

Some Properties of Post-excitatory Inhibition, studied in the Optic Nerve with Micro-electrodes.

By

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With 4 Figures in the text.

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When fibres of the optic nerve are isolated with the aid of micro-electrodes, introduced into the opened bulb of anaesthetized or decerebrate animals, spontaneous activity is often observed (see the figs. of this paper). The discharge is then recorded with amplifier and cathode ray oscillograph in the usual manner. The spontaneous activity provides a good background for discovering inhibitory phenomena which against a silent background would have escaped notice. In this manner post-excitatory inhibition was found and described in a recent paper by the author (GRANIT, 1944). As seen, *e. g.* in fig. 1, post-excitatory inhibition manifests itself as a pause after the discharge caused by stimulation of the retina with light. During this pause the spontaneous discharge is completely silenced, is then gradually resumed at a subnormal rate, and finally accelerates up to normal frequency. It was suggested that the phenomenon was homologous with the 'extinction' or 'post-stimulatory inactivation', found by DUSSEY DE BARENNE and McCULLOCH (1935) in the brain. In this, as in so many other respects, the retinal neurones seem to behave as other central structures.

This brief report describes some properties of post-excitatory inhibition, and also contains an attempt to differentiate it from pre-excitatory inhibition (GRANIT and THERMAN, 1935, HARTLINE, 1938). By pre-excitatory inhibition is meant the case when light, instead of exciting a fibre to discharge, causes

an inhibition, and removal of the stimulus elicits a vigorous off-effect signalling darkness. The off-effect is stopped by re-illumination. The presence of off-effects is thus a sign that illumination has caused pre-excitatory inhibition instead of pure excitation. An off-effect is shown in fig. 2 *a*.

Method.

The micro-electrode technique has been described in several earlier papers (see *e. g.*, GRANIT, 1941). If this electrode is successfully applied on the optic nerve fibres inside the bulb, single fibres or well synchronized fibres can be isolated (see the figs.). The electrodes are connected to amplifier and cathode ray oscillograph. The discharge is photographed on running film.

In this work some decerebrated cats have been used but chiefly guinea pigs and rats which have received a dose of from 2—6 cc of a 20 % solution of urethane intraperitoneally. The animals have been dark adapted.

Results.

A. Intensity, duration of exposure.

It seemed necessary first to investigate the effect of stimulus intensity and duration of exposure upon post-excitatory inhibition. A typical result is shown in fig. 1. Duration of exposure increases from *a* to *d*. In *c* and *d* merely the end of the long periods of illumination is marked by the light signal on the film. The inhibitory pause is seen to increase from *a* to *d*, and, after the longer exposures, the discharge is resumed at a slower rate.

The effect of an increase in intensity is shown in records *e* to *g*. Post-excitatory inhibition is very brief at 20.4 m.c. (*e*), somewhat longer at 149 m.c. (*f*), still longer at 490 m.c. In parallel with the lengthening of the inhibitory pause is seen a decreased initial rate of the frequency at which the spontaneous firing is resumed. Some other fibres, less well localized by the micro-electrode, are found to respond with an off-effect, particularly well marked in *d* and *g*. The characteristic effects of duration of exposure and stimulus intensity upon the inhibitory pause are not always as regular as in fig. 1.

It is well known that off-effects increase and, hence, that pre-excitatory inhibition too is favoured by a lengthening of duration of exposure or an increase of stimulus intensity (cf.

