

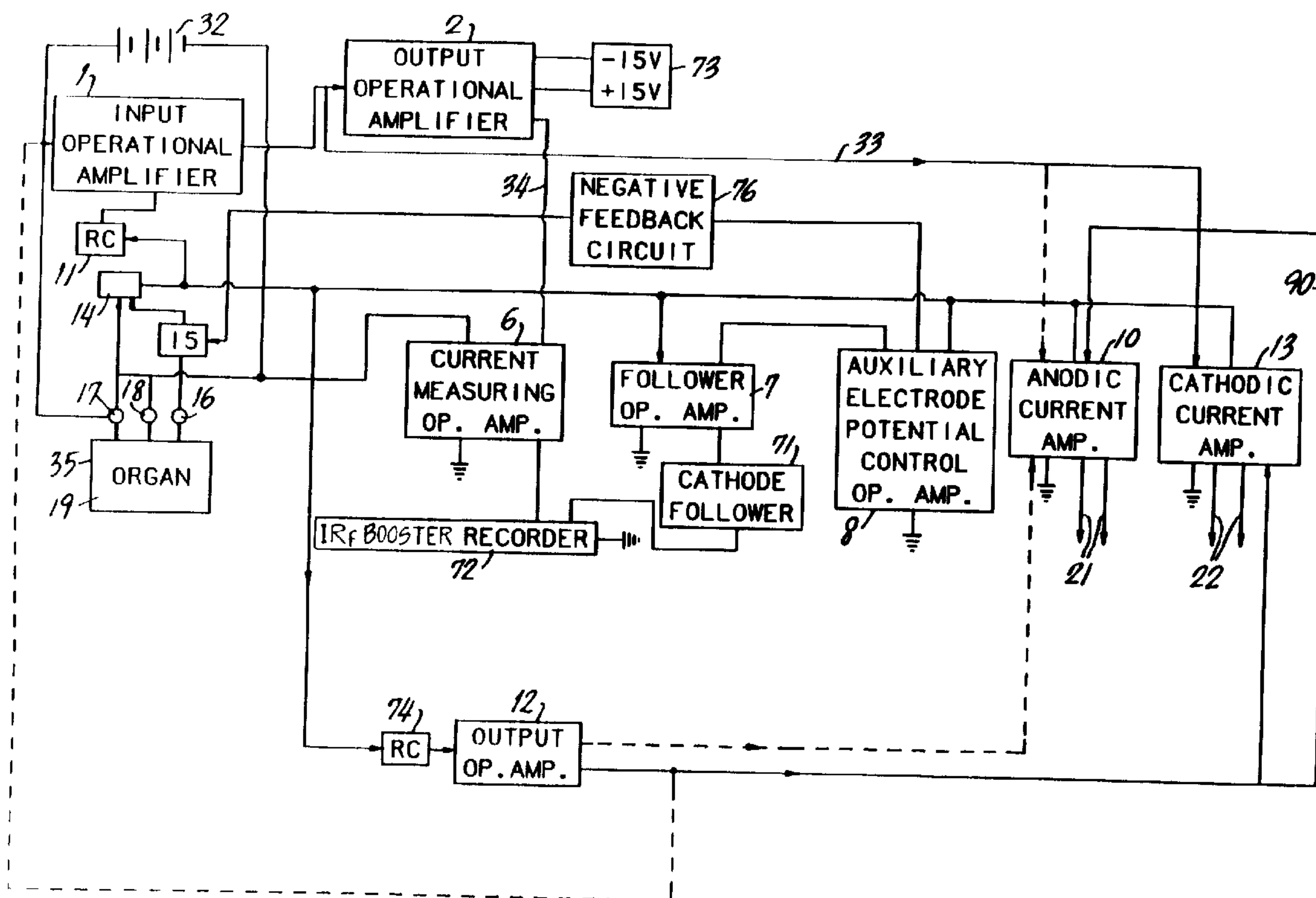


(86) Date de dépôt PCT/PCT Filing Date: 1990/08/14
(87) Date publication PCT/PCT Publication Date: 1991/03/07
(45) Date de délivrance/Issue Date: 2001/08/07
(85) Entrée phase nationale/National Entry: 1992/01/17
(86) N° demande PCT/PCT Application No.: US 90/04571
(87) N° publication PCT/PCT Publication No.: WO 91/02485
(30) Priorité/Priority: 1989/08/17 (395,431) US

(51) Cl.Int.⁵/Int.Cl.⁵ A61B 5/05
(72) Inventeur/Inventor:
Broderick, Patricia A., US
(73) Propriétaire/Owner:
RESEARCH FOUNDATION OF THE CITY UNIVERSITY
OF NEW YORK, US
(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : MICROELECTRODES ET LEUR USAGE DANS UNE INSTALLATION DE COURANT CATHODIQUE
ELECTROCHIMIQUE AYANT UNE APPLICATION TELEMETRIQUE

(54) Title: MICROELECTRODES AND THEIR USE IN CATHODIC ELECTROCHEMICAL CURRENT ARRANGEMENT
WITH TELEMETRIC APPLICATION



(57) Abrégé/Abstract:

This invention relates to a microelectrode comprising graphite, oil and, additionally, a compound selected from the group of lipids, glycolipids, lipoproteins, fatty acids, fatty acid derivatives, any water insoluble species and perfluorosulfonated compounds and salts thereof. This invention also relates to a method for using the microelectrode, a device that may be employed with the microelectrode, a method for making the microelectrode, and a method for using the device with the microelectrode.

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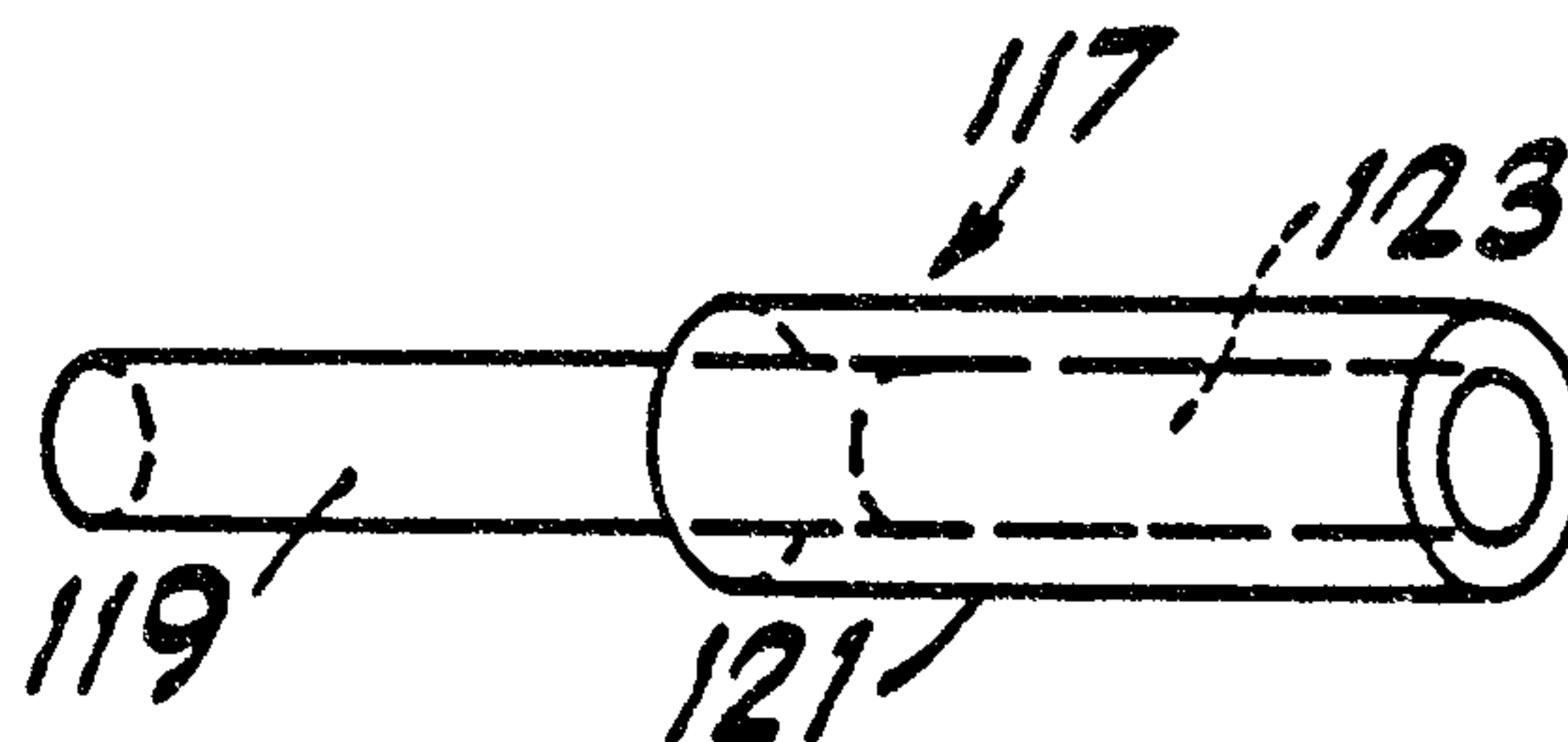
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61B 5/05, G01N 27/26	A1	(11) International Publication Number: WO 91/02485 (43) International Publication Date: 7 March 1991 (07.03.91)
(21) International Application Number: PCT/US90/04571 (22) International Filing Date: 14 August 1990 (14.08.90) (30) Priority data: 395,431 17 August 1989 (17.08.89) US (71) Applicant: RESEARCH FOUNDATION OF THE CITY UNIVERSITY OF NEW YORK [US/US]; 79 Fifth Avenue, New York, NY 10003 (US). (72) Inventor: BRODERICK, Patrica, A. ; 1110 Dean Avenue, Bronx, NY 10465 (US). (74) Agents: VENTURINO, Anthony, P. et al.; Morgan & Finnegan, 345 Park Avenue, New York, NY 10154 (US).		(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

