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Amplitude Modulation of the Retinal Ganglion Cell Impulses

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Zusammenfassung. Die Netzhaut decerebrierter Katzen wurde mit sinusförmig moduliertem Licht gereizt und die in den Ganglienzellen ausgelöste Erregung extracellulär registriert. Amplitude und momentane Frequenz der Aktionspotentiale ändern sich sinusförmig und besitzen zueinander eine Phasenverschiebung von 180°. Die Phasenverschiebung ist unabhängig von der Frequenz des Reizlichtes, die im Bereich von 0,1—10 Hz geändert wurde. Anhand von Kontrollmessungen wurde gezeigt, daß die Amplitudenänderung der gemessenen Aktionspotentiale auf Änderungen des Membranpotentials beruht.

Introduction

HUGHES and MAFFEI, 1966 have observed, that with sinusoidally modulated light stimuli, the amplitude of extracellularly recorded impulses of retinal ganglion cell is similarly modulated (Fig. 1).

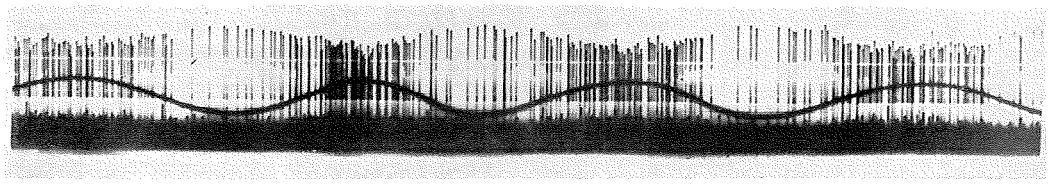

Fig. 1. Ganglion cell discharge under sinusoidal photic stimulation. The frequency of stimulation is 1 cps. The picture is the super position of the two beams on a dual beam CRO, one of which displays neural activity, and the other the time course of the stimulus

In this paper we present a quantitative description of the impulse amplitude modulation of retinal ganglion cells under sinusoidal photic stimulation, and experiments designed to investigate the origin of the phenomenon. From these data it will be argued that the impulse amplitude variations derive from the variations of the cell membrane potential. Thus it becomes possible to detect the variation of cell membrane potential by observing the amplitude of extracellular spikes.

I. Methods

Experiments were performed on decerebrate or pretrigeminal (BATINI et al., 1959) cats. The activity of ganglion cells was recorded using steel microelectrodes placed in the retina of the intact eye through a hole near the limbus of the sclera. Optic nerve units were detected from the optic tract.

Unit activity was analysed in different experimental situations of external stimulus and recorded on magnetic tape (Ampex FM 1100). The band-pass of the recording system was 200—5000 cps. The light source was a sylvania glow modulator tube fed by a linear preamplifier driven by a H. P. oscillator. Light sinewave stimulation at different frequencies and amplitudes was used.

II. Data Analysis

The activity recorded on magnetic tape was analysed by the analog to digital converter AD described by GERACE and GESTRI, 1963. The device measures the amplitude and time coordinates of the spikes exceeding an adjustable threshold, and transfers these data to a digital magnetic tape for input to the digital computer CEP (Pisa Electronic Computer). Data elaboration was performed by program on the digital computer. The time unit of the device AD is 100 μsec. The amplitude measured by the device is the peak amplitude of the spike from the base line. The amplitude measure is expressed by an integer number not greater than "15". The "0" reference point in the amplitude scale coincides with the threshold, which has the task to prevent conversion of useless data (background activity). The amplitude measure is schematized in Fig. 2.

III. Results

a) Definition of the Stimulus and of the Output Variables. The sinusoidal light stimulus variation was chosen both because it provides the necessary generality to describe any dynamic stimulus and because the sinusoidal transfer characteristics between light stimulus and retinal ganglion cell discharge are known (HUGHES and MAFFEI, 1966). The sinewave light stimulus $L(t)$ is given as follows:

$$L(t) = L_0 + L \sin 2\pi f t$$

where L_0 is the average light, L (with $L < L_0$) the amplitude of the sinusoidal variation, f is the frequency in cycles per second.