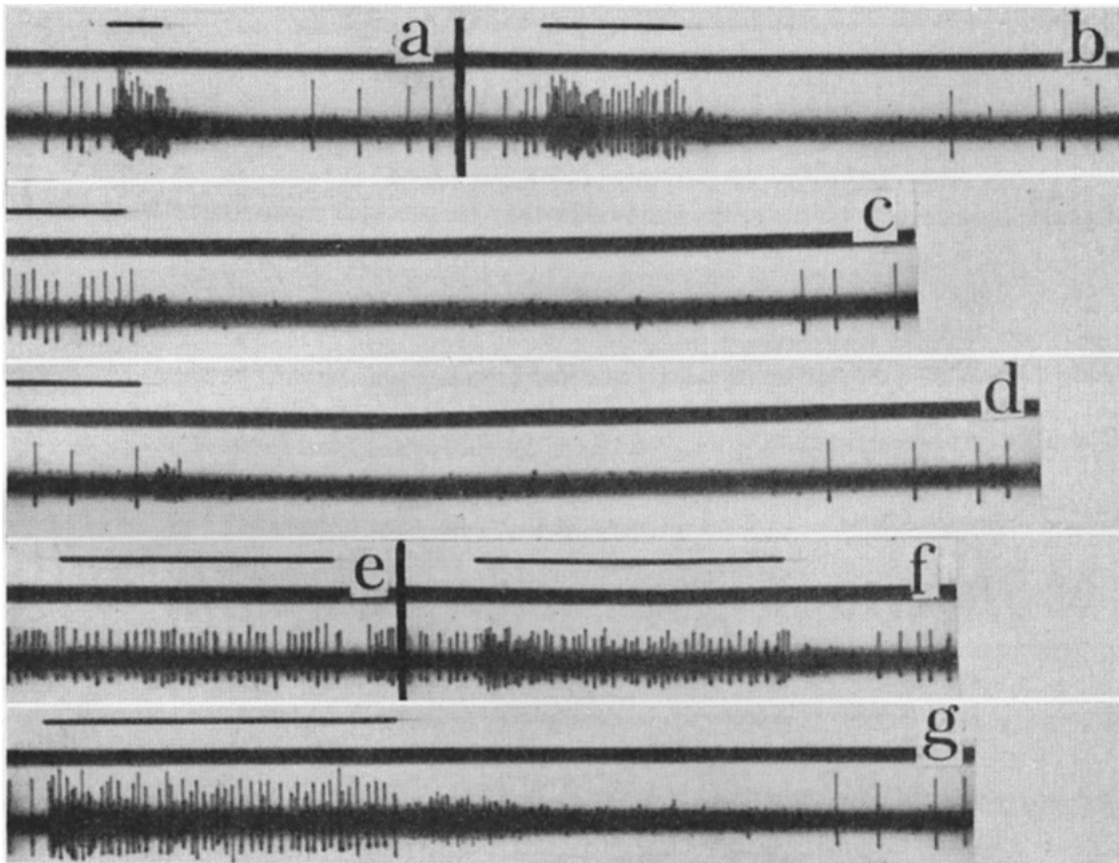


Fig. 1. Retina and optic nerve of guinea pig. Micro-electrode. *a—d*, variation of exposure. *a*, about 1/3 sec., *b*, 1 sec., *c*, end of exposure of 5 sec. duration, *d*, end of exposure of 20 sec. *e—g*, variation of stimulus intensity. *e*, 20.4 meter candles, *f*, 149 m.c., *g*, 490 m.c.

Time signal above record of spikes compressed at this slow film speed but light signal above each record also indicates time in terms of the 1 sec. light signal of record *b*.

fig. 1, records *d* and *g*). For this reason one must ask whether pre- and post-excitatory inhibition merely are different aspects of the same physiological process, say, that the fibres concerned were connected by reciprocal innervation, or whether they represent different processes. The easiest way of finding an answer to this question would seem to be the use of pharmacological test methods. The two forms of inhibition should be influenced in parallel if they are expressions of the same fundamental process. If not, pharmacology should differentiate between them.

B. Pharmacological tests.

General observations.

Ether was given the animals to inhale, the other substances were introduced directly into the bulb. Now it is impossible to make quantitative comparisons of doses by this method.

