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SPONTANEOUS BIOELECTRIC ACTIVITY OF CULTURED CEREBELLAR PURKINJE CELLS DURING EXPOSURE TO AGENTS WHICH PREVENT SYNAPTIC TRANSMISSION*

by

B.H. Gähwiler
A.M. Mamoon
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Introduction

Although spontaneous bioelectric activity of Purkinje cells has been recorded in a variety of preparations by different investigators (11, 19, 20, 28), more precise information is needed about the mechanism of spontaneous spike generation and cellular interactions. It is the purpose of this study to investigate the relative contributions of individual Purkinje cells and of their synaptic inputs to the recorded spontaneous bioelectric activity. Nerve tissue cultures provide a particularly suitable preparation since the average spike frequency and the discharge patterns remain constant over a period of at least one hour and spontaneous bioelectric activity can be recorded without the complicating effects of climbing and mossy fiber activation. In addition, agents which prevent synaptic transmission in vivo (29) can as easily be added as removed from the bathing solutions. The existence of functional interneuronal connections in cultures has been demonstrated by both electrophysiological (5, 8, 9, 25) and electron

*Running title: SPONTANEOUS ACTIVITY OF CULTURED NEURONS

microscope (3, 24) analyses. The generation of spontaneous complex synaptically-mediated discharges in cultures of rodent spinal cord and cerebral cortex has been reviewed by Crain (7). More detailed analyses of the organotypic temporal patterns of discharges in spinal cord and brain-stem explants have been reported more recently (4). The effect of different ions has been explored in a preliminary manner in cerebellar cultures (27).

Materials and methods.

Explants from the cerebellum of two- to four-day-old rats (Sprague-Dawley, Simonsen albino) were grown on glass coverslips in a plasma clot (Difco Laboratories, Ltd., liophilized chicken plasma and chicken embryo extract). The cultures were incubated on flying coverslips in roller tubes at a temperature of 37°C. The nutrient consisted of 50% Basal Medium Eagle (Grand Island Biological Company), 25% Hanks Balanced Salt Solution (Microbiological Associates, Inc.) and 25% calf serum (Grand Island Biological Company). The glucose concentration in the medium was increased to a net 6 mg/ml (21). Hanks balanced salt solution was used as bathing solution during electrophysiological experiments and contained the following ion concentrations expressed in millimoles/liter: Na^+(141.7), K^+(5.8), Cl^-(145.0), Ca^{2+}(1.3), Mg^{2+}(0.9), HCO_3^-(4.2), SO_4^{2-}(0.4), HPO_4^{2-}(0.2), $H_2PO_4^-$(0.4). The pH of the solution was kept close to 7.4 during all experiments. The electrophysiological methods applied have been described earlier (15, 16). Solutions were exchanged by means of a syringe feed system. When

a change was made, the bioelectric activity was disturbed temporarily, probably due to hydrodynamic effects. After about one minute it settled to a stable pattern. Electrical stimuli, 0.1 msec in duration and up to 30 uA in strength, were applied by a pair of micropipettes with five to ten-micrometer tips. All measurements were carried out at a temperature of 37°C ± 0.2°C.

Results

Analysis of spike trains recorded extracellularly at 37°C in Hanks balanced salt solution from the soma of large nerve cells (presumably Purkinje cells) reveals an average spike frequency of 17 ± 5 spikes/sec (16) and usually irregular interspike distribution (Fig. 1A). Less frequently, bursting activity (Fig. 1B) or regular discharge patterns (Fig. 1C) were observed. Purkinje cells can be recognized by their shape and the origin of dendritic arborization.