(54) Title: MICROELECTRODES AND THEIR USE IN A CATHODIC ELECTROCHEMICAL CURRENT ARRANGEMENT WITH TELEMETRIC APPLICATION**(57) Abstract**

This invention relates to a microelectrode comprising graphite, oil and, additionally, a compound selected from the group of lipids, glycolipids, lipoproteins, fatty acids, fatty acid derivatives, any water insoluble species and perfluorosulfonated compounds and salts thereof. This invention also relates to a method for using the microelectrode, a device that may be employed with the microelectrode, a method for making the microelectrode, and a method for using the device with the microelectrode.



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5 MICROELECTRODES AND THEIR USE IN A
CATHODIC ELECTROCHEMICAL CURRENT
ARRANGEMENT WITH TELEMETRIC APPLICATION

BACKGROUND OF THE INVENTION

10 I. FIELD OF THE INVENTION

This invention relates to the use of an in vivo electrochemical method (also called an in vivo voltammetric method or voltammetry) to measure the amounts of biogenic chemicals present in the body and brain of an animal or a human being. More particularly, it relates to the use of in vivo semiderivative voltammetric measurements of biogenic chemicals, particularly neurotransmitters, such as amines, amine metabolites, ascorbic acid, amino acids and neuropeptides produced in reaction to psychopharmacological agents and neuropsychopharmacological agents such as analgesics, antipsychotic drugs, antidepressants, and other modulators of brain and peripheral neurochemistry in diseased and healthy states.

25 II. DISCUSSION OF BACKGROUND

It has been known to those possessing ordinary skill in the art that it is possible to measure certain limited types of biogenic chemicals using in vivo electrochemistry in the brains and suborgans of nonhuman primates and other animals. This measurement has been accomplished using such facets of electrochemical measurements as chronoamperometry, differential pulse voltammetry, double differential pulse voltammetry, linear scan

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° voltammetry, and semiderivative voltammetry. In all of these methods, a working electrode, a reference electrode and an auxiliary electrode are attached to the brain or other organ of the animal to be studied. A controlled potential is applied to the working electrode and the current passing between the working electrode and the reference electrode is monitored as a signal and used to measure basal neurotransmitter release and any alterations in brain neurochemistry after pharmacological manipulation with drugs and other compounds and/or after a diseased state such as schizophrenia or Alzheimer's has been induced or mimicked. The signal is directly related to the chemical concentration of the neurotransmitter released from or through or taken up by or through the neuronal brain membrane (extracellularly) or the synaptic vesicles or other relevant organelles or cytoplasm (intracellularly). The mechanism of action can be presynaptic, or possibly postsynaptic and each signal represents an instantaneous readout of rate of neuronal mechanism. The signal may also be related to inhibition of normal reuptake of neurotransmitter at the neuronal membrane and may be a summation of release and reuptake processes, especially during treatment. The prior art teaches that this current is an anodic (oxidation) current, based on scientific principle. This signal is recorded as a graph indicating change in current with respect to time (chronoamperogram) or voltage (voltammogram).

30 It is known that voltammetric measurements can be used to detect certain biogenic substances in the brain of rats [Kissinger, P.T.; Hart, J.B.; Adams, R.N.; "Voltammetry in Brain Tissue - A New Neurophysiological Measurement", Brain Research, 55 (1973), p. 209.]. Other researchers have also

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° detected signals in the brains of living rats, as follows: McCreery, et al., Brain Res. Vol. 73 (1974), p. 23; Gonon, et al., Brain Res. Vol. 223 (1981), p. 69; Lane, et al., J. Electroanal. Chem., Vol. 95(1979), p. 117; Clemens and Phebus, Brain Res., Vol 267(1983), p. 183 and Millar, et al., Eur. J. Pharmacol. Vol. 109(1985), p. 341.

There has been, however, little or no description of circuitry for in vivo electrochemical circuits. However, certain improvements have been made for in vitro voltammetric measurements since the linear scan for in vivo electrochemistry method was first described for measuring biogenic chemicals. One such improvement was the in vitro processing of the linear scan current signal as the first half-derivative of the linear signal [Oldham, "Analytical Chemistry" Vol. 45 (1973) p. 39 and U.S. Patent No. 3,868,578; Kanazawa, U.S. Patent No. 4,449,552]. However, neither Oldham nor Kanazawa describe circuitry applicable for detection of organic materials in living organisms. Although they describe oxidation and reduction reaction species, they do not describe cathodic (reduction) currents in vivo. For the purposes of this application, cathodic current is defined as current based on the acquisition of electrons by neurochemicals within the organ or suborgan and flowing away from and/or on an indicator electrode situated within the organ or suborgan. Anodic current is defined as current based on the loss of electrons by neurochemicals in the organ or suborgan and flowing toward and/or on an indicator electrode situated within the organ or suborgan.

When applied to the brain, or other body organs, this type of processing (the first half derivative of the linear signal) should result in a

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° semidifferentiated voltammogram having sharper peaks, which then allows greater separation between peaks representing chemical substances and which are easier to read than previous, linear voltammograms. The older conventional methods did not allow sharply defined individual detection of amines because of similar electrochemical potentials for biogenic amines set by the catechol moiety of the amines. Chronoamperometry, for example, does not allow any direct, individual and simultaneous detection of the biogenic amines. Semiderivation or semidifferentiation of the signal briefly allowed somewhat better detection. Many practitioners, however, have found it difficult or impossible to obtain reproducible measurements routinely using what is known as the in vivo electrochemistry technique of semiderivative or semidifferential voltammetry.

Although telemetric devices have been produced in the past, as described in U.S. Patent No. 4,424,812 (Lesnick) and U.S. Patent No. 3,882,277 (DePedro), telemetric devices for monitoring brain signals have not been described. Neither of these patents describe monitoring signals produced electrochemically either in vivo, in vitro or in situ.

The wisdom of the prior art indicates that an oxidation current, or anodic current, should be used to detect biochemical species in the brain. Previous researchers assumed that in living systems, all chemical species which could be detected by electrochemical signals were converted into stable oxidized species. Most of the previous researchers also assumed that all biogenic chemical reactions produced oxidized species without producing stable reduced species. These assumptions have provided only a limited tool for diagnosing the mental and physiological states of living organisms, as only a

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- ° limited number of biogenic chemicals can be detected using prior art methods.

