CONSTANCY OF HIPPOCAMPAL AFTERDISCHARGE UNDER VARIOUS CONDITIONS OF STIMULATION[1]

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Afterdischarge (AD) following electrical stimulation of various cerebral structures has been known for a long time (Gibbs and Gibbs 1936). The effect of excitation of the CNS usually depends on the parameters of stimulation, such as frequency and voltage; *e.g.*, Rosenblueth and Cannon (1941, 1942) have demonstrated that cortical AD is progressively prolonged by a gradual increase in the intensity or frequency of stimulation. They also found an optimal duration of stimulation, beyond which the AD declines.

The influence of stimulation parameters on the intensity and duration of central nystagmus (Bergmann *et al.* 1961) and of blood pressure changes due to brain-stem stimulation (Gutman *et al.* 1962), has been reported from this laboratory. We have now extended these experiments to hippocampal afterdischarge (HAD). Liberson and Cadilhac (1953) have previously studied the variations of the threshold for HAD as a function of stimulation parameters. Thus, in guinea pigs, no difference in threshold voltage was found over a wide frequency range (20–300 c/sec). However, these authors did not include in their investigation other characteristics of the HAD.

It appeared of interest whether the hippocampus would show regularities of the afterdischarge, similar to those found by Rosenblueth and Cannon (1941, 1942) for cortical stimulation. We have therefore explored the factors defining the duration of HAD.

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METHODS

Thirty-three rabbits of either sex were used. During the experiment, the animal was placed in a hammock-like cage. Electrodes were introduced under local anesthesia (2% xylocaine) through a stereotaxic soclet, according to the method of Hess (1932) and the atlas of Monnier and Gangloff (1961).

Stimulation of the dorsal hippocampus and recording from various subcortical structures were carried out with concentric stainless steel electrodes, made of an outer tube of 0.7 mm diameter and an inner electrode, protruding 0.5–1 mm. The electrodes were completely insulated except at their tips.

For cortical recording, silver screw electrodes were used. These were usually placed over the sensory-motor, associative and optic areas (Longo 1962).

Electrical stimulation was provided by a Grass model 4S stimulator. The parameters — frequency, voltage, pulse duration and stimulation period — were varied as specified under Results. The interval between subsequent stimulations was 5–20 min, depending on the extension of the afterdischarge.

At the end of each experiment the animal was sacrificed under deep anesthesia. The head was then perfused with formalin through the carotid arteries. After 7 days of fixation in formalin (4%) the brain was cut into transverse sections of 80 μ thickness in order to identify the position of the electrode tip. The trajectory of the electrode was easily seen in the sections even without staining. The location of the tip was mapped according to the charts of the atlas of Monnier and Gangloff (1961).