After increasing the concentration of Mg^{2+} in the bathing solution from 0.9 millimoles/liter to 8 millimoles/liter, about half of the Purkinje cells stopped bioelectric activity (17). The effect of Mg^{2+} upon the discharge pattern of the other Purkinje cells which kept firing in high concentration of Mg^{2+} is demonstrated in Fig. 2. The firing is characterized by a significant regularization as indicated by a decrease of the coefficient of variation (Table 1). In addition, the average spike frequency decreased to a smaller value than obtained in normal balanced salt solution. There was in no case an irregular interspike interval distribution

observed in solutions with high concentration of Mg^{2+}. All cells resumed normal bioelectric activity after transfer to normal balanced salt solution. In order to exclude the possible effects caused by an increased osmolarity due to addition of $MgCl_2$ to the balanced salt solution, glucose (leading to the same increase in osmolarity) was added to the bathing solution. There was no effect on the discharge pattern and average spike frequency detectable. It should be noted, however, that both the concentration of Mg^{2+} and Cl^- is changed when adding $MgCl_2$ to the balanced salt solution. Whereas the concentration of Cl^- is changed only by about 10%, the concentration of Mg^{2+} is increased by about 900%.

Addition of 10 micrograms/milliliter d-tubocurarine, or 10 micrograms/milliliter atropine, to the bathing solution had very little effect on the average spike frequency and on the discharge patterns. If synaptic activity was mediated by acetylcholine then blocking would be expected in a manner similar to a high concentration of Mg^{2+}. Addition of 20 micrograms/milliliter pentobarbitol led, however, to regularization of firing (Table I) as observed in vivo (23). The changes in average spike frequency and interspike interval distribution are very similar to the alterations observed when increasing the concentration of Mg^{2+}. Removal of Ca^{2+} from the bathing solution had initially the same effect of regularization of firing, but soon led to irreversible changes.

It is remarkable that isolated Purkinje cells in peripheral areas of the culture (Fig. 3), which have fewer or no synaptic connections to other neurons (19), fire regularly in solutions of

normal balanced salt solution as well as high concentrations of Mg^{2+}. The average spike frequency decreases, however, by about 20% when the concentration of Mg^{2+} is raised from 0.9 millimoles/liter to 8 millimoles/liter. Electrical stimulation some distance away from a spontaneously regular-firing neuron evoked an action potential that is possibly due to direct stimulation of dendrites or antidromic stimulation (Fig. 4B). In contrast, stimulation of nerve cells from dense areas (irregular discharge pattern) of the culture gave rise to a synaptically evoked action potential, suggesting stimulation of a pathway (Fig. 4A). Increasing the concentration of Mg^{2+} to 8 millimoles/liter abolishes only the evoked action potential, as in Fig. 4A. This gives further proof that synaptic transmission is effectively blocked by increasing the concentration of Mg^{2+} to 8 millimoles/liter.

Discussion

The regular discharge patterns during exposure to solutions with high concentration of Mg^{2+} and the observation of regularly firing Purkinje cells in peripheral areas suggests that about half of the tested Purkinje cells behave in culture like pacemaker neurons. Superposition of synaptic inputs may in normal balanced salt solution lead to the irregular interspike distribution observed. The spontaneous bioelectric activity of the other Purkinje cells that stop firing during exposure to solutions with high concentrations of Mg^{2+} is assumed to be wholly synaptic in origin. A similar selective and reversible blockade of synaptically mediated

bioelectric activity after acute as well as chronic exposure of spinal cord and cerebral cortex cultures to high concentrations of Mg^{2+} has been reported (6, 10).

Regularization of firing has also been noticed in most Purkinje cells tested two weeks after exposure of fresh explants to large doses of x-irradiation (22). These observations are consistent with the experiments described before, since it is known from recent morphological studies that an x-ray dose of 20 krad causes severe damage to synapses (14).

Spontaneous bioelectric activity has been frequently measured in situ (2, 12, 18), and it has even been observed in the chronically deafferented cerebellum (13). Fairly regular discharge patterns have been measured from Purkinje cells of adult animals in situ, but are generally attributed to mechanical injury by the microelectrode (13, 26). Purkinje cells of 7-day-old rats display, however, a regular singlet pattern which is very similar to the regular discharge patterns observed during exposure to solutions of high concentration of Mg^{2+} (11, 30). It is nevertheless not possible to exclude the possibility that the "intrinsic rhythm" observed in vitro is a property of nerve cells in culture caused by an increased excitability of neuronal membranes. Moreover, it is not yet known how the superposition of the spontaneous activity with both excitatory and inhibitory inputs is functioning and, clearly, there is need for further studies which should include stimulation of the granule cell/parallel fiber pathway and stimulation of basket and stellate cells.

