Central nervous tissue: an excitable medium. 
A study using the retinal spreading depression as a tool

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According to its physicochemical properties, neuronal tissue, including the central nervous system (CNS) and thus the human brain, is an excitable medium, which consequently exhibits, among other things, self-organization, pattern formation and propagating waves. Furthermore, such systems can be controlled by weak external forces. The spreading depression (SD), a propagating wave of excitation–depression, is such an event, which is additionally linked to a variety of medically important situations, classical migraine being just one example. Especially in retinal tissue, a true part of the CNS, the SD can be observed very easily with the naked eye and by video imaging techniques due to its big intrinsic optical signal.

We have investigated the retinal SD and its control by external physical parameters such as gravity and temperature. Beyond this, especially due to its medical relevance, the control of CNS excitability by pharmacological tools is of specific interest, and we have studied this question in detail using the retinal SD as an experimental tool to collect information about the control of CNS tissue excitability.

Keywords: retinal spreading depression; excitable media; small external forces

1. Introduction

The visual scotomas of migraine and the spreading depression (SD) in neuronal tissue have been studied since the 1940s (Lashley 1941; Leao 1944). Their relation and the medical relevance of SD then became obvious in the late 1950s (Milner 1958). Their interpretations in terms of nonlinear thermodynamic theories started somewhat later (for a summary see: Fernandes de Lima et al. 1999) and among other things led to the interpretation of the CNS and thus the human brain being an excitable medium, with the consequence that its behaviour can be controlled by small external forces (Hanke et al. 2001). Such forces can be of physical (i.e. temperature or gravity) or chemical nature (i.e. pharmacological active drugs). The latter point is becoming more and more important under medical aspects, as it has been proven that the SD is related to a variety

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of pathophysiological events in the human brain, including for example classical migraine, stroke, transient global amnesia and certain forms of epilepsy (Welch et al. 1990).

Especially, the retinal SD in chicken eyecups has been proven to have a variety of experimental advantages, especially due to the enormous intrinsic optical signal (IOS) accompanying this event, which allows its study by classical video imaging techniques (i.e. Fernandes de Lima et al. 1999). We have used the intrinsic optical signal of the retinal SD to investigate the dependence of SD parameters on physical and chemical control mechanisms.

In this paper, we want to outline some general aspects of the IOS of the retinal SD and its mechanistic basis as a useful tool to study the control of brain excitability by weak external forces. Some mechanistic aspects can be assigned to the IOS, as shown in figure 1.

Thus we want to show in some examples how parameters of the retinal SD and especially the IOS change under the influence of small external physical forces, especially gravity, and of some pharmacologically important substances, and how conclusions can be formed about changes in cellular, membrane and molecular actions. Finally, we will briefly outline how these changes might be interpreted in mechanistic terms.

2. Materials and methods

In brief, 1-day-old chickens were bought at a local dealer and kept in our facilities until use. The retinas of 7- to 21-day-old chickens were examined in the experiments. The chickens were killed by decapitation and the eyes were removed from the skull immediately. Then the eyes were cut parallel to the equator, the vitreous humour was removed and the posterior part (eyecup) with the retina was transferred to a Petri dish, typically containing the following Ringer solution: 100 mM NaCl; 6 mM KCl; 1 mM MgSO$_4$; 1 mM CaCl$_2$; 1 mM NaH$_2$PO$_4$; 30 mM

\[
\text{mechanism} \quad \text{phase of IOS}
\]

\begin{itemize}
  \item set-up of SD wave and breakdown of ion homeostasis
  \item re-establishment of ion gradients
  \item consumption of ATP by pumps
  \item re-establishment of ATP level
  \item consumption of glucose
\end{itemize}

Figure 1. Mechanistic interpretation of the different phases of an intrinsic optical signal of a spreading depression wave in retinal tissue. The typical duration of the event is approximately 15 min and is given as a function of brightness of tissue over time. IOS, intrinsic optical signal.
NaHCO₃; 10 mM Tris; and 30 mM glucose. The pH was adjusted to 7.4 by titration with HCl. The eyecup was glued to the bottom of a Petri dish of 8 ml volume with cyanoacrylate glue; the dish was perfused continuously with 1–4 ml min⁻¹ during the entire experiments. The temperature of the dish was kept constant at 30°C. Drugs were added to this Petri dish in concentrations as given in §3. More details about the preparation can be found in Fernandes de Lima et al. (1999).