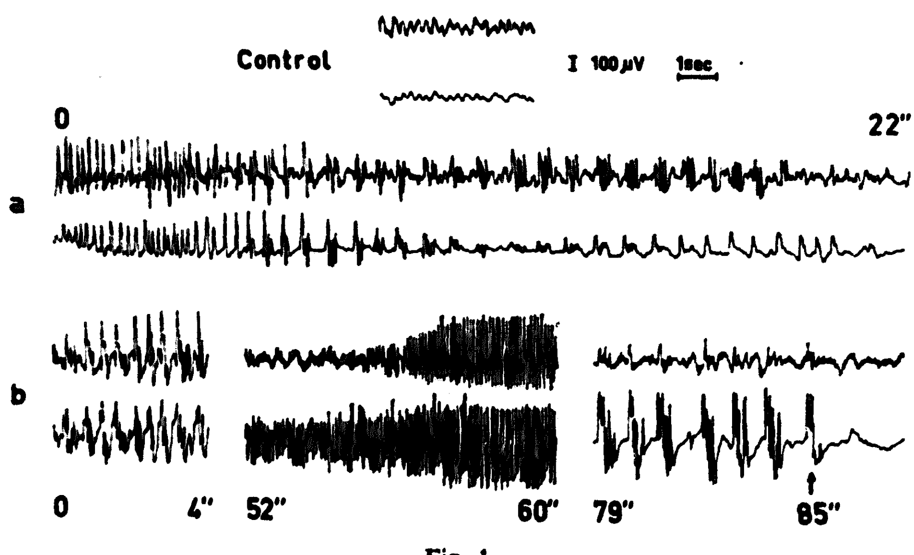

Fig. 1

Extent of hippocampal afterdischarge at the beginning and at the end of an experimental series. Rabbit 107. Stimulation of left dorsal hippocampus at 40 c/sec; 3 V; pulse duration 2 msec; stimulation period 10 sec. Recording from associative cortex (upper tracing) and from dorsal hippocampus (lower tracing). Amplitude calibration and time scale in right upper corner. Numbers on EEG records denote time (sec) from end of stimulation. Control: Cortical and hippocampal tracings at rest. *a*: Afterdischarge following the first stimulation of the hippocampus in this series. *b*: Afterdischarge under the same conditions of stimulation after 25 stimulations applied during 4 h.

RESULTS

1. *Hippocampal afterdischarge under constant conditions of stimulation*

In order to evaluate the factors which determine the extension of HAD, it is a prerequisite that this response should remain stable as long as the parameters of stimulation are kept constant. We have however repeatedly observed that, in spite of constant conditions, the duration of HAD increased on repeated stimulations applied to an animal during a single day. Thus, at the beginning of a series, HAD periods were below 30 sec in 90 per cent of all experiments, while towards the end of the day about 40 per cent showed an HAD in excess of 50 sec (Table I).

TABLE I

Duration of hippocampal afterdischarge (HAD) under constant conditions of stimulation.

	Duration of HAD (sec)		
	⩽30	30–50	⩾50
A	88%	9%	3%
B	42%	16%	42%

The rabbits were stimulated in the dorsal hippocampus at 40 c/sec; supra-threshold intensity; pulse duration 2 msec; stimulation period 10 sec. The numbers denote per cent of all experiments.

A: Distribution of duration of HAD following the first stimulation within each series.

B: Distribution of duration of HAD at the end of the stimulation series.

A typical example is shown in Fig. 1, in which an AD of 22 sec at the beginning of the experiment became prolonged to 85 sec after 25 stimulation periods during 4 h. It is noteworthy that when the AD was prolonged, more variety of *form* of discharge was displayed. Variations in the form of the potentials during a single afterdischarge period have been previously reported (Corell and Ingram 1956; Morin and Green 1953). In the present experiments we have frequently observed during extended HADs the appearance of "perfect synchronization periods"[1], which were absent at the beginning (Fig. 1, *b*).

In the present study, results were evaluated only when standard tests under constant conditions of stimulation, carried out at the be-

[1] This phenomenon has been previously termed by Green and Shimamoto (1953) "jack-in-a-box" and by Liberson and Akert (1955) "tonic phase".