The experiments described suggest that, during exposure to agents which prevent synaptic transmission in our cultures, about half of the Purkinje cells behave in vitro pacemaker-like, and half of them are silent. Other experiments in this laboratory indicate that this ratio may change with external factors such as temperature (16). Longterm experiments may help determine whether individual cells without synaptic inputs are either completely silent or spontaneously active, or whether each Purkinje cell has two stable states, one spontaneously active, the other silent, as suggested by Albus (1).

Summary

Analysis of the spontaneous activity recorded extracellularly from cultured Purkinje cells in Hanks balanced salt solution at 37°C reveals an average spike frequency of 17 ± 5 spikes/sec and usually irregular interspike interval histograms. Increasing the concentration of Mg^{2+} from 0.9 to 8 millimoles/liter leads to a significant regularization of firing of about half of the tested Purkinje cells, whereas the others stopped bioelectric activity. Pentobarbital (20 micrograms/milliliter) had the same effect, but addition of d-tubocurarine (10 micrograms/milliliter) or atropine (10 micrograms/milliliter) had no detectable effect on the rate and temporal distribution of the spontaneous activity. Neurons in peripheral areas of cultures fire regularly in solutions of normal as well as high concentrations of Mg^{2+}.

Acknowledgments

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TABLE I

FIRING FREQUENCIES OF PURKINJE CELLS

UNDER VARIOUS CONDITIONS

	BSS	8mM/l Mg^{2+}	Pentobarbital (20µg/ml)
Number of cells	35	25	10
Average (imp/sec)	17	10.8	8.2
Standard deviation (imp/sec)	23	2.3	3.1
Coefficient of variation	1.35	0.21	0.38

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FIGURE CAPTIONS

Figure 1

Extracellularly recorded spontaneous action potentials of neuron soma from cerebellar cultures.

Figure 1A: Sample of most often observed discharge pattern.

Figure 1B: Sometimes, spikes occur in trains, with silent periods between the bursts.

Figure 1C: Nerve cells in peripheral areas of cultures exhibit regular discharge patterns.

Figure 2

Effects of a high concentration of Mg^{2+} on the discharge pattern of Punkinje cells.

Upper picture: Interspike distribution as recorded in Hanks Balanced Salt solution (0.9 mM/1 Mg^{2+}).

Lower picture: Interspike distribution recorded from the same neuron in a solution with 8 mM/1 Mg^{2+}. Time between the two measurements: 10 min. Bin with: 4 msec. Total counts: 3000.

Figure 3

Living large nerve cells (presumably Purkinje cells) in cultures from cerebellum of newborn rats.

Upper picture: Row of large nerve cells in dense area of culture. 17 days in vitro. Phase contrast.