SUMMARY OF THE INVENTION

5 It is an object of this invention to provide a method for measuring biogenic chemicals.

It is another object of this invention to provide an in vivo, in situ or in vitro electrochemical method for measuring biogenic chemicals.

10 It is another object of this invention to provide a useful way to measure biogenic chemicals using the semiderivative or semidifferential voltammetric technique to produce a cathodic current.

15 It is another object of this invention to provide the circuitry for such a cathodic current.

It is another object of this invention to provide a cyclic voltammogram within the context of the cathodic current.

20 It is another object of this invention to provide an in vivo electrochemical method for measuring biogenic amines, amine metabolites, ascorbic acid, amino acids and neuropeptides and other neurotransmitters and modulators of brain neurochemistry.

25 It is another object of this invention to provide an in vivo electrochemical method for measuring alterations in biogenic brain chemicals in relation to the administration, both peripheral and central, of psychopharmacological agents and
30 neuropsychopharmacological agents such as antidepressants, analgesics, antianxiety agents, anti-panic agents, anti-manic/depressive agents, calcium blocking agents, agents of addiction, other neuropeptides, enkephalinamides, dynorphin and other
35 potential modulators of brain neurochemistry.

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° It is another object to provide an in vivo electrochemical method for interpreting these alterations in brain neurochemistry for diagnosing mental illness, Alzheimer's disease, and other diseased states caused by or which involve these neurochemical alterations, vis-a-vis healthy states, and developing new and more effective psychotherapeutic agents and other clinical applications.

10 It is another object of this invention to provide an in vivo electrochemical method for measuring the levels and dynamic changes of biogenic chemicals with an instantaneous readout of rate in humans, in vivo.

15 It is another object of this invention to provide a means for studying the dynamic levels, reuptake and/or release of biogenic chemicals produced during certain behavioral manifestations and thus provide a method for determining the causes of these manifestations.

20 It is another object of this invention to provide a method by which to correlate the production of certain biogenic chemicals with electrophysiological measurements and other measurements such as dialysis measurements.

25 It is another object of this invention to develop electrodes which are extremely selective to biogenic chemicals, which could be and are biological markers.

30 It is another object of this invention to provide a method of making reference, indicator (working) and auxiliary electrodes.

35 It is another object of this invention to provide a means for diagnosing illness in vivo, as opposed to the current manner of diagnosing illness from in vitro markers, i.e. post-mortem, from frozen

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° brains and body organs.

It is another object of this invention to provide a telemetric method of diagnosing dynamic release mechanisms of biogenic chemicals such that a human patient may be continuously monitored without the impediment of wires.

It is another object of this invention to describe peaks representing biogenic chemicals and neurotransmitters which have heretofore not been described and which influence behavior.

It is another object of this invention to provide a means for automating the in vivo monitoring of an animal or patient.

It is another object of this invention to provide neurochemical profiles from different brain regions, providing a neurochemical mapping device for diagnosis.

Unexpectedly, the inventor has found that a vast number of biochemical reactions produce a large number of biogenic chemical species heretofore unknown and undetectable. Using the method of this invention, it has now been found that semiderivative (semidifferential) voltammetry can be used for measuring concentrations and an instantaneous readout of rates of release and/or reuptake of biogenic chemicals in vivo in a reliable and repeatable manner according to the method of this invention. More particularly, it has been found, also unexpectedly, that the current produced by biogenic chemicals when monitored by a semiderivative electrochemistry device is only measurable by circuitry which provides a cathodic current. Thus, the method of this invention relates to the measurement of a current produced in the body of an animal or a human being, the signal of which is processed by a cathodic current vis-a-vis previous circuits which are anodic. This permits the

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- ° measurement of stable reduced and oxidized species in vivo with a cathodic current, heretofore inconceivable.

5 The method of this invention is particularly well-suited to measuring biogenic chemicals in vivo, that is, in a living animal or human being, although this method can be used to measure such chemicals in vitro. It was previously thought that biochemical reactions, in vitro, and quintessentially in vivo could only be measured by anodic currents. However, 10 using the method of this invention, one can routinely detect oxidized and reduced species, with cathodic currents, expanding the range of detection considerably for the field of diagnosis in pharmacotherapeutics and medicine.

15 The present invention pertains to electrodes; an electronic circuit arrangement, which includes the electrodes, acting as an electrochemical cell to effectuate a cathodic (reduction) current in vivo, in vitro and in situ, which can be faradaic in nature; methods for using the circuit; and methods 20 for making the electrodes. In situ is defined as taking a specimen of living cells, or an organ containing living cells, from a body and analyzing the specimen in a glass, or similar vessel. An example of an in situ specimen is a Pap Smear. The 25 cathodic current can be derived from an electrochemical cell; the cathodic current with semidifferential voltammetry is unknown within the current concepts of brain neurophysiology and in vivo electrochemical reactions. The cathodic current 30 serves as a specific detector for recognizing electroactive materials (and even non-electroactive materials with the use of antibodies) for biomedical diagnosis of diseased states, vis-a-vis healthy states. Cathodic analysis and detection of 35

reversible and irreversible reduced or oxidized compounds, directly applicable to pharmacotherapeutics in biomedical research, were heretofore precluded.

The invention provides a microelectrode for inserting in vivo, in situ or in vitro into a warm-blooded or cold-blooded animal brain or body, or extra-corporeally of said animal brain or body and measuring, in vivo, in situ, or in vitro, intracellular and/or extracellular concentrations and/or release and/or reuptake inhibition of one or more biogenic chemicals, comprising, a mixture of graphite, oil and at least one compound selected from the group consisting of complex lipids and simple lipids and salts thereof and wherein the complex lipid is selected from the group consisting of acylglycerols, phosphoglycerides, sphingolipids, glycolipids, lipoproteins and waxes and the simple lipid is selected from the group consisting of terpenes, steroids and prostaglandins, said graphite, said oil, and the compound being present in said microelectrode in a ratio of graphite, in weight in grams, oil, in volume, in cubic centimeters, and compound, in weight in grams, of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5, respectively, the diameter of the microelectrode ranging from about 0.001 μm to about 10 mm, and said oil has a specific gravity from 0.5 to 2.

The invention also provides a microelectrode for inserting in vivo, in situ and in vitro into a warm-blooded or cold-blooded animal brain or body, or extra-corporeally of said animal brain or body and measuring, in vivo, in situ, or in vitro intracellular and/or extracellular concentrations and/or release and/or reuptake inhibition of one or more biogenic chemicals, comprising, a mixture of graphite, oil and at least two compounds, the first being arachidic acid and the second being stearic acid, said at least two compounds being employed in a 1:1 weight ratio with respect to each other, said mixture having a ratio of graphite, in weight in grams, oil, in volume in cubic centimeters, and combined compound weight in grams of 1.0 to 2.0:0.1 to 5.0:0.01 to 0.5,

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respectively, and the diameter of said microelectrode ranging from about 2 μm to about 1 mm.

The invention further provides a microelectrode for inserting in vivo, in vitro or in situ into a warm-blooded or cold-blooded animal brain or body, or extra-corporeally and measuring intracellular and/or extracellular concentration and/or release and/or reuptake of one or more biogenic chemicals while measuring said chemical in situ, or in vivo or in vitro and said microelectrode comprises a mixture of graphite, oil and at least one compound selected from the group consisting of palmitoleic acid, oleic acid, linoleic acid, arachidonic acid, elaidic acid, and transhexadecenoic acid and salts thereof and in which the graphite, oil and compound are present in a ratio of graphite, weight in grams, oil volume in cubic centimeters, compound, weight in grams, of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5 and said oil has a specific gravity from 0.5 to 2, and the diameter of said micro-electrode ranges from 0.001 μm to about 10 mm.

The invention additionally provides a microelectrode for inserting in vivo, in vitro or in situ into a warm-blooded or cold-blooded animal brain or body and measuring intracellular and/or extracellular concentrations and/or release and/or reuptake of one or more biogenic chemicals while measuring said chemicals in situ, or in vivo or in vitro and said microelectrode comprising a mixture of graphite, oil and at least one compound selected from the group consisting of acetic acid, propionic acid, butyric acid, pentanoic acid, caproic acid, caprylic acid, capric acid, tridecanoic acid, pentadecanoic acid, heptadecanoic acid, nonadecanoic acid, lauric acid, myristic acid, arachidic acid, lignoceric acid, lignoceric acid, cerebronic acid, lactobacillic acid and salts thereof, the graphite, oil and compound being in a ratio of graphite, weight in grams, oil, volume in cubic centimeters, compound, weight in grams, of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5, respectively, said oil having a specific gravity from 0.5 to 2, and the diameter of said microelectrode ranges from about 0.001 μm to about 10 mm.