Two variables are defined to characterize the discharge of the unit under consideration, the average impulse amplitude $A(t)$ and the average firing rate $R(t)$. These variables are defined as follows: the temporal sequence of the discharge is divided in intervals each of length t_1, where t_1 is one tenth of the stimulus period. $R(t)$ and $A(t)$ represent respectively

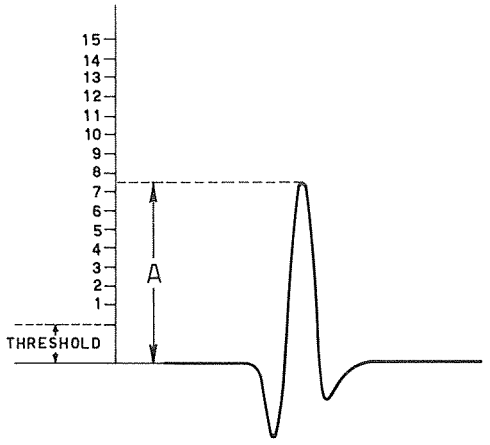

Fig. 2. Scheme of the technique for measuring the spike amplitude

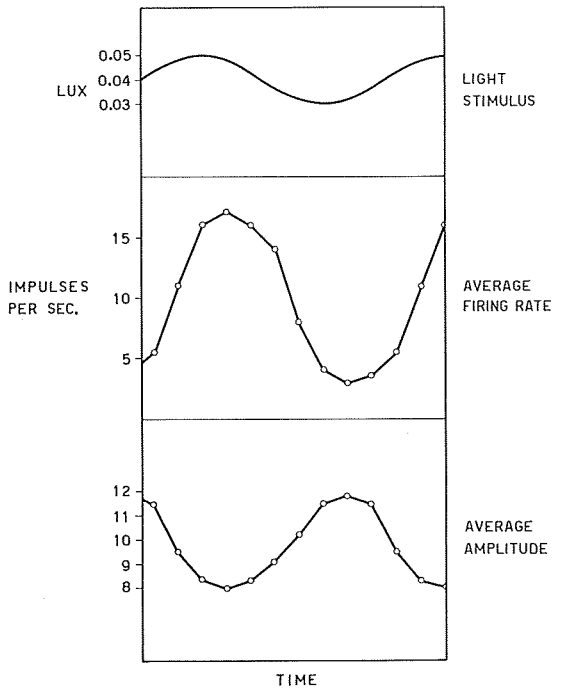

Fig. 3. Firing rate and amplitude modulations of a ganglion cell. The frequency of stimulation is 0.5 cps

the number and the average amplitude of the impulses in each interval, both averaged on a number of stimulus cycles so that the total number of impulses considered is in the range 2000—3000.

It can be noticed that $R(t)$ is defined in the same way as in the previous study of Hughes and Maffei (1966).

b) Recordings from Ganglion Cells. Twenty retinal ganglion cells have been analysed. Fig. 3 shows the results of an experiment, with $f = 0.5$ cps. According with the findings described in Hughes and Maffei, 1966, the rate of cell discharge is almost sinusoidally modulated:

$$R(t) = R_0 + R_1 \operatorname{Sin}(2\pi f t + \varphi). \quad (1)$$

Furthermore, as it can be seen from the figure, also the average impulse amplitude is almost sinusoidally modulated:

$$A(t) = A_0 + A_1 \operatorname{Sin}(2\pi f t + \varphi_1). \quad (2)$$

The sinusoidal modulation of the average impulse amplitude has been observed in all the units analysed, and for all the stimulus frequencies texted (range 0.1 to 10 cps). Between $R(t)$ and $A(t)$ a phase difference of 180° was observed for all units and at all frequencies of stimulation. So we can rewrite relation (2) as follows:

$$A(t) = A_0 - A_1 \operatorname{Sin}(2\pi f t + \varphi) \quad (3)$$

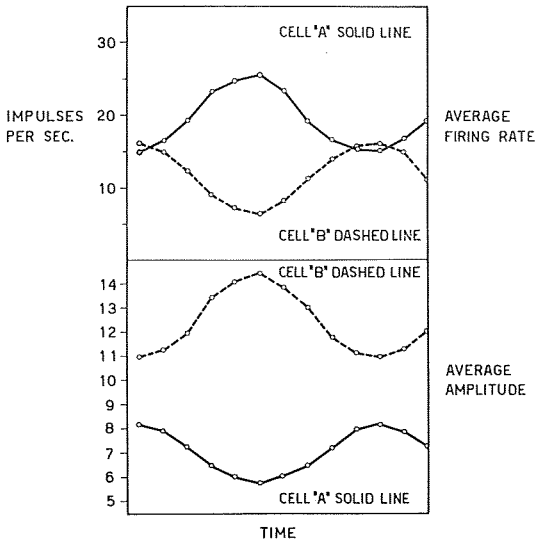

Fig. 4. Firing rate and average amplitude of an optic nerve fiber

where for the same cell and the same frequency f, φ is the same as in relation (1). The amplitude modulation $\alpha_1 = A_1/A_0$ was near 0.15 for a frequency modulation $\alpha_2 = R_1/R_0$ near 0.50. The ratio α_2/α_1 between frequency and amplitude modulation was found, like the phase shift, to be independent on the frequency of stimulation.

c) Recordings from the Optic Tract. Thus far the results described have been obtained by recording impulse activity in the retina near the ganglion cell bodies. Other experiments were conducted to see if the amplitude modulation phenomenon could be detected from ganglion cell axones. Ten units recorded from the optic tract were analyzed and amplitude modulation was never found at this level. Fig. 4 shows the results of such experiment. It is evident from the figure that the firing rate is sinusoidally modulated, while the average amplitude is not.

d) Control Experiments. From the results reported in the section III-b, it is evident that the amplitude of the ganglion cell impulses depends on the sinewave changes of light. This could be, however, a secondary effect of the frequency modulation. In fact, suppose that the amplitude of an impulse were proportional to the time interval between it and the preceding one.

Then an amplitude modulation would result from a frequency modulation, and exactly with a phase shift of 180 degrees. To control this possibility, the following analysis was performed:

$A(t)$ was calculated taking not all the impulses of the discharge, but only the impulses whose distance from the preceding one of the discharge was nearly the same, for istance in the range 8—10 msec.

If the amplitude modulation were a secondary effect of the frequency modulation, no amplitude modulation would be present in these samples. The results of the above analysis, on the contrary, have shown that the sinusoidal amplitude modulation is still present, and therefore the amplitude modulation is not a consequence of the frequency modulation.

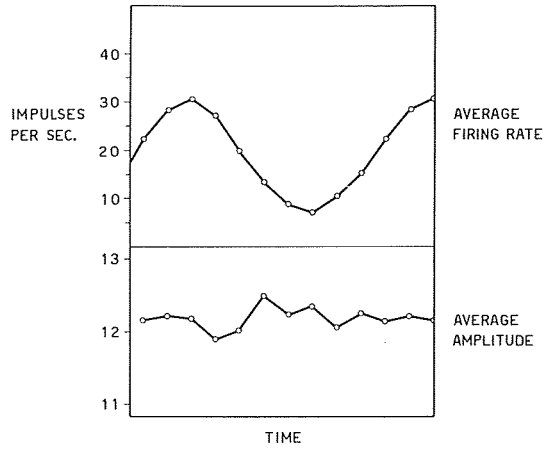

Fig. 5. Firing rate and amplitude modulations of two ganglion cells in the same time. See text for details