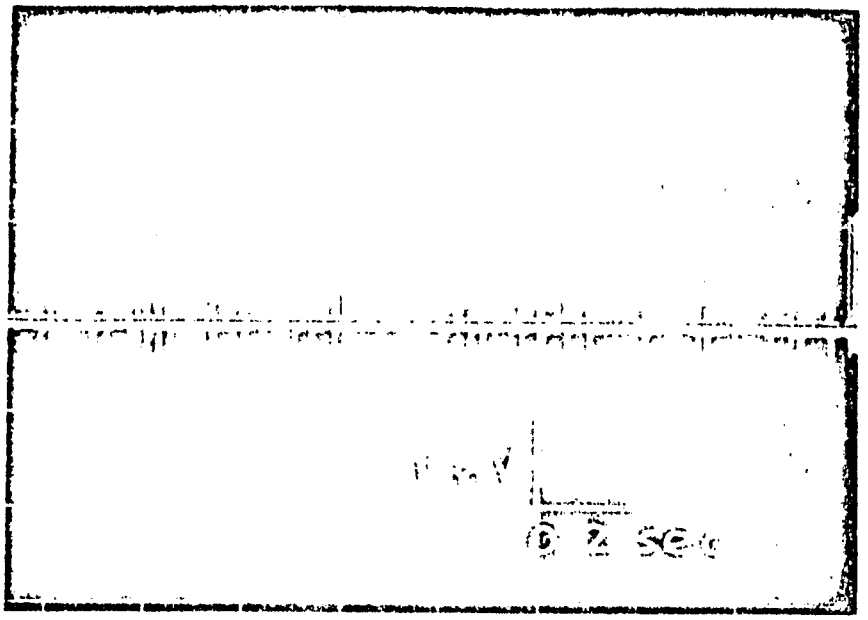
Lower picture: Purkinje cell in a peripheral area of a culture. A microelectrode is positioned close to the soma of the neuron. 20 days in vitro.

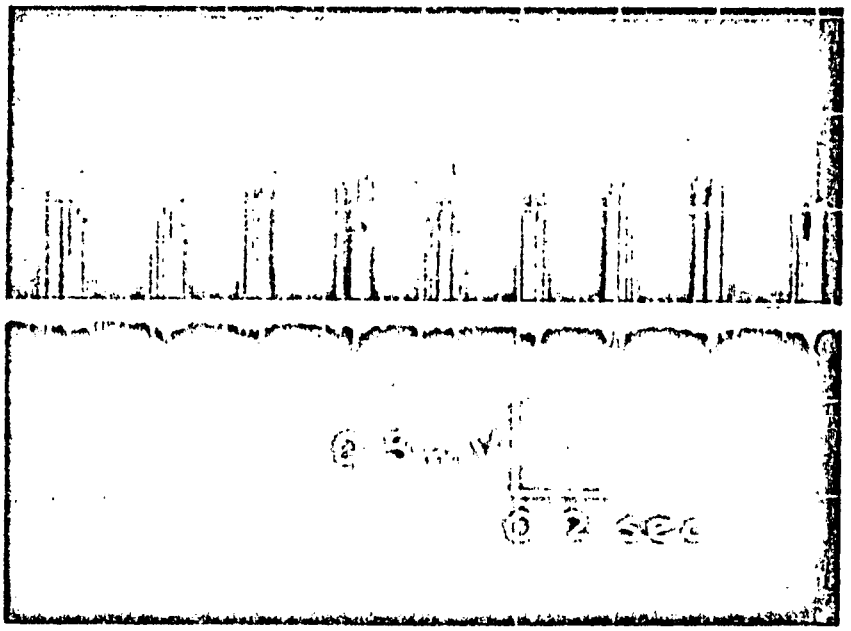
Figure 4

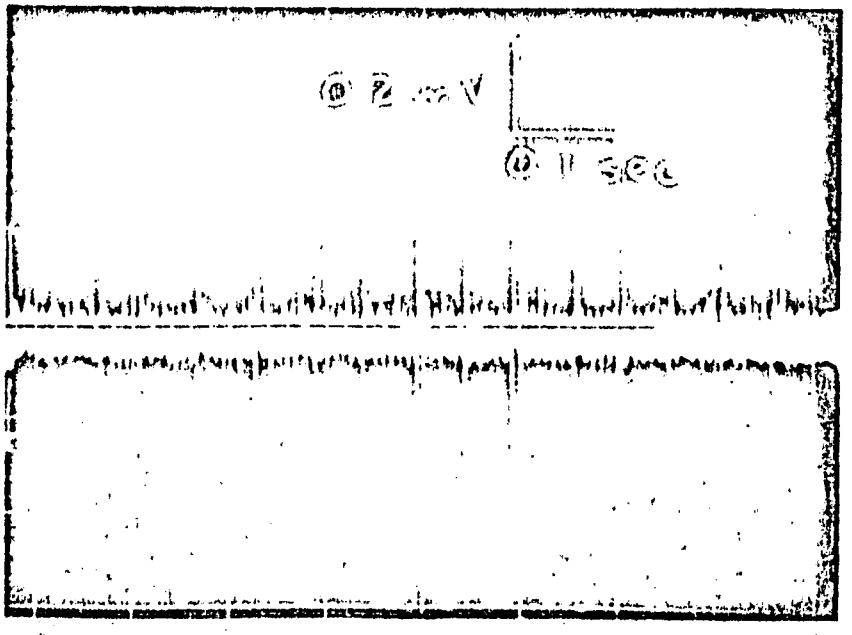
Response of Purkinje cells to electrical stimulation approximately 100μ away from the cell soma.