Retinal SD waves were elicited mechanically by gently touching the retina with a fine tungsten needle. The retina was recorded with a colour video camera from before the initiation of the wave up to 30 min after wave appearance, and the data were stored on a DVD recorder or a computer by a frame grabber for later evaluation of the velocity of the propagating waves, some features of the IOS, and the time between stimulus and wave onset.

Changes of gravity were applied to the system either by a laboratory centrifuge (macro-g at values up to 8g and for periods up to some 10 min, even hours, are possible) or by micro-g facilities provided by the German space agency, parabolic flights with micro-g periods of approximately 25 s, and sounding rocket missions with micro-g periods up to approximately 6 min (Hanke et al. 2006; Wiedemann et al. 2006). In addition, the parabolic flight delivers 1g control phases and 1.8g macro-g phases, and the sounding rocket during the launch period delivers up to 12g.

3. Results

To demonstrate the experimental basis of our experiments, in figure 2 a series of photos of a propagating retinal SD wave after a mechanical stimulus are shown. From this montage, a velocity of the propagating wave of approximately 4 mm min⁻¹ can be extracted. Additionally, the time interval between stimulus and wave onset can be taken from such recordings, reflecting the excitability of the neuronal tissue (Weimer & Hanke 2005). Either from such consecutive photos of a video recording or by directly recording the brightness of a spot of defined size of the retinal tissue as a function of time, using for example a photomultiplier, an IOS as shown in figure 3 can be measured. The typical transient (this means, intact tissue after a certain time completely recovers from the effects of the passing wave) two-peak structure of this IOS delivers indirect information about a variety of mechanistic events concomitant with a propagating SD. The rising phase of the first peak mainly reflects the opening of ion channels (resulting in osmotic changes) due to which the ion gradients across the membranes are significantly reduced. In intact tissue, metabolic energy is present, and thus the falling phase of the first peak of the IOS mainly reflects the re-build-up of the ion gradients by the action of pumps which use ATP. This can be further verified by recording the electrical extracellular potential during the passage of an SD wave with a glass microelectrode. Such a recording is also given in figure 3 on a somewhat extended time scale. As can be seen in the electrical recording directly, during wave passage a transient depolarization of the tissue is given which is re-established on a minute time scale. These electrical events are almost parallel in time and shape to the first peak of the IOS of a propagating SD wave, as can be seen by a direct comparison of the two traces presented in figure 3.

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Figure 2. Series of photos with a time difference of 2 s between consecutive frames, showing the propagation of an SD wave in a retinal eyecup after a mechanical stimulus. The stimulus is marked in the figure, and the wave itself can be seen as a milky circle with increasing diameter, which is centred around the place of stimulation.

(a) relative brightness

(b) relative potential change (mV)

Figure 3. (a) A complete retinal IOS recorded with a photomultiplier during the passage of an SD wave. It has two peaks, the first reflecting mainly membrane transport processes lasting about a minute, and the second lasting some 10 min and reflecting mainly metabolic processes. The stimulus artefact can be seen at approximately \( t=2 \) min. (b) The recording of the extracellular potential of an SD wave, as recorded with a glass microelectrode. It is obvious that the measured depolarization according to its time scale qualitatively goes together with the first phase of the IOS.
In the IOS, a significant second peak can then be found, lasting for some 15 min in the presented example, which mainly reflects metabolic events (Dahlem & Hanke 2005) including the re-build-up of the ATP level after a period of increased ion pump activity with high ATP consumption. This second IOS peak has no equivalent in the electrical recording, where only a single peak can be seen.