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The invention also provides a microelectrode for inserting in vivo, in vitro or in situ and measuring intracellular and/or extracellular concentrations and/or reuptake of one or more biogenic chemicals while measuring said chemicals in situ or in vivo or in vitro and said microelectrode comprising a mixture of graphite, oil and stearic acid, said graphite, said oil and said stearic acid being present in said microelectrode in a ratio of graphite, weight in grams, oil, volume in cubic centimeters, stearic acid, weight in grams, of 1.0 to 1.5:1.19 to 5.0:0.01 to 0.5, respectively, and in which the oil has a specific gravity from 0.5 to 2, and the diameter of said microelectrode ranges from about 2 microns to about 1 mm.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1 and 2 are schematic representations of two circuits which can be used in attempting to establish a semidifferential voltammetric signal.

Figures 3A, 3B and 3C are representations of graphs showing signals which may be generated by linear and semidifferential scanning. Figure 3A depicts a graph drawn according to conventional electrochemical format wherein the axis indicating anodic (oxidation) current points downward and the axis indicating positive voltage points to the left. Figure 3B and 3C depict semiderivative voltammetry signals drawn according to nonconventional format, wherein the axes indicating anodic (oxidation) current points upward and the axes indicating positive voltage points to the right.

Figure 4 is a representation of semiderivative voltammograms obtained from the tuberculum olfactorium of the rat brain before and after systemic treatment with D-Ala²-D-Pro⁵-enkephalinamide monoacetate (DAP), a compound similar to an endogenous opiate.

Figure 5 is a representation of semiderivative voltammograms obtained from nucleus accumbens and striatum of the rat brain before and after treatment with DAP. These beginning neurochemical profiles from different brain regions

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can provide a means for neurochemical mapping for diagnosis as also exemplified in figure 6 below, so that the electrode placement can be ascertained by

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the signal profile displayed on a recorder, printer, or computer monitor.

Figure 6 is a representation of semiderivative voltammograms obtained prior to and 1, 2 and 3 hours after treatment with morphine, the results being directly related to underlying neurochemical addiction and pain processes.

Figure 7 is a representation of the results of the signal obtained using the method of this invention of endogenously released striatal dopamine and serotonin in acutely and chronically diabetic rats. Results are expressed as percent change from endogenously released dopamine and serotonin in the non-diabetic rat, matched for age, sex, food and weight. Bars represent the mean values derived from six to eight rats.

Figure 8 is a representation of the signals, obtained from using the method of this invention, of rat striatal extracellular dopamine in hypoxic hypoxia using the method of this invention. Results are expressed as percent change from basal extracellular dopamine in the same animals under normal and abnormal conditions of O₂ (oxygen) availability. Bars represent mean values derived from six rats.

Figure 9a represents a semidifferential voltammogram showing basal dopamine and serotonin release from rat striatum in vivo prior to treatment with dynorphin. Figure 9b represents a semidifferential voltammogram showing dopamine and serotonin release from rat striatum in vivo after pharmacological manipulation with dynorphin (1-13) 1.5 mg/kg administered subcutaneously. The figure uses nonconventional electrochemical notation.

Figure 10a illustrates a line graph showing the time course characteristics of the effect of

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° cocaine (20 mg/kg injected subcutaneously) on dopamine release from rat striatum. The x axis represents time (in minutes) before and after the administration of cocaine. The y axis represents dopamine release as percent of control. The percent
5 of controls were calculated by averaging the first four scans and dividing all values by that average. Figure 10b illustrates semiderivative voltammograms showing post-mortem differences between dopamine release in cocaine treated (left voltammogram) and
10 untreated animals (right voltammogram). The x axis represents increasing oxidation potentials in volts; the y axis represents current in nA sec^{-1} . The electrochemical signal for dopamine (1) represents dopamine release pre-mortem. The electrochemical
15 signals for dopamine (2,3,4,5) represent dopamine release post-mortem, (ten minute intervals), in a cocaine treated animal. In contrast, the right voltammogram represents dopamine release ten minutes post-mortem in an untreated animal. The figure uses
20 non-conventional electrochemical notation.

Figure 11 is a representation of a typical linear scan cyclic voltammogram for a reversible redox reaction by electrochemical convention.

25 Figure 12 is a representation of a typical linear scan cyclic voltammogram for an irreversible redox reaction producing a stable oxidized species only.

30 Figure 13 is a representation of a typical linear scan voltammogram producing stable reduced and oxidized species.

35 Figure 14 is a representation of semiintegrated and semiderivative signals wherein curve A in amperes represents unchanged current and curve B in ampombs represents a semiintegrated curve, the latter providing for a longer lasting

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° signal.

Figure 15 is a schematic diagram illustrating an embodiment of a circuit arrangement according to the method of this invention. The diagram illustrates the circuitry for producing cathodic (reduction) currents to distinguish these from anodic (oxidation) currents which are used in other forms of electrochemical detection such as linear scan and chronoamperometry.

Figure 16 is a diagram illustrating a linear scan voltammogram showing the production of biogenic chemicals using the conventional electrochemical notation.

Figure 17 illustrates a semidifferential voltammogram showing the production of biogenic chemical peaks at 150, 290, 520, 690, 790, 820 and 910 mv. This Figure illustrates the usefulness of the methods of this invention in medical diagnosis, as the peaks represent neurotransmitter and neurotransmitter-like peptides as detected by cathodic currents in accordance with the method of this invention with semidifferential processing according to the invention herein using conventional electrochemical notation and describing peaks not heretofore described.

Figure 18 is a schematic diagram of the three electrode system used in accordance with the method of this invention to generate such signals from living organisms and its relationship to the operational amplifier circuitry needed for semidifferential processing of current in addition to other types of electroanalysis.

Figure 19(a) and 19(b) are diagrams illustrating that the difference between linear scanning techniques (b) and semiintegral and semidifferential processing (a) of current are not a

function of polarity.

Figures 20(a) and 20(b) are diagrams illustrating that the differences between linear scanning techniques (b) and semiintegral and semidifferential processing (a) of current are not a function of the directionality of E_{app} (applied potential).

Figure 21 is a diagram illustrating recording results from a semidifferential voltammogram processed with an anodic (oxidation) current, from the brain. The recording is unrecognizable.

Figure 22 is a diagram illustrating a recording from a semidifferential voltammogram processed with a cathodic (reduction) current according to the method of this invention.

Figure 23 is a diagram illustrating a recording from a semidifferential voltammogram processed with a cathodic (reduction) current with an electrode which had not been brain-treated; e.g., whose adsorption properties at the diffuse double layer of the electrode surface (the capacitance or barrier layer) had not overcome any possible column effects.

Figure 24 is a diagram illustrating a recording from a semidifferential voltammogram, processed with a cathodic (reduction) current, with a brain fluid-adsorbed electrode treated with fatty acids to selectively detect cations or anions for medical diagnosis.

Figure 25 is a diagram illustrating a recording from a semidifferential voltammogram, processed with a cathodic (reduction) current according to the method of this invention, with a brain fluid adsorbed electrode, treated with fatty acids, in vitro in phosphate buffer (0.01M PO_4

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buffer made with monobasic and dibasic phosphate) with dopamine and serotonin hydrochloride, said biogenic chemicals correlating to the same peaks in the brain, thus showing the detection of said biogenic chemicals as attainable for purposes of future discoveries in treatment.

Figure 26 is a schematic drawing illustrating the telemetric application to the electrochemical process of this invention, using a channel 8 or 9 television band in the transmitter to detect biogenic and other chemicals useful in biomedical diagnosis.

Figure 27 is a schematic drawing illustrating a system for interfacing the detector with a computer.

Figures 28(a) and 28(b) are computer programs to direct a modified Bioanalytical System (BAS) brand DCV5 detector or similar detectors to scan for oxidation or reduction currents in vivo (Computer Program 1) and in vitro (Computer Program 2), dependent on the type of electrochemical technique used.