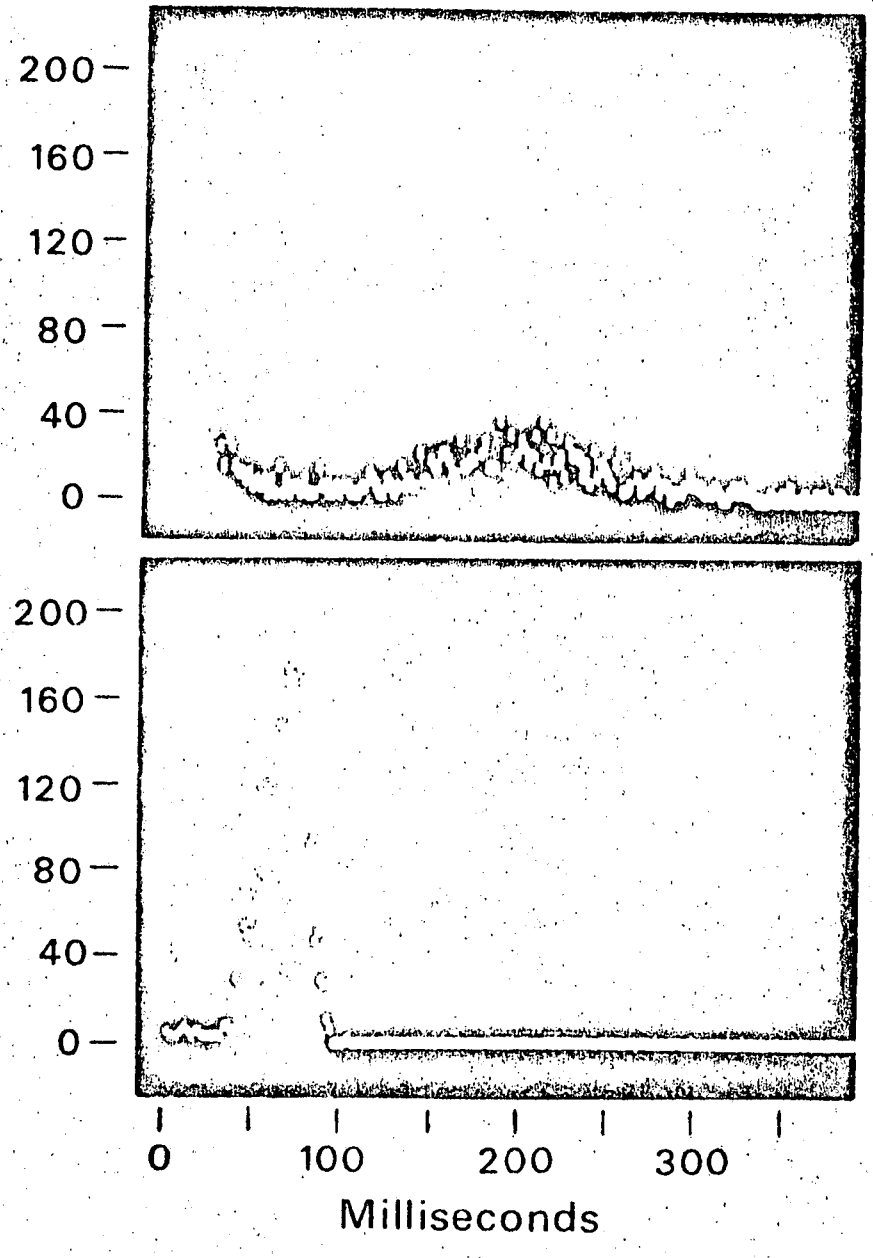
Figure 4A: Evoked action potential in irregularly firing nerve cell. This action pontential was eliminated when the concentration of Mg^{2+} was raised.