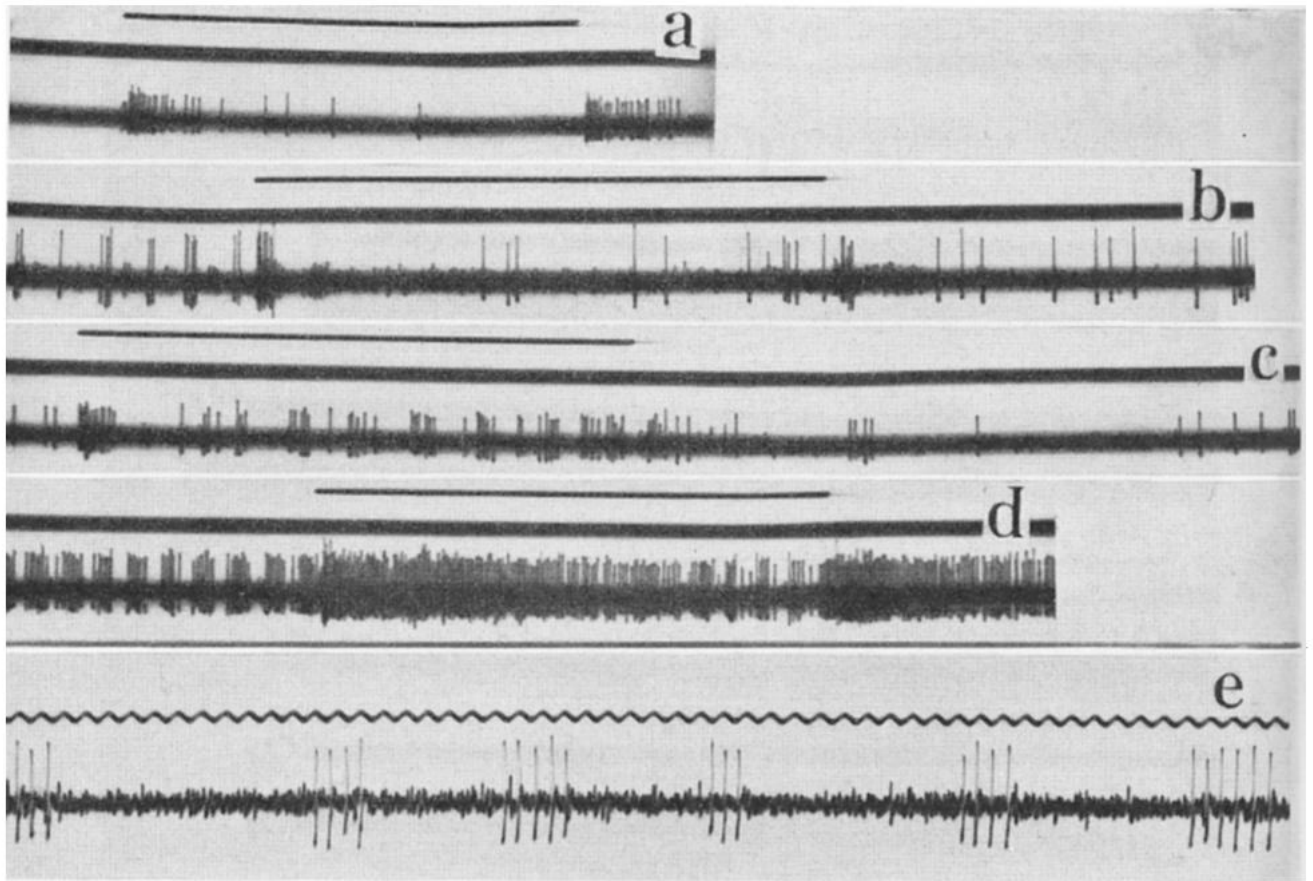


Fig. 2. The effect of strychnine or citrate on the discharge from the optic nerve. *a—d* in cat, *e*, in guinea pig. In *a* typical on-off-element responding to onset and cessation of illumination, marked by light signal above time record. Strychnine rhythms in different parts of the retina in *b—d*. Last record, *e*, at faster speed of film to show frequency of spontaneous rhythms initiated by citrate dropped into the bulb. Time in 1/50 sec. above spike record.

The drug is diluted by the aqueous body to an unknown degree. Further complications are introduced by the formation of membranes around the electrode and elsewhere. Thus, for instance, concentrated doses of citrate have been seen to be without an effect owing to the formation of such coagulates. But from the point of view of the problem concerned the absolute magnitude of the dose is of little interest. The magnitude of the effect can always be estimated and so can the *difference* in the effect with respect to pre- and post-excitatory inhibition.

Many substances, alcohol, citrate, strychnine, are excitants in small or moderated doses and in large doses have an initial excitatory effect succeeded by depression. If the dose happens to be too large most substances set up a transient rhythm, synchronized over the whole retina, before the eye ceases to respond. A specific and permanent effect of this nature is