Figure 29 (a) and (b) are schematic diagrams of (a) a generalized operational amplifier circuit and (b) an operational amplifier integrator.

Figure 30 is a schematic diagram of a direct coupling amplifier having two triodes.

Figure 31 represents:

(a) A semidifferential voltammogram showing the dopamine (DA) signal on the first trial of electrode conditioning in phosphate buffer containing 5uM of each of dopamine (DA), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), 3-4 dihydroxyphenylacetic acid (DOPAC), uric acid (UA) and ascorbic acid (AA).

(b) A semidifferential voltammogram showing

the 5-HT signal on the first trial of electrode conditioning in phosphate buffer containing 5uM of each of DA, 5-HT, 5-HIAA, DOPAC, UA and AA.

(c) A semidifferential voltammogram showing no signal in phosphate buffer having a pH of 7.4 with no added chemicals.

Figure 32 is a semidifferential voltammogram derived from the graphite stearate electrode in phosphate buffer having a pH of 7.4 containing 5uM of each of DA, 5-HT, DOPAC, 5-HIAA, AA and UA on the third trial of electrode conditioning. Only DA and 5-HT were detected. DOPAC, 5-HIAA, AA and UA, did not alter either the DA signal or the 5-HT signal.

Figure 33 is a semidifferential voltammogram derived from the graphite stearate electrode in phosphate buffer having a pH of 7.4 containing 5uM of each of DA, 5-HT, DOPAC, 5-HIAA, AA and UA on the fifth trial of electrode conditioning. Only DA and 5HT were detected. Homovanillic acid (HVA) can be detected between 0.4 and 0.5 V. DOPAC, 5-HIAA, AA and UA did not alter either the DA signal or the 5-HT signal.

Figure 34 is a semidifferential voltammogram which shows the behavior of the electrode when the paste modification with stearate is incorrect. There is no significantly defined difference between the dopamine and serotonin peaks. The semiderivative voltammogram is representative of the fourth electrode conditioning, at which time the 20uM dopamine (1) peak and the 20uM serotonin (2) peak are significantly defined, when the correct paste modification is used. Additionally, the ratio between peaks and the amplitude of each peak is incorrect.

Figure 35 is another semidifferential voltammogram which shows the behavior of the

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electrode when paste modification with stearate is incorrect. In this case, there was no significantly defined difference between norepinephrine (NE) and serotonin (5-HT) peaks. The semiderivative voltammogram is representative of the fourth electrode conditioning, at which time the 20uM norepinephrine (1) peak and 20uM serotonin (2) peak should be significantly defined when the correct paste modification is used.

Figure 36 is a semidifferential voltammogram which shows the behavior of the electrode when paste modification with stearate is correct. The semiderivative voltammogram is representative of a second electrode conditioning. The concentration of biochemicals [5uM AA, 10uM DA, and 10uM 5-HT] is less than in Figs. 34 and 35. Also, the number of conditionings is less. However, the voltammogram is correct and dramatically better than Figs. 34 and 35. There is a clear, significant difference between the peaks of dopamine, serotonin and ascorbic acid.

Figure 37 is a semidifferential voltammogram showing a clear, significant difference between the peaks of ascorbic acid (1) and homovanillic acid (4), in addition to dopamine (2) and serotonin (3). This shows that ascorbic acid and homovanillic acid can be detected at high concentrations.

Figure 38 is a semidifferential voltammogram obtained from the analysis a solution of 5uM of each of DA, 5-HT, DOPAC, 5-HIAA, AA, UA and HVA in phosphate buffer. Only DA and 5-HT are detected. Stearate graphite electrodes were previously exposed to extracellular fluid in the nucleus accumbens region of a rat brain, in vivo.

Figure 39 is a semidifferential voltammogram obtained from the analysis of 1uM NE and 1uM 5-HT, in phosphate buffer.

Figure 40 is a semidifferential voltammogram obtained from the analysis of NE and 5-HT, in phosphate buffer, using DA-pretreated graphite stearate electrodes. The dashed line represents 5uM NE and 5uM 5-HT. The solid line represents 5uM NE and 1uM 5-HT.

Figure 41 is a semidifferential voltammogram obtained from the analysis of NE and 5-HT, in phosphate buffer, using DA-pretreated graphite stearate electrodes. The dashed line represents 10uM NE and 10uM 5-HT. The solid line represents 10uM NE and 5uM 5-HT.

Figure 42 is a semidifferential voltammogram obtained from the analysis of NE and 5-HT in phosphate buffer using DA-pretreated graphite stearate electrodes which were previously exposed to extracellular fluid in hippocampal CA1 brain region of rat, in vivo. The --- --- line represents 20uM NE and 20uM 5-HT. The - - - line represents 20uM NE and 15uM 5-HT. The ----- line represents 20uM NE and 10uM 5-HT. The _____ line represents 20uM NE and 5uM 5-HT.

Figure 43 is a semiderivative voltammogram showing the detection of ascorbic acid (1) (greater than 200uM), dopamine (2) (greater than 200uM) and serotonin (3) (greater than 200uM) in phosphate buffer solution. At high uM concentrations of DA, 5-HT and AA, three clearly singular peaks occur.

Figure 44 is a schematic diagram of a working electrode.

Figure 45 is a semidifferential (semiderivative) voltammogram showing the in vitro detection of dopamine and serotonin (20 uM) in PO₄ (phosphate) Buffer containing DA (dopamine), 5-HT (serotonin), DOPAC (3, 4-dihydroxyphenylacetic acid), 5-HIAA (5-hydroxyindoleacetic acid), AA (ascorbic

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acid), UA (uric acid) and HVA (homovanillic acid), at a pH of 7.4 with a stearic acid electrode made from a mixture of 1.5g carbon (graphite), 1.00 cc extra heavy Nujol, and 100 mg stearic acid.

Figure 46 is a semidifferential (semiderivative) voltammogram showing the in vitro detection of dopamine and serotonin (20 uM) in PO₄ buffer containing DA, 5-HT, DOPAC, 5-HIAA, AA, UA and HVA, pH 7.4 with an arachidic acid electrode made from a mixture of 1.5g carbon (graphite), 1.24 cc extra heavy Nujol and 100 mg. arachidic acid.

Figure 47 is a semidifferential (semiderivative) voltammogram showing the in vitro detection of dopamine and serotonin (5 uM) in PO₄ Buffer containing DA and 5-HT, pH 7.4 with a stearyl cerebroside electrode made from a mixture of 0.075g carbon (graphite), 0.05 cc extra heavy Nujol and 0.5 mg stearyl cerebroside.

Figure 48 is a semidifferential (semiderivative) voltammogram showing the in vitro detection of dopamine and serotonin (20 uM) in PO₄ buffer containing DA, 5-HT, DOPAC, 5-HIAA, AA, UA and HVA, pH 7.4 with a stearic acid electrode made from a mixture of 1.5g carbon (graphite), 1.00 cc extra heavy Nujol and 100 mg stearic acid, with electrode conditioning taking place over a period of nine months. The typical period is 1 to 2 weeks.

Figure 49 is a semidifferential (semiderivative) voltammogram showing the in vitro detection of dopamine and serotonin (20 uM) in PO₄ buffer containing DA, 5-HT, DOPAC, 5-HIAA, AA, UA and HVA, pH 7.4 with an arachidic acid electrode made from a mixture of 1.5g carbon (graphite), 1.24 cc extra heavy Nujol, and 100 mg arachidic acid, with the electrode conditioning taking place over a period of nine months. The typical period is 1-2 weeks.

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Figure 50 is a semidifferential
(semiderivative) voltammogram showing in vivo
detection of dopamine (1), wherein the in vivo signal
is enhanced by a palmitate vehicle. The electrode
used to obtain this signal was implanted in the
5 ventral tegmentum.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The method of this invention involves
10 implanting, preferably, three electrodes in the body,
preferably the brain: a reference electrode, an
auxiliary electrode and a working or indicator
electrode. The reference electrode provides a zero
voltage point. The auxiliary electrode maintains the
15 current, with a potential control operational
amplifier, providing a sort of "ground" insofar as
"drift" is eliminated, which maintains the voltage
applied between the working and the reference
electrode as a constant within the time limits of
20 detection. The working or indicator electrode is
used to vary the electrical potential with respect to
the reference electrode.