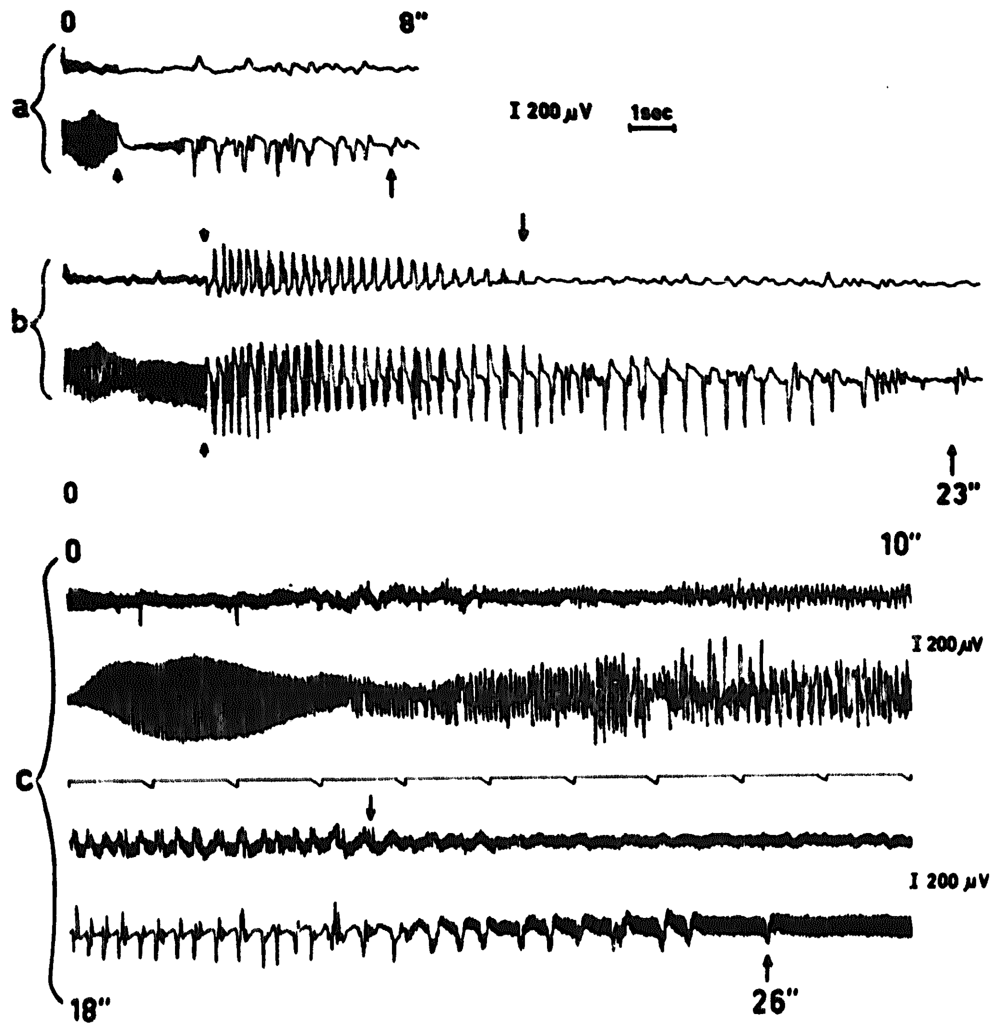

Fig. 2

Duration of hippocampal discharge as function of length of stimulation period. Rabbit 117. Stimulation of right dorsal hippocampus at 40 c/sec; 2 V; pulse duration 2 msec. ↑ End of stimulation period. ↑ End of hippocampal discharge (lower tracing). ↓ End of cortical discharge (upper tracing). Numbers on EEG records indicate time (sec) from *onset of stimulation*. *a*: 1 sec stimulation. Note absence of cortical discharge. *b*: 3 sec stimulation. Cortical discharge terminates before hippocampal discharge. Amplitude calibration and time scale for *a* and *b* in right upper corner. *c*: 40 sec stimulation. Time scale, *different from a and b*, is indicated between upper and lower pair of tracings. Note early emergence of discharge potentials, differing in frequency and amplitude from the stimulus artefact. Hippocampal discharge dies out while stimulation goes on. When the discharge fades (after about 22 sec of continuous stimulation), the stimulus artefact is again discernible in the hippocampal record (lower tracing). The total discharge period of the dorsal hippocampus in *b* and *c* is approximately of the same duration.

ginning and end of the experimental period, produced HADs of identical length.

II. *Effect of duration of stimulation on HAD*

The response of the hippocampus to stimulation is usually determined as the discharge *following* cessation of stimulation. However, if the stimulus artefact is not too large, hippocampal potentials, appearing already *during* the stimulation period, are easily recognizable by their different frequency (Fig. 2, *c*). This is particularly evident in the contralateral hippocampus. We may presume, therefore, that hippocampal discharge starts even earlier than the first distinct potential, but is obscured in the beginning because of the relatively large amplitude of the stimulus artefact. Separation of early hippocampal potentials from the stimulus artefact will be further studied with oscilloscopic techniques.

To simplify the comparison of HAD under varying conditions of stimulation, we have arbitrarily assumed the beginning of the discharge to coincide with the start of stimulation (a procedure which may introduce an error of a few seconds). The period from the start of stimulation to the end of the afterdischarge may be called total discharge (TD). The total discharge is thus composed of one part coinciding with the stimulation period and another part occurring after the end of stimulation.

Fig. 2 shows the effect of graded prolongation of the stimulation period on the *duration* of TD. With very short stimulation (1 sec), the TD was also shortlived (8 sec) and was confined to the hippocampus alone (Fig. 2, *a*). Prolongation of the stimulation from 1 to 3 sec resulted in an extension of TD from 8 to 23 sec and in spread of the discharge to other structures, such as the associative cortex (Fig. 2, *b*). But further lengthening of the stimulation period to 40 sec had no significant effect on the *duration* of TD. Furthermore, with a stimulation period of 40 sec the discharge clearly stopped *before* the end of the stimulation (Fig. 2, *c*).