Furthermore, the amplitude modulation cannot be a result of the noise from neighboring units, or of some other kind of noise. To give origin to the amplitude modulation observed, noise must be out of phase of 180 degrees from the unit discharge for all the observed units and at all the frequencies tested. This can be excluded by the results of experiments (five) where two units recorded with the same microelectrode were clearly distinguishable by the difference in the amplitudes of their impulses. Fig. 5 shows the results of such an experiment. In this experiment the phase of the modulation of the firing frequencies of the two units is nearly 180° different. The impulse amplitude of each cell is, as usually, 180 degrees out of phase from its firing frequency. It is clear that noise cannot be sinusoidally modulated and 180 degrees out of phase with respect to firing frequencies of both cells, which are 180 degrees out of phase to each other.

e) Analysis of the Shape of the Impulses. Photografic records of the impulses have been analysed to see whether or not the shape of the impulses recorded under sinusoidal light stimulation from a given cell is related to their amplitude. It was found that the shape of the impulses does not change with the amplitude, that is, two impulses with different amplitudes become pratically identical when the amplitude scale of one of the two impulses is adjusted so that its amplitude matches the other.

Two impulses from the same cell are shown in Fig. 6. One impulse belongs to a time interval where $A(t)$ is maximum, the other to a time interval where $A(t)$ is minimum. Note that the time course of the two is identical.

IV. Discussion

It is well known (Tasaki, 1959) that the extracellular spike is a linear function, involving time derivatives, of the intracellular action potential.

We have observed that, although the amplitude of extracellularly recorded action potential of retinal ganglion cells is modulated under light stimulation, the shape of this potential does not vary. Therefore

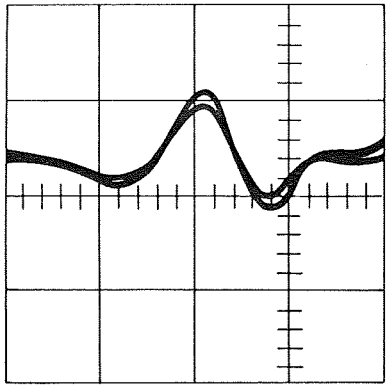

Fig. 6. Analysis of the shape of the spikes of a ganglion cell. The picture is the superposition of two spikes of the same cell, one occurring in a time interval where the amplitude was maximum, and the other in a time interval where the amplitude was minimum

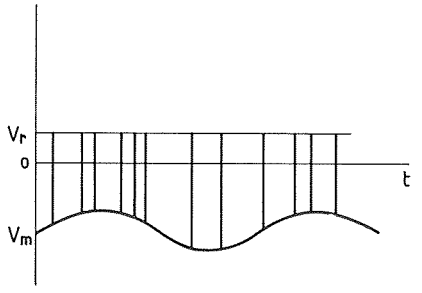

Fig. 7. Relation between amplitude of the spike and membrane potential. V_m represents the membrane potential, V_r the amplitude of the reversal of the membrane potential during activity

the same must hold for the shape of the intracellular action potential. If the shape of the intracellular spike does not change, the amplitude of the impulse recorded extracellularly is proportional to the amplitude of the intracellular action potential. This amplitude is given by the absolute value of the membrane potential at the time of firing plus the amplitude of the reversal of the membrane potential during activity. This reversal is rather constant for small depolarisations (see for istance Eccles, 1957). Therefore it can be argued that the variations in the amplitude of the impulse recorded extracellulary describe, a part a scale factor, the variations of the absolute value of the membrane potential. This is schematized in Fig. 7.

We have shown that the amplitude of the pulse recorded extracellularly from the ganglion cells is almost sinusoidally modulated under sinewave modu-

lation of light stimulus in the frequency range 0.1—10 cps, and that in this range the amplitude modulation is very closely related to the frequency modulation, with a phase shift of 180°, independent of the frequency of stimulation. Since the membrane potential is negative, inside with respect to outside, to obtain its variations we must change the sign of the variations of its absolute value (Fig. 7). Reversing the sign of the impulse amplitude variations, the sinusoidal shape observed is obviously maintened, but the phase is shifted 180°, so that the sinusoid is now in phase with the firing rate. Therefore we can reformulate our results as follows: in the retinal ganglion cells

i) the membrane potential is almost sinusoidally modulated under sinusoidal light stimulation, in the range 0.1—10 cps.

ii) the firing rate is linearly related to the membrane potential. More precisely, membrane potential and firing rate are directly proportional. To this relationship between membrane potential and firing rate we shall not give the name of "transfer function" because the frequency modulation may be, but may also not be, the effect of the membrane potential modulation.