The current signal emitted by any biological
system is produced by the charge flowing, or current,
25 between the reference and working electrodes. The
current varies dependent on the electrochemical
reactions taking place within the organ or suborgan.
The signal is preferably processed with at least two
operational amplifiers, one for the indicator, a
30 current measurer, and one for the reference, a
follower operational amplifier, processing the
current between the two electrodes. The
semiderivative circuit is an electrochemical circuit
which is added to a circuit for linear scanning. The
35 semiderivative circuit is made up of a series of

ladder networks of resistors (from about 2 kilohms to about 2 megohms) and capacitors (from about 5×10^4 to 5×10^{-2} microfarads) which process the current signal as the analog of the first one-half derivative of the undifferentiated signal. The resistive capacitive network that produces the semidifferentiated signal is described in detail in "Semiintegral Electroanalysis: Analog Implementation", Analytical Chemistry, Vol. 45, No. 1 January 1973, by Keith Oldham.

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The novel circuitry of this invention provides the production of a reduction, or cathodic, current, which allows detection of both reduced and oxidized species heretofore undetected and precluded within these detection limits.

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The electrodes should be situated in such a way as to accommodate a cathodic, or reduction, current from, for example, the brain. The electrodes can be situated in any specific region, such as the striatum, tuberculum olfactorium or nucleus accumbens. Figure 1 shows a very simplified schematic view of the circuitry as it should appear in accordance with the method of this invention. The semiderivative circuit, 107, is connected to the brain, 101, via two leads: the brain lead, 103, attached to the working electrode, 104, and the reference lead, 105, which is attached to the reference electrode, 109. The current being emitted from the brain is a cathodic current, positive for reduction. Thus, in order to obtain a reliable measurement, the positive terminal of the voltammetry control should receive the emitted current signal from the brain lead which is attached to the working electrode. No signal would be produced if the leads were connected as shown in Figure 2, which is the

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° circuit configuration which would conventionally be expected to measure current generated by biogenic brain species. In Figure 2, the working electrode 204 is shown implanted in the brain, 201. The brain lead, 203, is connected to the working electrode and
5 the negative terminal of the semiderivative voltammeter, 207. This system, which produces anodic current, does not generate a recognizable signal. Ultimately, a cathodic current must be produced.

Conceptually, the indicator electrode of
10 this invention is an inert surface which serves as an electron source or sink. The indicator electrode can be placed in specific parts of an organism or suborganism for diagnosis of diseases which range from mental and neurological problems to aging
15 processes and malignant cervical cancers and other types of cancers, for example. As the potential is applied, the electrons from molecules or molecules of biogenic chemicals near the electrode surface can either gain electrons from the body, or source
20 (cathodic reduction) or lose them to the indicator electrode sink (anodic oxidation). The current is dependent on the number of molecules which undergo electron transfer at the electrode surface; this is an accurate indication of the increase of species
25 concentration in solution, in addition to an accurate assessment of the rate at which electricity is moved across the electrode and converted into charge. An instantaneous readout of rate is a unique feature of electrochemistry; this reflects in vivo release
30 and/or reuptake mechanisms.

For the purposes of illustration, parts of Figures 3, 11 and ff. (and the following) are provided to delineate the conventional means for interpreting electrochemical measurements with the
35 use of drawings according to electrochemical

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convention. By conventional electrochemical notation, oxidation (anodic) currents are plotted downward and cathodic (reduction) currents are plotted upward (See Figure 11).

5 In the brain, in specific areas such as caudate, cerebral cortex and hippocampus, some biochemical reactions are irreversible in that they do not yield reproducible stable species, in range. This is indicated by the lack of reduced species on the reverse half cycle, as shown by Figure 12, a
10 linear scan cyclic voltammogram.

Some organic material shows reversibility in brain tissue such as the oxidation-reduction behavior of injected 6-hydroxydopamine (6OHDA) and the injected quinone 6-hydroxyquinone (6OHQ). This
15 reversible reaction produces both stable reduced species (6OHDA) and stable oxidized species (6OHQ). Figure 13 illustrates this reaction as a linear scan. Peaks appear at the voltages at which these species are, respectively, reduced and oxidized. This
20 is an example of the use of the apparatus of the prior art to measure by oxidation current.

The application of a potential, between 0 and + 1000 mv, tests for stable oxidation species; application of a potential between 0 and -1000 mv
25 detects reducible species. This is a useful test to distinguish reversible from irreversible oxidation reactions in all types of electrochemical experimentation.

An electrical circuit for providing an
30 output signal having a mathematical relationship in operation to an input signal can be semiintegrated or semidifferentiated. The semiintegration circuit consists of an electrical transmission line in the input to the operational amplifier; the
35 semidifferentiated circuit has such a line in the

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° feedback loop of an operational amplifier.

Semiintegral and semidifferential electroanalysis diminishes non-faradaic current by the addition of analysis time. A graph comparing results obtained from electroanalytical apparatus where the indicator (working) electrode current $i(t)$ has been recorded (a) unchanged (Curve A in amperes) and (b) semi-integrated (Curve B in amplombs) is shown in Figure 14. The amplomb measurement on the y axis is the sum of ampere $\text{sec}^{1/2}$ and coulomb $\text{sec}^{1/2}$. The full current curve (A) contains a preliminary peak followed by a falling off, making integration difficult. However, the semiintegrated curve (B) rises to a steady state value which can be used to determine concentration in dynamic levels because the signal lasts longer than that of linear scanning techniques.

It is postulated that, due to the speed at which the semiderivative circuitry is able to process signals, the reduced and oxidized species in the brain are detected using cathodic current according to the method of this invention. Conventional brain electrochemical scans have only processed oxidation currents. It is a novel aspect of this invention that cathodic reduction currents can be used to detect neurotransmitters and other chemicals in the brain and other sub-organisms.

The method of this invention describes a circuitry which can differentiate specific chemicals as biological markers of diseased states for purposes of psychotherapeutics, i.e. irreversible and reversible substances in the diagnosis of mental disease such as Alzheimer's and schizophrenia, mood disorders relating to diseases such as diabetes, and peripheral disease such as uterine or cervical cancer, diabetes, and the subsequent applications of

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- ° the principles of telemetry thereof and the automation of the technique.

The method of this invention distinguishes semiintegral and semidifferential electroanalysis from other types of electrochemical analysis such as linear scan techniques within the concepts of cathodic and anodic currents. In the circuitry of the method of this invention, a cathodic (reduction) current is used to detect reversible and irreversible oxidizable and reducible species to delineate specific chemicals in disease vis-a-vis the anodic (oxidation) current taught in the prior art. Anodic currents taught in the prior art are unusable for semidifferential techniques in brain and other sub-organisms. They are unstable and not reproducible. Cathodic currents with semidifferential circuits are stable, reproducible and irreversible and not amenable to cyclic voltammetry. Another embodiment of the method of this invention would be the insertion of a differential operational amplifier to process the first derivative of the output current so as to provide a signal that would be cyclic in nature. This could also be a signal inversion circuit.

When monitoring the reaction of biological systems to the administration of a particular stimulus, such as a drug, a baseline value should first be obtained by measuring the current generated with respect to different potentials without the stimulus. The voltammeter is used to obtain a baseline value for certain biogenic chemicals by measuring the current generated from the brain with respect to the application of varying potentials or voltages. Different applied potentials can range from about -200 mv to about -1000 or from about 0 to about +1000 mv or any combination thereof may be

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° applied. The scan rate, or rate at which the potentials are applied, is preferably in the range of about 5 to 30 mv-sec⁻¹, most preferably about 10 mv-sec⁻¹. Sensitivity, or amplification of the signal, can be in the range between about 0.1 and about 150nAs^{-1/2} inch⁻¹ -1 volt, preferably about 5-20nAs^{-1/2} inch⁻¹ volt⁻¹ in vivo and about 1-1- nAs^{-1/2} inch⁻¹ volt⁻¹ in vitro with time constants at any point or range between about 0.001 and about 10 seconds. Preferably, for in vivo measurements, the time constant is in the range from about 1.0 to about 5.0 seconds, most preferably about 5.0 seconds. Preferably, for in vivo measurements, the temperature should be about body temperature, i.e. for mammals, 37.0°C ± 0.5°C. For in vitro measurements, the temperature of the phosphate buffer electrochemical cell should be from about 20°C - about 25°C. It is important to remember that oxidation potentials can and will undergo lateral shifts dependent on temperature changes, time constant charges and/or changes in resistance, capacitance and electrochemical parameters.