Figure 4B: Evoked action potential in regularly firing neuron. This action pontential is not affected by a change in the concentration of Mg^{2+} and is probably due to direct stimulation of dendrites or antidromic stimulation.





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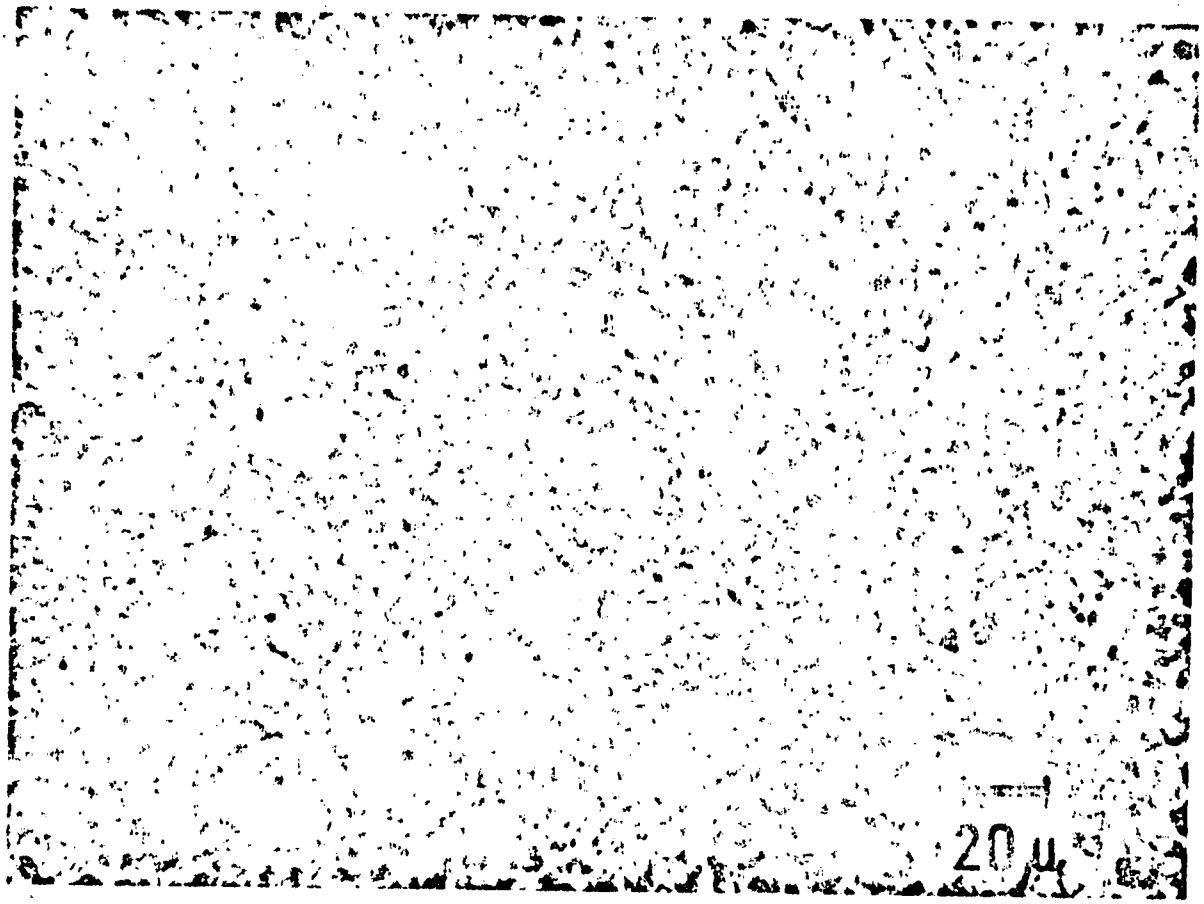