Table II shows again that the total discharge

TABLE II

Effect of stimulation period on duration of hippocampal discharges. Rabbit 113.

Stimulation-period (sec)	Duration of discharge after the end of stimulation (sec)	Duration of total discharge* (sec)
3	0	0
4	5	9
5	26	31
7	24	31
10	19	29
20	8	28
30	1	31
40	0**	31

Stimulation in the right dorsal hippocampus at 40 c/sec; 4 V; pulse duration 2 msec. * Total discharge refers to the period from onset of stimulation to termination of hippocampal discharge. ** Here, the discharge stopped 9 sec before the termination of stimulation. For further discussion see text.

was constant although the "afterdischarge", following cessation of stimulation, might become shorter and even disappear completely on lengthening of the stimulation period. Although the total duration of HAD varied from one rabbit to the other, in the same animal it was constant for stimulation periods of different lengths. This was confirmed in ten animals exposed to these tests.

III. *Effect of intensity of stimulation on HAD*

The foregoing experiments demonstrate that the duration of stimulation has no effect on HAD and thus suggest that some intrinsic mechanism determines the duration of AD. The effect of increasing the intensity of stimulation on the duration of HAD is a suitable means to test this suggestion. If the HAD is produced only by the limited group of cells that is directly stimulated, then stepwise increase in intensity should — by spread of the excitation to a larger number of neurons — gradually prolong the AD, in analogy to the findings of Rosenblueth and Cannon (1941, 1942) for the cat's cortex. If, however, the afterdischarge is produced by a structure larger than that directly stimulated, then the duration of HAD should not be proportional to the voltage applied.

In the experiment shown in Fig. 3, *a*, threshold stimulation (at 3.5 V) produced a short HAD of 8 sec. Increasing the intensity from 3.5 to 4 V, while all other parameters of stimulation were kept constant, resulted in prolongation of the HAD from 9 to 19 sec (Fig. 3, *b*). No further increase in *duration* of HAD was observed even after quadrupling the intensity of stimulation (Fig. 3, *c*). Throughout a series of experiments, which included eleven animals, consistent results were obtained in each case. Although the duration of HAD differed from one rabbit to the other, in the same animal the HAD period was constant over a wide range of voltages used for stimulation. Thus, the influence of stimulus strength resembled the effect of length of stimulation period on the duration of TD.

It is noteworthy that in many experiments the difference between the threshold voltage and the minimal intensity which produced a fully developed HAD was only 0.1–0.2 V. Sometimes

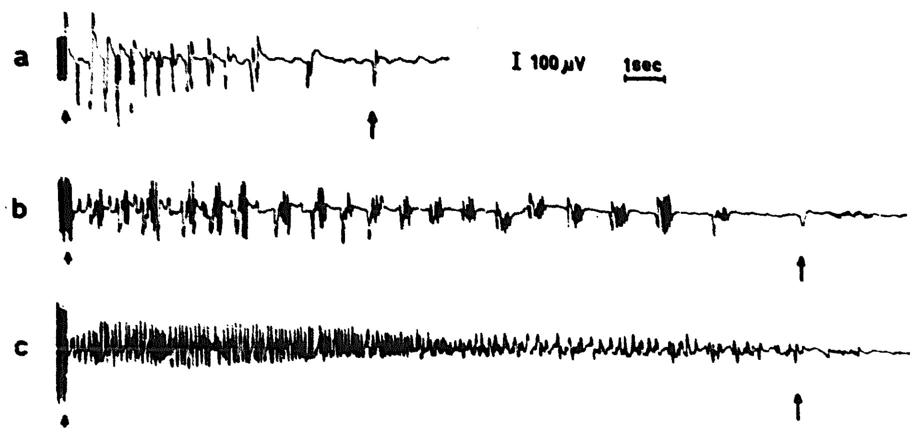

Fig. 3
Hippocampal afterdischarge (HAD) under different intensities of stimulation. Rabbit 114. Stimulation in left dorsal hippocampus at 40 c/sec; pulse duration 2 msec; stimulation period 10 sec. Amplitude calibration and time scale at upper right corner. ↑ End of stimulation period. ↑ End of HAD. *a*: 3.5 V (threshold); *b*: 4 V; *c*: 15 V. In the same series, stimulation at 6, 8 and 10 V resulted in HAD of 22, 23 and 23 sec duration, respectively. Variations in the *form* of the afterdischarge, as seen in records *b* and *c*, were also observed under repeated stimulation at constant voltage.

it was altogether impossible to obtain a graded response as the slightest change on the voltage dial would transform an ineffective stimulus into one eliciting an HAD of maximal duration.