The reaction to the administration of a stimulus can be measured after baseline values are recorded. After administration of the stimulus, potentials are applied and the current is measured with respect to the changing potentials. A comparison between voltammograms obtained prior to and after the stimulus will indicate the changes in production of biogenic chemicals and hence the presence of diseased states, which can even lead to bizarre behavior.

The method of this invention can be used for chronic studies, which take place over a relatively long time period, e.g. three to eight months and even longer, or for acute studies, in which values are

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° taken over a short time period or only a few times or even once or during one study. The integrity of the electrode over a long time period is extremely useful and there is no problem discerning electrode placement in specific and minute areas of brain
5 because of the neurochemical mapping profile described in this invention.

Preferably, the reference electrode used should be Ag/AgCl. The auxiliary electrode can be a platinum or a stainless steel electrode. Various
10 modifications, however, may be made. The working electrode, which is also known in the art as a probe or sensor, if used for acute or chronic studies in a freely-moving animal, is preferably composed of a teflon-coated stainless steel microelectrode
15 homogeneously packed with graphite paste, which is comprised of graphite and nujol or mineral oil or silicon oil. Most preferably the nujol has a specific gravity from about 0.8 to about 1. Commercial nujol typically includes DL-a- tocopherol
20 as a stabilizer. A milliliter of nujol may contain up to 0.02 international units of DL-a-tocopherol. For the purpose of this specification, weight or volume of nujol means the total weight or volume, including additives which are typically in
25 commercially sold nujol. The graphite paste also comprises an additional chemical compound which can serve as a modifier, selected from the group of complex lipids (e.g. acylglycerols, phosphoglycerides, sphingolipids and waxes) and
30 simple lipids (e.g. prostaglandins, steroids and terpenes), glycolipids, lipoproteins, fatty acids such as, but not limited, to lauric acid, myristic acid, palmitic acid, stearic acid, arachidic, lignoceric acid, palmitoleic acid, oleic acid,
35 linoleic acid, linolenic acid, arachidonic acid,

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° tuberculostearic acid, lactobacillic acid, elaidic acid, cerebronic acid, trans-hexadecenoic acid and the like, fatty acid derivatives, cerebrosides, and salts thereof.

5 The mixture should not come into contact with any substance other than an inert substance (for example, plastic, polypropylene and the like and glass) while it is being mixed or admixed. The graphite, oil and additional chemical compound should be in a container made of an inert substance and
10 stirred or mixed with an implement made of an inert substance. For example, the graphite, oil and additional chemical compound could be in a glass container such as a mortar and pestle and stirred with a glass implement. The mixing typically occurs
15 as the mixture is heated over a period of about 5 to about 20 minutes from a starting temperature of about 10° to about 25°C to a final temperature of about 50° to about 55°C. Preferably the heating occurs from about 25°C to about 55°C. Preferably the time period
20 is from about 5 to about 10 minutes. The graphite paste can be used immediately after it is formed or it can be stored indefinitely. If it is stored, it should not be permitted to come in contact with any light. Typically, the graphite paste is stored for
25 about 5 days - 2 weeks prior to use to allow it to age. The graphite paste should be capable of being stored indefinitely as long as it remains impermeable to contamination with any other substance such as dust, etc., and light.

30 The microelectrode of the present invention can be used in any one or all of the techniques employed for in vivo electrochemical measurements including but not limited to square wave, amperometric measurements, semidifferential
35 voltammetry, linear scanning, chronoamperometry,

° differential pulse and double differential pulse.
The electrode made in this manner allows the subject to move without breaking the electrode. When the microelectrode is used for acute or chronic studies in an anesthetized animal, either glass or stainless steel teflon coated microelectrodes may be used. If it is desired to measure biogenic chemicals such as dopamine with or without the appearance of certain acids such as ascorbic acid or the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), or to measure serotonin (5-HT) with or without uric acid (UA) or 5-hydroxyindoleacetic acid (5-HIAA), the serotonin metabolite, and if it is further desired to have even greater selectivity, e.g., to determine norepinephrine selectively, the graphite paste should be modified. (Stearate modification has been shown to detect serotonin without detecting 5-hydroxyindoleacetic acid or uric acid in the same electrochemical signal or at the same oxidation potential) (Broderick 1986, 1987, 1988, 1989).
Broderick, P.A., Neuropeptides 10 (1987) 369-386,
Broderick, P.A., Neurosci. Lett. 95 (1988) 275-280,
Broderick, P.A., Brain Res. 495(1) (1989) 115-121.
Unmodified paste which is known, consists of graphite (carbon) and Nujol* in a 3:2 ratio (Adams 1973). When paste is modified with an additional chemical compound, in accordance with the present invention, using stearic acid (stearate), for example, 0.7477 g graphite, 0.4503 g Nujol (extra heavy), 0.0502 g stearic acid are used. The weight ratio of graphite, oil and added compound or chemical can be in the range of, but not limited to, 0.15 - 7.5: 0.1 - 5: 0.01 - 0.5, respectively. Preferably the ratio of graphite weight in grams: oil volume in cc's: compound weight in grams is 1.0 to 2.0: 1.0 to 2.0: 0.01 to 0.4, respectively; most preferably 1.0 to

*Trade-mark

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- ° 1.5: 1.14 to 1.34: 0.01 to 0.10, respectively. The density of the oil typically ranges from 0.5g/cc - 2g/cc.

Where arachidic acid (or less preferably stearic acid) is the modifying compound a preferable ratio is a weight of graphite in gms: volume of oil in cc's: and weight of acid in grams, respectively, of 1.0-1.5: 1.14-1.34: 0.10. More preferably the ratio is 1.0-1.5: 1.19-1.34: 0.10. Most preferably the ratio is 1.0-1.15: 1.19-1.29: 0.10.

10 Where the modifying compound is stearyl cerebroside a preferable ratio is a weight of graphite in grams: volume of oil in cubic centimeter: and weight of cerebroside in grams, respectively, of 1.0-1.5: 1.14-1.34: 0.01-0.05.

15 Where the modifying compound is a mixture of arachidic acid and stearic acid in a 1:1 weight ratio, then the weight ratio of mixture is preferably 0.15 to 7.5 graphite: 0.1 to 5.0 oil: 0.01 to 0.25 arachidic acid.

20 The voltammogram shown in Figure 35 is incorrect, came about by admixing 0.5072 g graphite, 0.4804 g Nujol (extra heavy), and 0.0526 g stearic acid. The density of Nujol may range from about 0.8 to about 1.5 gm/cc, preferably about 0.8 to about 1.2 gm/cc. Most preferably about 0.8 to about 1.0 gm/cc. Nujol is a type of mineral oil. Mineral oil preferably ranges from about 0.8 g/cc to about 1.2 g/cc. Silicon oil ranges from about 0.9 g/cc to about 1.2 g/cc.

30 Correct paste composition can and should be verified by combustion analysis and gas chromatographical analysis. Combustion analysis and gas chromatographical analysis were performed on 5mg synthetic graphite stearate paste mixtures to
35 determine the accuracy of the content of graphite and

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° stearate acid. The graphite content was determined by combustion analysis and the stearic acid content was determined by gas chromatographical analysis. The level of Nujol can be calculated by the difference. These methods are important in the verification of paste composition.

It is noteworthy that another unexpected breakthrough in the distinction between the catecholamines, dopamine and norepinephrine has been accomplished with the stearate modification described in this invention (Broderick, P.A., Neurosci. Lett. 95 (1988) 275-280). These catecholamines are very important physiological brain neurotransmitters which oxidation potentials were too close in proximity to distinguish. We distinguish them here in Figures 38-42. Each of these catecholamines are involved in diseased states such as Alzheimers, sleep disorders, schizophrenia, drug abuse, anxiety disorders and others. Furthermore, one is more important than the other in specific disorders, e.g., norepinephrine may well be more important than dopamine in anxiety disorders.