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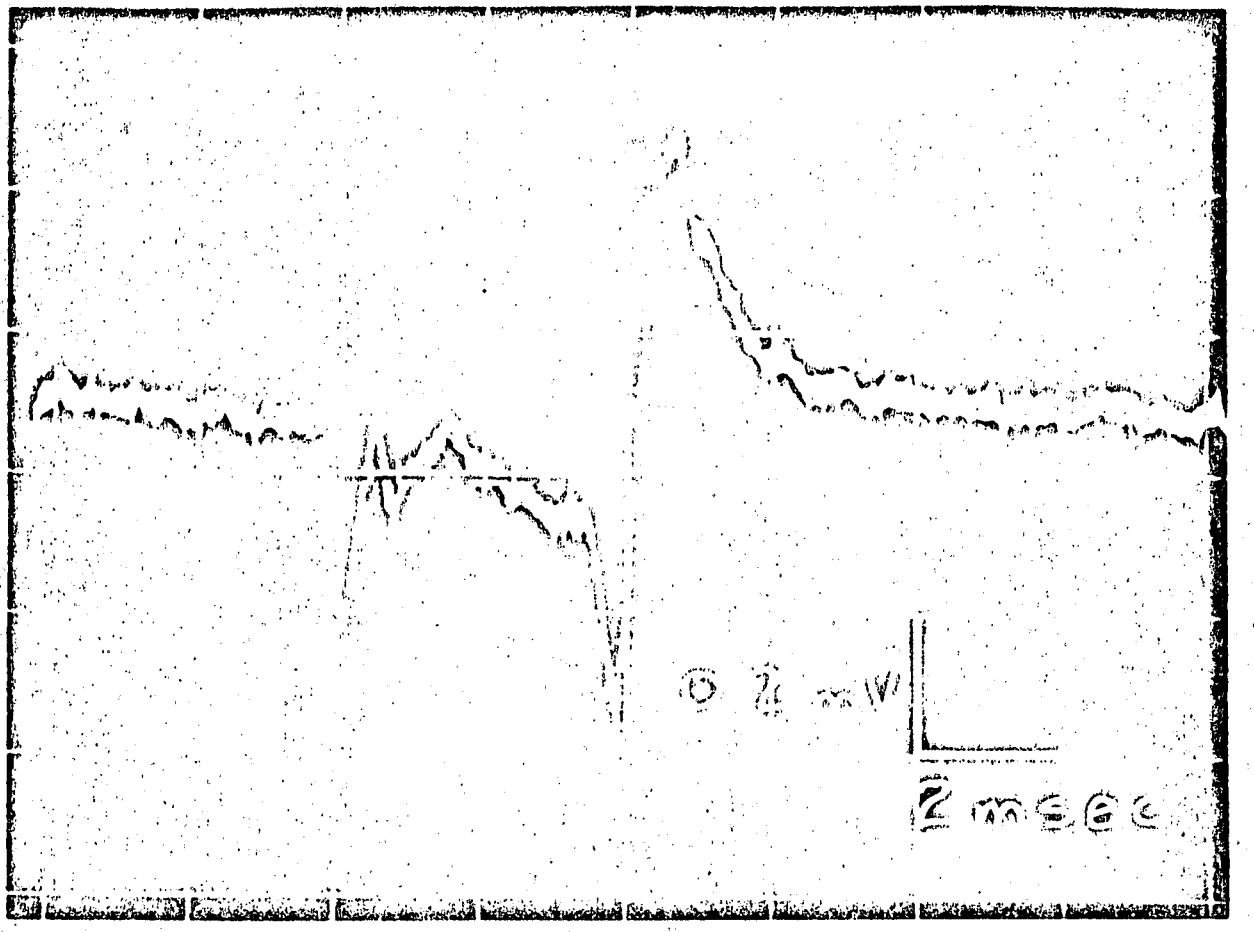