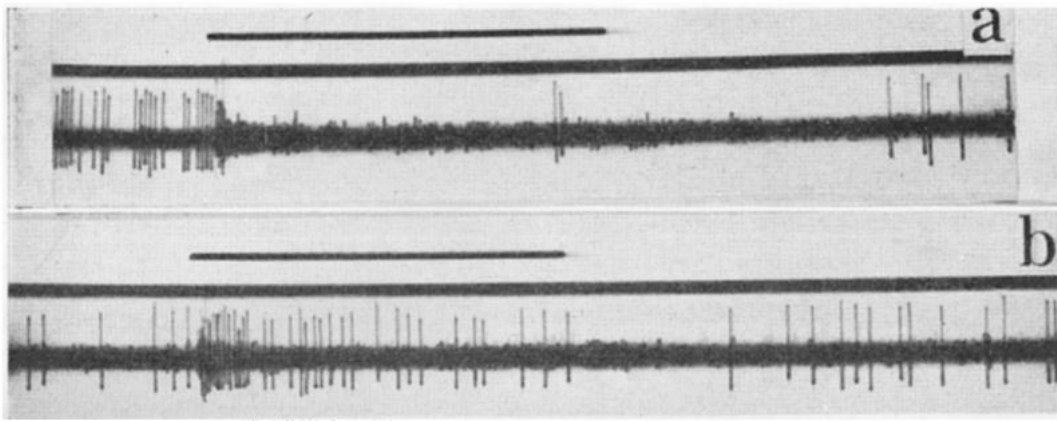


Fig. 3. The effect of small dose of strychnine on an active unit in the rat's optic nerve. In *a* this unit responds to illumination (light signal above record) by inhibition, after strychnine, in *b*, it responds with excitation, 12 min. later.

given by strychnine, as first shown for the whole optic nerve by ADRIAN and MATTHEWS (1928). In a steadily increasing number of papers from different laboratories this effect of strychnine has been confirmed for other central structures such as brain and spinal cord.

Strychnine rhythms in relatively well isolated elements are shown in fig. 2. The records *a* to *d* (cat) refer to the same eye, *a* being a control before strychnine. It is, like most elements in the cat's eye, an on-off-unit. Some 10 min. after strychnine the records *b* to *d* are taken from different places in the retina. Record *e* shows the same effect in the retina of the guinea pig, taken with a higher speed of the film in order to show the time relations.

Changes in type of discharge.

The main question in this connexion is whether under the influence of pharmaca the general pattern of discharge can be shown to alter in type. Fig. 3 (rat) shows a case in which an element with a high frequency spontaneous discharge was inhibited by illumination (*a*). Strychnine (1:1000) was dropped into the eye and 6 min. later the effect of stimulation was pure excitation with a brief post-excitatory pause of inhibition (*b*). This dose was small to judge by the fact that the spontaneous rhythm decreased and that no spontaneous beating occurred. Single experiments are, of course, not conclusive but it has been observed several times that small or moderate doses of strychnine can remove inhibitory effects of illumination and supplant them by excitatory effects. Post-excitatory inhibition is far more sensitive to such influences than pre-excitatory inhibition.