IV. *Effect of the frequency of stimulation on HAD*

The next parameter studied was the frequency of stimulation. When voltage, pulse duration and length of stimulation period were constant, then variation of the frequency over a wide range produced the same duration of HAD. Identical results were obtained in twelve rabbits used for this type of test. With a pulse duration of 2 msec the threshold frequency was usually at 10–15 c/sec, and frequencies up to 100 c/sec did not evoke a longer HAD. No specific relation of the form of HAD to the rate of stimulation was found.

A representative experiment is shown in Table III. Over the range of 15–100 c/sec the HAD extended approximately to 30 sec. With the same pulse duration, however, the duration of HAD shrank at frequencies beyond 120–150 c/sec. This decline of the HAD could be prevented if the pulse duration was reduced. In keeping with this observation the HAD disappeared completely, even with low frequencies (10–20 c/sec), when the pulse duration was expanded to 15–30 msec. Thus it seems that when current flow occupies about 20–30 per cent of the total stimulation period[1] the HAD declines and finally vanishes.

TABLE III
Effect of frequency of stimulation on duration of hippocampal afterdischarge (HAD). Rabbit 45.

Frequency of stimulation (c/sec)	Duration of HAD (sec)
15	32
20	31
40	32
70	31
100	35

Stimulation in the right dorsal hippocampus at 3 V; pulse duration 2 msec; stimulation period 10 sec.

V. *HAD in response to stimulation of various points in the dorsal hippocampus*

The results of the above experiments indicate that the duration of HAD depends relatively little on variation of frequency, intensity and length of stimulation, even if these parameters are varied over a wide range. The extent of the HAD seems to be regulated by some intrinsic mechanism of the structure involved. If this is

[1] 100 c/sec × 2 msec = 200 msec/sec = 20%; 10 c/sec × 30 msec = 300 msec/sec = 30% of the total stimulation period.

the case, the duration of HAD should not vary when the dorsal hippocampus is stimulated at different points. In each of three rabbits, examined for this purpose, four concentric electrodes were introduced into the dorsal hippocampus, two on each side. The electrodes were fixed in bC and bD (see atlas of Monnier and Gangloff 1961), their tips reaching 8 mm below the surface of the skull. Although the absolute length of the HAD periods differed from one rabbit to the other, in a single animal stimulation at any one of the four electrodes resulted in an HAD of nearly the same duration. Variations were well within the range observed for repeated stimulation of a single hippocampal site. A representative example is given in Table IV.

TABLE IV

Duration of afterdischarge following stimulation of different points in the dorsal hippocampus. Rabbit 117.

		Duration of HAD (sec)
Right hippocampus	rostral electrode*	24
	caudal electrode	30
Left hippocampus	rostral electrode	25
	caudal electrode	29

Stimulation at 40 c/sec; 2 V; pulse duration 1 msec; stimulation period 5 sec. * Rostral electrodes were introduced in bC and caudal ones at bD, according to the coordinates of Monnier and Gangloff (1961).

DISCUSSION

Various aspects of HAD, such as the discharge patterns and the propagation of the potentials from hippocampus to other structures, have been thoroughly studied (Correll and Ingram 1956; Creutzfeldt and Meyer-Mickeleit 1953; Green and Shimamoto 1953; Liberson and Akert 1955; Liberson and Cadilhac 1953; Morin and Green 1953). However, the factors regulating the *duration* of HAD have not yet been subjected to systematic analysis.