A detailed study of the relation between IOS and electrical recordings is in progress and will deliver further information about processes during the SD wave, as well as a study of the spectral distribution of the IOS when taken under white illumination.

Each of the parameters of propagating waves, the IOS and the electrical recordings briefly discussed above and a variety of other parameters of such waves that are not discussed here depend on the influence of external forces of either physical or chemical nature. In the following, two such physical examples will be briefly presented.

A physical parameter always present on Earth is gravity (1\(g\)), but micro-\(g\) or macro-\(g\) can significantly influence neuronal tissue as has been tested using the retinal SD (Wiedemann et al. 2005; Hanke et al. 2006) in parabolic flights and sounding rocket missions as well as in a laboratory centrifuge. It has been shown that the waves significantly slow down under micro-\(g\) and accelerate at higher \(g\)-values. Additionally, the latency—the time interval between stimulus and wave onset—becomes much longer under micro-\(g\). As discussed (Weimer & Hanke 2005), this points to a lower excitability of the neuronal tissue under micro-\(g\) conditions; in a somewhat populist view, attacks of classical migraine, for example, will be less probable under micro-\(g\) conditions. Nevertheless, for future long-lasting manned spaceflights, such effects on neuronal tissue might be of importance.

In studies investigating the influence of gravity on the oscillating chemical Belousov–Zhabotinsky reaction (Belousov 1959; Zaitkin & Zhabotinsky 1970), which, in a variety of parameters and in the theoretical framework used to describe it mathematically, is very similar to the SD, comparable results were found (Wiedemann et al. 2002).

A medically and pharmacologically even more important question is about the influence of drugs on neuronal tissue. Here, especially the influence of substances on intact tissue has to be studied, as it can be completely different from single cell results. Besides the retinal SD model, not very many other tissue models are known that can be used in pharmacological studies.

One possible classification in such experiments is to distinguish between excitatory, inhibitory and modulating substances. Indeed, excitatory substances, e.g. acetylcholine (Sheardon 1997) or glutamate (see later), often even induce SD waves but usually have only smaller additional effects on wave parameters at low concentrations, whereas inhibitory substances, i.e. ketamine, usually reduce wave activity, prolong latency and slow down the velocity of propagating waves. Additionally, a variety of inhibitory substances have been shown to be neuroprotective, whereas excitatory substances often are also neurotoxic (Lipton & Rosenberg 1994; Wiedemann & Hanke 1997).

It is discussed in the literature that substances being effective on glutamatergic receptors may possibly be important in medical therapy, for example related to stroke events. De facto, a variety of such substances have even been used pharmacologically in the field for many years (i.e. Buchan et al. 1991, 1993;...
Eng et al. 1992). In the retinal SD, a typical glutamatergic agonist, usually stated to be an excitatory substance, namely NMDA, has been shown at moderate concentrations to only slow down SD velocity slightly, to have no significant effect on the latency and to only moderately modify the IOS at lower concentrations. However, as we have investigated in detail in our laboratory, NMDA is strictly neurotoxic at higher concentrations in retinal tissue (Piffel 2005).

A substance similar in its action to NMDA and which is an NMDA-receptor agonist, chinolinic acid, at moderate concentration, for example, induces repetitive spontaneous waves in retinal tissue (figure 4), indicating, among other things, a significant change in tissue excitability, without being as neurotoxic as NMDA, at least at moderate concentrations. This is a somewhat typical behaviour for the action of the majority of excitatory substances on the retinal SD.

Ketamine, a blocker of glutamate receptors (NMDA-receptor antagonist), thus being an inhibitory substance, on the other hand, has drastic effects on all parameters of the retinal SD as shown in figure 5 in an IOS recording without and with the substance. Besides reducing the amplitude of both peaks of the IOS, ketamine also changes the relative proportion of the first peak compared with the second one, indicating a reduced membrane transport activity during passage of the wave compared with the control situation. Additionally, ketamine reduces the propagation velocity of retinal SD waves in a concentration-dependent manner (at 300 μM about 50% reduction); however, it does not significantly change the latency of waves.