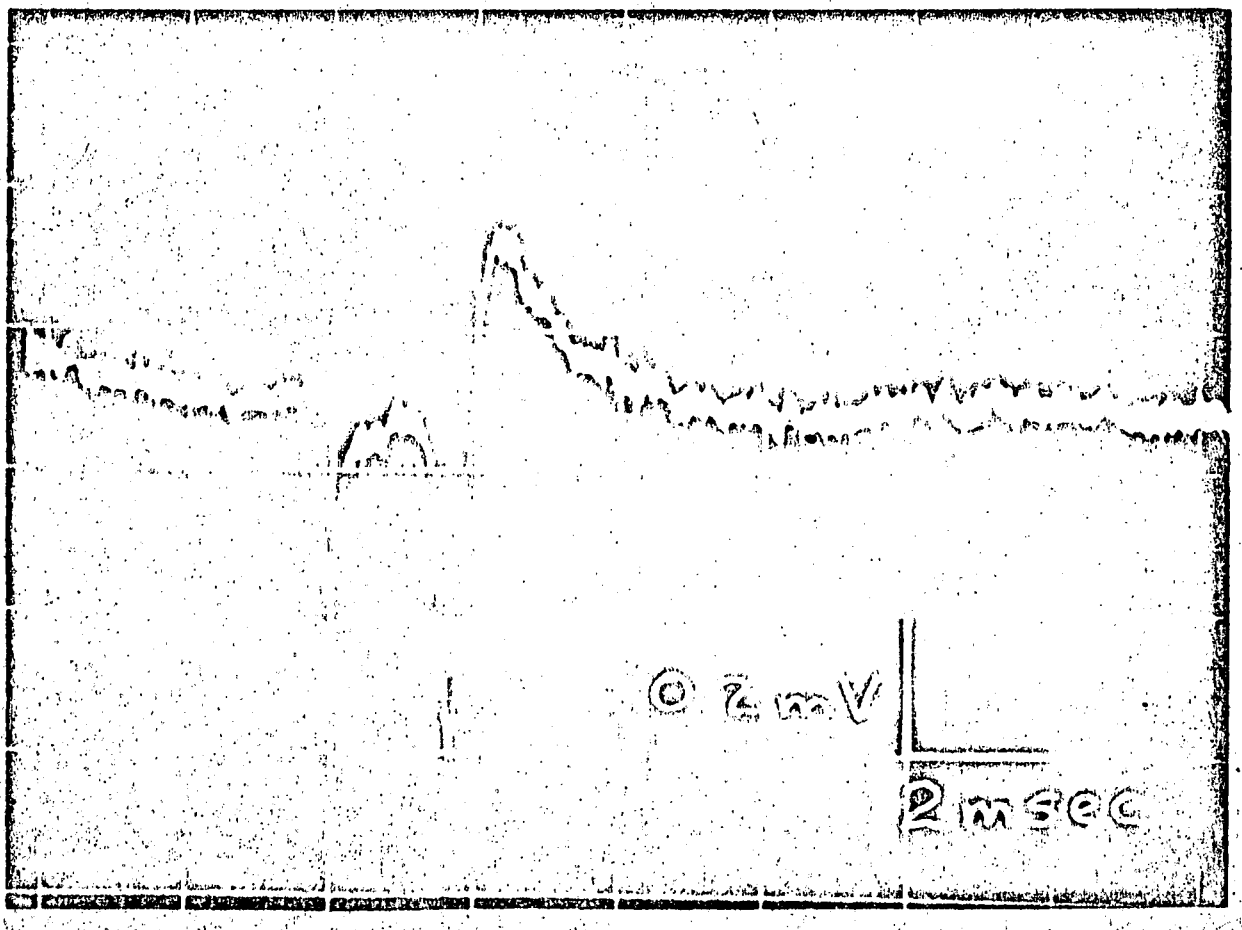

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