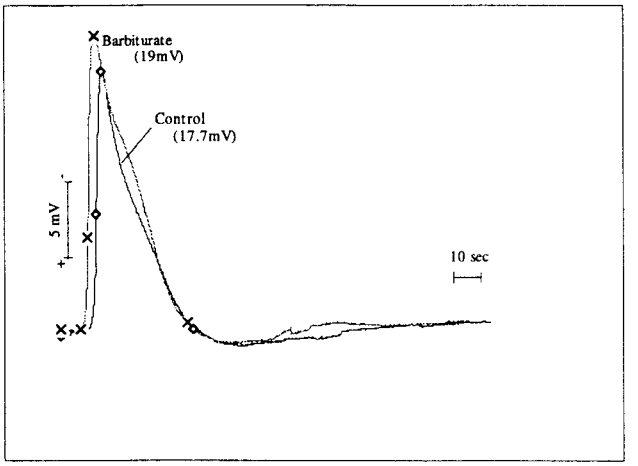

Figure 5. Slow potential shift of a control wave and during perfusion with natrium-barbital. Amplitudes are indicated near the waves.

4: Discussion

The simultaneous recording of electrophysiological and optical parameters of retinal spreading depression, RSDs, permitted an undoubtful demonstration of the relationship between the part of the ionic flux responsible for the electrical concomitant of RSDs and the intrinsic optical changes associated with them. It is clear that the electrode senses the electrical field 3 to 4 seconds before the region is invaded by the optical changes and that at the peak of the derivative of the electrical wave the IOS is at the pipette tip. This is a confirmation of the customarily accepted premise that the sensitivity of the electrode is a spherical volume. The time course of recovery appears to be independent of the two parameters analysed here. If the time derivative is interpreted as the resultant coherent ionic movement (resultant sink) in the extracellular space, then it follows that only a small part of the total flux gives rise to the field potential. It also seems that the the strength of the peak of the derivative, i.e., coherent ionic flux is a component of the propagation mechanisms: blockage of the field depresses velocity of propagation and increases in the peak derivative is compatible with higher velocities. The two parameters are not independent but the functional relationship between them is still an open question.

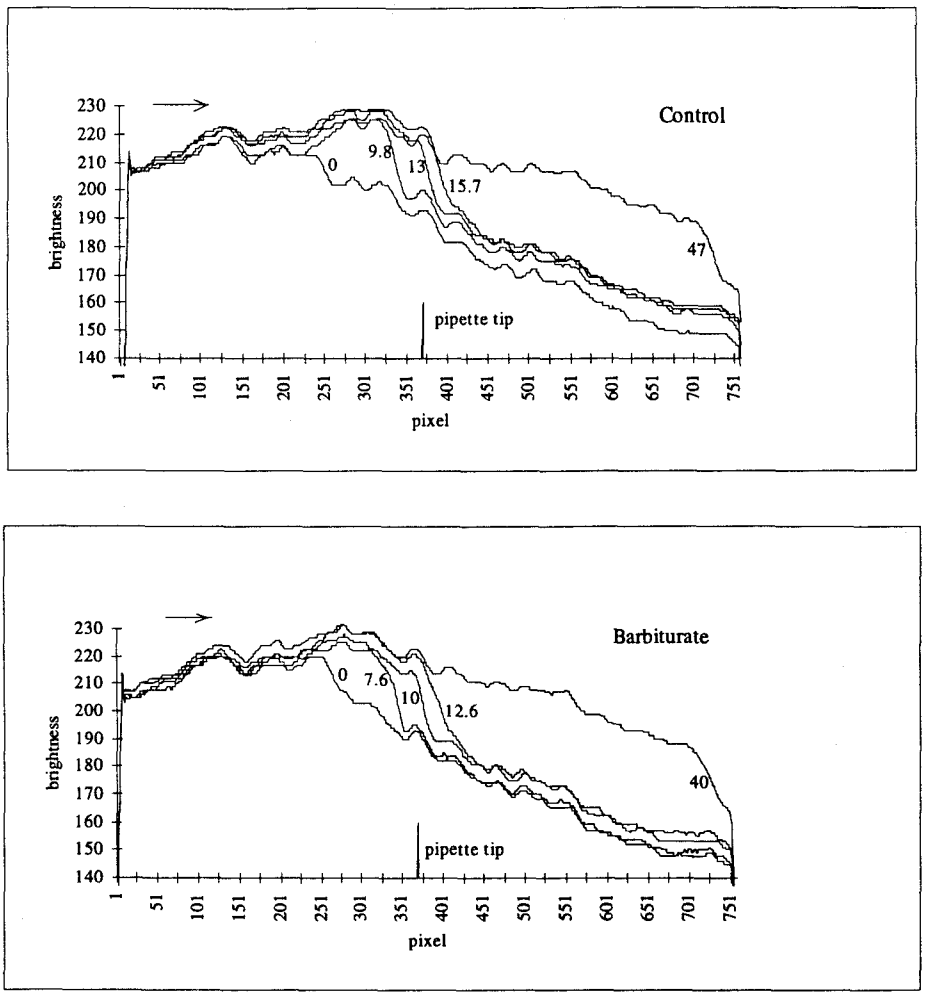

Figure 6. Brightness profiles of the waves from Fig. 5. Arrow indicates the direction of the wave.

5: Conclusions

Concomitant recording of optical and electrophysiological signals during RSDs permitted the demonstration of the separation of physical processes behind these macroscopic variables. The field potential appears to be an important modulator of the wave propagation. On the other hand, the IOS appears to follow the triggering events, and hence is poorly correlated to the propagation velocity.

6: References

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