Many responses involving stimulation of the CNS depend in a typical way on the parameters of stimulation. For example, the rate of the nystagmoid movements evoked by stimulation of the nystagmogenic area (Bergmann *et al.* 1961) varies with the frequency, exhibiting an optimum on either side of which the response declines sharply. Blood pressure responses to stimulation in the brain-stem are also frequency-dependent, the blood pressure rising with increasing frequency (Hare and Geohegan 1941; Gutman *et al.* 1962). Rosenblueth and Cannon (1941, 1942) have shown a stepwise increase in duration of *cortical* afterdischarge on increasing the frequency from 10 to 120 c/sec, as well as on augmenting the intensity of stimulation. In general, a gradual increase of response with increasing intensity of stimulation is widely known in physiology. In this respect the present experiments demonstrate a fundamental difference between HAD and other central reactions. The HAD, once initiated, is not dependent on the frequency or intensity of stimulation, but is maintained by some intrinsic mechanism. It appears that hippocampal stimulation triggers a chain-reaction which, once activated, proceeds to completion, without regard to the character of the initiator. Therefore, HAD may stop while stimulation goes on (see Fig. 2, *c*). On the other hand, nystagmus can be driven for minutes and hours, as long as stimulation continues (Bergmann *et al.* 1961).

The present observations on the progressive lengthening of HAD, during a series of stimulations, find a certain counterpart in the cat experiments reported by Delgado and Sevillano (1961) which showed that daily repetition of hippocampal stimulation prolonged the duration of afterdischarge and behavioural phenomena progressively, over a month, while stimulation conditions were kept constant. In the present experiments, however, prolongation of HAD was observed in a series of stimulations carried out on a single day. This prolongation may be due to involvement of extra-hippocampal centres, as suggested by Delgado and Sevillano (1961) for behavioral phenomena in the cat.

Finally, it is of interest to recall the importance attributed to afterdischarge by Penfield and Jasper (1954) as an essential characteristic of experimental epilepsy. AD, in the general sense of a response outlasting the stimulus, is encountered in many central reactions. However, the AD often depends on the stimulation characteristics (Bergmann *et al.* 1961; Gutman *et al.* 1962; Rosenblueth and Cannon 1941, 1942).

Therefore it may be possible to classify central responses according to the relationship of the AD to conditions of stimulation. Investigations in this direction may help to define more clearly the phenomena described so far rather vaguely as experimental epilepsy.

SUMMARY

The effect of stimulation parameters on duration of afterdischarge (AD) evoked from the dorsal hippocampus has been studied in the non-anaesthetized rabbit.

Repeated hippocampal stimulation of an animal on a single day frequently resulted in prolongation of AD in spite of constant conditions of stimulation.

Within short series of stimulations, in which the response remained constant, the following observations were made:

1. Variation of the frequency of stimulation from 10–100 c/sec resulted in AD of the same duration.
2. Increase in voltage, beyond the threshold, did not substantially influence the duration of AD.
3. Hippocampal discharge is observed *during* stimulation. Under prolonged stimulation, hippocampal discharge comes to an end while stimulation continues. The period from the beginning of stimulation to the end of the hippocampal discharge is constant and not dependent on duration of stimulation.
4. In a given animal AD evoked from different points of both dorsal hippocampi is of th same duration.
5. The duration of hippocampal AD appears to be independent of stimulation parameters over a wide range. It is suggested that this may be a general characteristic of epileptic afterdischarge.

RÉSUMÉ

L'ORDRE INVARIABLE DE LA POST-DÉCHARGE HIPPOCAMPIQUE SOUS DIFFÉRENTES CONDITIONS DE STIMULATION

L'effet des paramètres de la stimulation électrique de l'hippocampe dorsal sur la post-décharge a été étudié chez le lapin non anesthésié.

Dans une série de stimulations répétées le même jour la post-décharge hippocampique est fréquemment prolongée chez le même animal malgré des conditions constantes.

Les résultats suivants ont été observés pendant des séries de courte durée dans lesquelles la post-décharge reste constante:

1. La variation de la fréquence de stimulation de 10–100 c/sec n'a pas modifié la durée de la post-décharge hippocampique.
2. L'élévation de la tension au-dessus du seuil n'a pas affecté de façon importante la durée de la post-décharge.
3. La décharge hippocampique devient déjà visible *au cours de* la stimulation. Les potentiels de décharge hippocampiques disparaissent pendant la stimulation électrique quand celle-ci est assez prolongée. L'intervalle entre le commencement de la stimulation et la disparition des potentiels de décharge hippocampiques est constant et n'est pas fonction de la durée de stimulation.
4. La durée de la post-décharge ne varie pas quand elle est évoquée par stimulation de différents points des deux hippocampes dorsaux chez le même animal.
5. En résumé, il semble que la durée de la post-décharge hippocampique est relativement indépendante des paramètres de stimulation. Vraisemblablement, c'est là un trait caractéristique de la post-décharge épileptique en général.

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