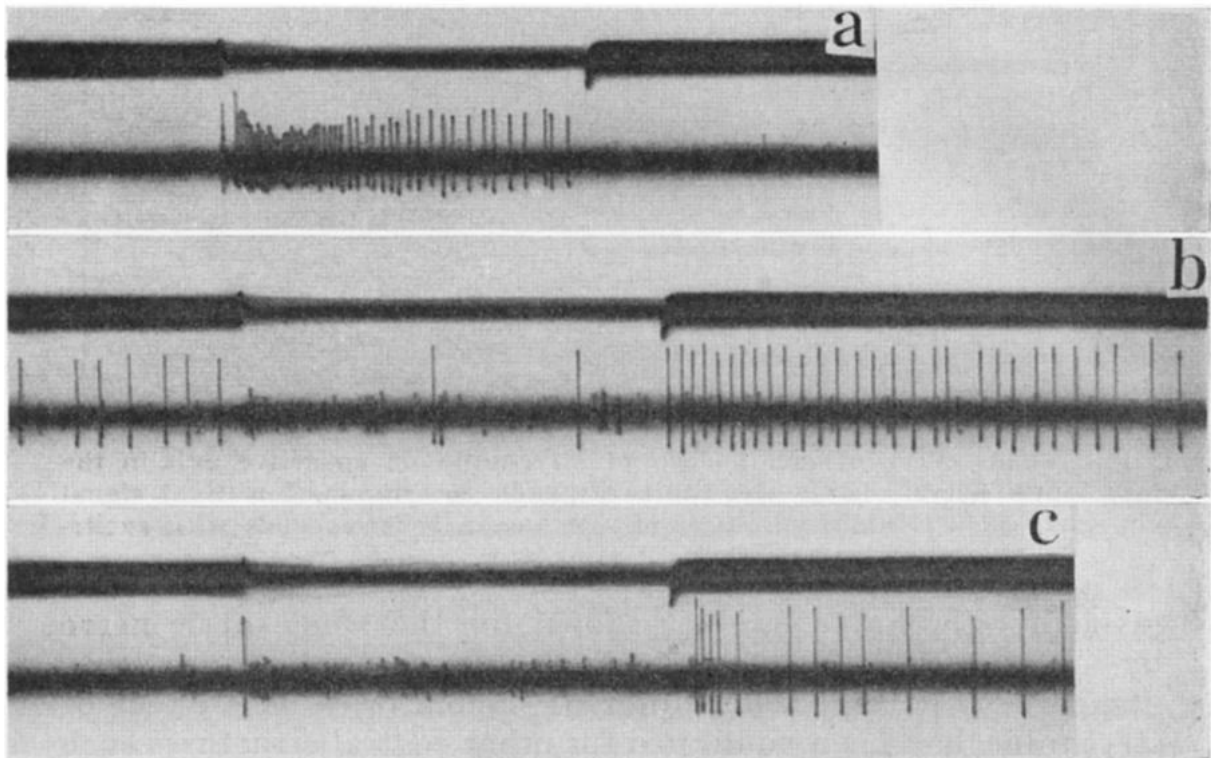


Fig. 4. Isolated element in retina of guinea pig responding in *a* to illumination, signalled by compression of time record above spike record, with pure excitation. In *b* and *c* the gradually developing late effect of a considerable dose of citrate. The unit recorded from ends by responding with pure pre-excitatory inhibition and off-effect. Stimulation for 2 sec.

The length of the inhibitory pause after excitation is very much dependent upon the general state of excitability. When the spontaneous discharge diminishes in frequency after inhalation of ether this is generally accompanied by a very considerable lengthening of the post-excitatory inhibition. Similarly, when small doses of citrate accelerate the spontaneous discharge, the inhibitory pause is shortened. It has not been possible to demonstrate convincingly that pre-excitatory inhibition would reflect general excitability in an equally close manner. It has not been possible to change on-off-elements (in cats) into on-elements by using pharmaca.

On the other hand, it has been a common finding that the pure on-type (pure excitation type) of discharge which characterizes most elements in rats and guinea pigs (GRANIT, 1944) is superseded by an on-off-type of discharge after larger doses of excitants such as citrate, sometimes also after strychnine. This late effect is preceded by transient hyperexcitation. In fig. 4 is shown an on-element in the eye of the guinea pig (*a*). The effect of citrate (*b*, *c*) is seen to consist in the appearance of spontaneous activity (*b*), inhibited by illumination, and increased into an off-effect at cessation of illumination. Finally

the spontaneous activity again diminishes in *c* but there is still left a vigorous off-effect.

In such experiments it may be difficult to be certain that the off-effect that turns up as a late citrate-effect actually is delivered by the same fibre that began by giving a pure on-discharge of excitation. If several fibres co-operate in close synchronization a redistribution of the elements engaged together may take place if some are suppressed and others excited. Nevertheless there is no way of escaping from the general conclusion that after a large dose of citrate off-effects may be obtained nearly everywhere in retinae of rats and guinea pigs in which before this treatment they were rare. Again, if off-effects were present from the beginning, they became accentuated by the treatment. Now off-effects are a sign of pre-excitatory inhibition, and, since they turned up without corresponding changes in post-excitatory inhibition it must be concluded that under such circumstances these two forms of inhibition have responded in a different manner to the substance introduced into the eye.

Conclusion.

The hypothesis tested in these experiments consists in the assumption that post-excitatory inhibition might be an effect, connected by reciprocal innervation with pre-excitatory inhibition in such a manner that the more extended the off-effect the longer the post-excitatory pause of inhibition in adjacent neurones. This hypothesis may still be held to contain an element of truth but it cannot fully explain post-excitatory inhibition since the two forms of inhibition need not vary in parallel when the retinal excitability is altered by the introduction of various pharmaca into the eye.

Summary.

Single fibres in the optic nerves of cats, rats, and guinea pigs have been isolated with micro-electrodes and the discharge led to amplifier and cathode ray oscillograph.

The effect of stimulus strength and duration upon post-excitatory inhibition, which is a period of silence after illumination, has been studied. Increased duration or strength of stimulus has been found to augment the inhibitory pause.

Tests with various pharmaca such as alcohol, ether, strychnine, citrate have been carried out and been found to influence

pre- and post-excitatory inhibition differentially. These and other general effects of the pharmaca are described in the paper.

Cand. med. BÖRJE LÖFGREN has co-operated in some of the citrate experiments.

References.

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