Ketamine is medically and pharmacologically used in anaesthesia, stroke and trauma treatment and as a hallucinogenic drug. Again, a lot of inhibitory substances behave comparably in retinal SD experiments.

As has been mentioned already above, additional information can be obtained from the optical signals, especially, the latency of the appearance of the wave after a given stimulus. The situation is depicted in some detail in figure 6.
A line through a video frame is stacked from consecutive frames in time, which allows one to observe wave propagation in a condensed two-dimensional presentation.

Figure 5. Action of ketamine, a glutamatergic antagonist, on the IOS of the retinal SD is shown. (a) Illustration of the control IOS; (b) control IOS after the addition of the drug. As can be seen, ketamine significantly changes the IOS, mainly reducing the amplitudes of the two peaks and modifying the relation of the first to the second peak.

Figure 6. Principles of a stack of a propagating SD wave in retinal tissue. The latency is the difference in time between stimulus and appearance of the wave. Some more details about the interpretation of such a stack are given in the text.

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The latency has been shown to be coupled with the excitability of the tissue (Weimer & Hanke 2005). Of specific medical interest in migraine research is the fact that attacks of classical migraine that are the consequence of SD waves in the CNS (i.e. Milner 1958; Welch et al. 1990) usually occur spontaneously on unknown low stimuli. A reduction of the excitability would simply reduce the probability of a migraine attack. Thus, one aim of pharmacological research is to find substances that reduce CNS excitability or in terms of our experiments prolong the latency of SD waves. An example is given in figure 7, where it is demonstrated that acetylsalicylic acid (ASA, also known as aspirin) introduces a significantly longer latency (and reduces propagation velocity of SD waves). As a consequence, it can be argued that application of ASA is not only analgesic, but can also be used prophylactically against classical migraine.

4. Discussion

As briefly shown in §3 the retinal SD is an excellent tool to study the excitability of neuronal tissue and its control by small external forces. Especially, the big IOS of the SD wave in the retinal tissue makes its observation by classical video imaging technology quite easy. Typically in a first simple approach, the velocity of retinal SD waves can be measured as a function of external impact, and we have shown, for example, that the velocity is reduced under microgravity. This might be a consequence of changed ion channel behaviour in the cellular membranes, as has been found in other experiments (Wiedemann et al. 2003). A similar behaviour has been found with propagating waves in other excitable media, for example the special Belousov–Zhabotinsky reaction (Wiedemann et al. 2002), or with propagating action potentials (Meissner & Hanke 2005). Additionally, our experiments have shown that the excitability of neuronal tissue itself is affected by gravity.
It is obvious that the typical control mechanisms for neuronal tissue are of chemical nature (Fernandes de Lima et al. 1999). Thus, the application of substances should also change their behaviour in terms of nonlinear thermodynamics and consequently parameters of the retinal SD should depend on the addition of drugs, which has been demonstrated indeed in detail (Fernandes de Lima et al. 1999). Here we have just argued in terms of a classification of drugs that one group raises excitability, mainly excitatory neurotransmitters, which on the other hand have often been shown to be neurotoxic at high concentrations. Antagonists of these excitatory neurotransmitters usually have much more pronounced effects on the parameters of the propagating SD waves. A very basic reason could be that, at the front of a propagating wave, all synaptic stores are emptied due to membrane depolarization, and that therefore additional excitatory substances only give a small add-on, whereas an antagonist of such neurotransmitters may significantly reduce membrane transport processes, and thus affect much more the parameters of the propagating waves. A possible consequence for pharmacological research would be that, at least for a variety of neuropathologically relevant syndromes, antagonists of excitatory or modifying neurotransmitters will be more effective than agonists for the related receptors.

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References