We plan to extend this investigation to higher frequency of photic stimulation, since the present results cover only the range up to 10 cps.

The fact that the amplitude modulation is not observed when recordings are taken in the optic nerve is easily explained. In fact, the variations in the membrane potential decay exponentially from the site of origin, i. e. the cell, and are no longer detectable a few space costants along the axon.

The linear relation between membrane potential and firing rate is not surprising, since it has been observed in frog's sensory terminals (KATZ, 1950) as well as in Limulus eccentric cells (MACNICHOL, 1956; FUORTES, 1959), under natural stimulation. These results are, however, limited to stationary conditions of stimulus. Only recently an investigation with application of intracellular current has been performed in a dynamic situation in the cray fish (TERZUOLO et al., 1966). It is relevant to notice that the linear relationship between membrane potential and firing rate is not related to the statistical properties of the firing itself. The discharges of muscle sensory terminals and of Limulus eccentric cells are quite regular (see for istance FUORTES and MANTE GAZZINI, 1962), i. e. the variance of the interpulse intervals is small with respect to the mean interval, while the ganglion cell retinal discharge is quite irregular (KUFFLER et al., 1957).

Our results have shown the possibility of observing the variations in the membrane potential by extracellular recordings. Obviosly by this way it is not possible to obtain the absolute values of the membrane potentials and of its variations, but only the relative variations. Furthermore this technique requires averaging of a large amount of data. In fact the impulses take the rule of sampling impulses for what the membrane potential observation is concerned, and average of a large number of data is necessary for reducing the effect of noise in the impulse amplitude.

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References. BATINI, C., G. MORUZZI, M. PALESTINI, G. F. ROSSI, and A. ZANCHETTI: Effects of complete pontine transaction on the sleep wakefulness rhythm: the midpontine petrigeminal preparation. Arch. ital. Biol. **97**, 1—12 (1959). ECCLES, J. C.: The physiology of nerve cells. Baltimore: Johns Hopkins University Press 1957. — FUORTES, M. G. F.: Initiation of impulses in the visual cell of limulus. J. Physiol. (Lond.) **148**, 14—28 (1959). — FUORTES, M. G. F., and F. MANTEGAZZINI: Interpretation of the repetitive firing of nerve cells. J. gen. Physiol. **45**, 1163—1179 (1962). — GERACE, G. B., e G. GESTRI: Un sistema automatico per l'analisi dell'attività nervosa. Alta Frequenza **32**, 639—644 (1963). — HUGHES, G. W., and L. MAFFEI: Retinal ganglion cell response to sinusoidal light stimulation. J. Neurophysiol. **29**, 333—352 (1966). — KATZ, B.: Depolarization of sensory terminals and the initiations of impulses in the muscle-spindle. J. Physiol. (Lond.) **111**, 261—278 (1950). — KUFFLER, S. W., R. FITZHUGH, and H. B. BARLOW: Maintained activity in cat's retina in light and darkness. J. gen. Physiol. **40**, 683—702 (1957). — MACNICHOL jr., E. F.: Visual receptors as biological transducer. In: Molecular structure and function activity of nerve cells. Washington: American Institute of Biological Sciences 1956. — TASAKI, I.: Conduction of the nerve impulse. In: Handbook of Physiology-Neurophysiology, vol. I. American Physiological Society Washington 1959. — TERZUOLO, C. A., R. PURPLE, E. BAILY, and H. HENDELMAN: Effect of inhibition upon the transducer and encoder nervous systems of 3d Int. Congr. of Neurobiology. Stokkolm 1966. In press.

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