A semidifferential voltammogram obtained from the analysis of a series of chemical tests with the graphite stearate electrode is shown in Fig. 38. Stearate graphite electrodes were exposed to DA, 5-HT, DOPAC, 5-HIAA, HVA, AA and UA. Only dopamine and serotonin were detected. Increasing amounts of DOPAC, 5-HIAA, HVA, AA and UA did not increase either the dopamine or serotonin signal. It should be noted that oxidation potential may shift laterally, dependent on concurrent shifts in pH, temperature, time constants, scan rate or resistance characteristics. The detection of norepinephrine (NE) and 5-HT, using graphite stearate electrodes in combination with semidifferential electroanalysis, is

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° shown in Fig. 39. The oxidation potentials are $0.155 \text{ V} \pm 0.015 \text{ V}$ and $0.290 \text{ V} \pm 0.015 \text{ V}$, respectively. The electrochemical signal and oxidation potential remains the same for 5-HT no matter which catecholamine is undergoing testing. The

5 electrochemical signal for the catecholamine NE, on the other hand, shows a different electrochemical signature from that electrochemical signal for DA. The oxidation potential for NE is 30 mV higher than that for DA. As concentrations of NE and 5-HT

10 increase in amount, and if the electrode is pretreated with DA, the NE signature consists of a double peak. The earlier peak occurs at $+0.030 \text{ V}$ and the later one occurs at $+0.140 \text{ V}$ (Fig. 40). Notably, the later oxidation potential for NE is approximately

15 15 mV less than that for NE when the electrode is not pretreated with DA, and when sensitivity, scan rate, pH, time constants, resistance and other relevant parameters are exactly the same. Progressive and upward amounts of NE result in an increased double NE

20 peak. This effect is shown in Fig. 41. The addition of 5-HT insignificantly increases the double peak signature of NE. Graphite stearate electrodes, which have not been exposed to the catecholamine DA, do not exhibit the double peak NE signature.

25 Finally, a semidifferential voltammogram showing in vitro detection of NE and 5-HT, using graphite stearate electrodes, which have been exposed to hippocampus of rat brain, is shown in Fig. 42. 5-HT is detected without interference from its

30 metabolite 5-HIAA and its purported contaminant, UA. The electrochemical signature for NE in vitro after hippocampal exposure exhibits a higher oxidation potential than that seen for prehippocampal exposure and displays the NE double peak. These electrodes

35 were pretreated with DA before their implantation in

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discrete hippocampal rat brain CA1 regions. Graphite stearate electrodes, not previously exposed to DA before hippocampal CA1 insertion, do not exhibit the double-peak signature. Whether or not the stearate graphite electrode has been previously exposed to DA either in vivo or in vitro, the electrochemical signature of NE shows an oxidation potential for NE which is greater than that for DA. Importantly, when the stearate graphite electrode detects NE with an electrode that is pretreated with DA, the NE peak will be earlier than that seen for the detection of NE alone, yet later than that seen for DA alone. The signal for the combination of NE, DA, and 5-HT differs both from the DA-5HT combination and from the NE-5HT combination.

The coulombic efficiency of the graphite stearate electrode for NE vis-a-vis that for DA is similar. The graphite stearate electrode is 2- to 3-fold more responsive to serotonin than it is to the catecholamines, DA and NE, at equimolar concentrations. The interactive effects of 5-HT on the NE signal are again similar to the serotonergic effects on the catecholamine DA, i.e., increasing μM amounts of 5-HT insignificantly increase the catecholamine signal.

Summarily, the electrochemical signal for NE is approximately 0.03 V greater than that for DA. For graphite stearate electrodes pretreated with dopamine, the electrochemical signature for NE is a double peak. Given the caveat then, that all electrochemical parameters are the same, the NE signature will have an oxidation potential that is greater than that for DA, whether or not the electrode is DA-conditioned. These data may have relevance to neurophysiological studies in vivo. It is entirely possible that the underlying principles

- ° can be extended to other types of electrochemical techniques in vitro and in vivo.

Blaha and Lane, in Brain Research Bulletin, Vol. 10, (1983) p. 861 have suggested modification with stearate but their report describes that
5 detection of ascorbic acid is not possible with their assay. It is believed that the same kind of detection can be achieved using nafion-coated electrodes or other coatings known to those of ordinary skill in the art, Gerhardt, GA et al. Brain
10 Res. 290 (1984) 390-395 [Adams, 1984].

However, the microelectrode described in the present invention overcomes these selectivity problems. The graphite and oil may also be modified with fatty acids (preferably other than stearic
15 acid), fatty acid derivatives (including stearic acid derivatives), simple lipids, complex lipids such as sphingolipids and waxes, glycolipids, or lipoproteins. The microelectrode may be modified by a cerebroside, sphingosine, ceramide,
20 galactocerebroside, sulfatide, cerebronic acid, psychosine, dihexoside, trihexoside, and tetrahexoside. The fatty acids differ in the complex lipids which contain backbone structure to which the fatty acids are covalently joined. Fatty acid
25 modifications produce fine selectivity and specific fatty acid paste mixtures are stearic acid, arachidic acids, N-stearoyl-DL-dihydrophingosine, arachidric acid stearyl ester, stearic acid arachidyl ester and stearyl cerebroside.

30 Various modifications on the indicator electrode can be made. The indicator electrode can be made a variable oxidant so that selectivity for anionic and cationic species can be made such that previously unidentified biological markers can be
35 detected by electrostatic repulsion,

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electrocatalysis, catalysis or other means, between the electrode surface and the molecule of interest. Modifications other than the fatty acid derived modifications not specifically noted are not precluded.

5 Complex lipids have fatty acids, simple lipids do not have fatty acids. There are two kinds of fatty acids: saturated and unsaturated. Stearic acid is saturated whereas arachidic acid is unsaturated. Thus, stearic acid and arachidic acid
10 can ionize to different extents when subjected to a given voltage and temperature. While not being bound by the following theory, these ionization differences appear to result in enhanced selectivity and amplification of sensitivity. Unsaturated fatty
15 acids have lower melting points than do fatty acids. Unsaturated fatty acids have non-rotating double bonds. Thus they are very rigid. They may have a "receptor" type reaction at the electrode diffuse double layer.

20 Indeed, although strides in the delineation of electrochemical signals have been made in the past few years, there remains a gap in the present literature in the description of electrochemical electrodes, which involves the conventional
25 electrochemical limitations regarding dopamine (DA), 3-4-dihydroxyphenylacetic acid (DOPAC) and ascorbate (ascorbic acid-AA). These problems exist insofar as these chemical substances have similar oxidation potentials and have not previously been readily
30 discerned one from the other. Moreover, these problems exist insofar as the electrocatalytic regeneration of dopamine by the reducing properties of ascorbate are concerned.

 The present invention addresses the
35 DA-AA-DOPAC question by defining and characterizing

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the stearate probe within the context of microelectrodes in vitro, in order to assist their use in electrochemical and neurophysiological studies. Additionally, the present invention addresses selectivity problems at indoleaminergic oxidation potentials, which are further positive than the catecholaminergic-ascorbate potentials. These are peak oxidation potentials at which serotonin, 5-hydroxyindoleacetic acid and uric acid can conceivably oxidize. The present invention describes semidifferential treatment of voltammetric data at small graphite stearate electrodes, in vitro. The findings show a distinct separate and simultaneous detection of dopamine and serotonin without interference from dopaminergic and serotonergic metabolites and other purported contributions to the dopamine and serotonin signal, i.e., ascorbic acid and uric acid. Ascorbic acid can be distinguished separately. This can be seen in schematic drawing Figure 37.

Conditioning of the electrode in vitro is all important in the appropriate detection of the neurotransmitters and metabolites by electrochemical signals in vivo and in vitro. Indeed, brain fluid-adsorbed electrodes or brain treated electrodes, which can be inserted into the extracellular fluid of the brain to increase sensitivity need not be used because the conditioning process (described here) on the electrode inherently enhances sensitivity. Importantly, it should be noted that any residual current is not responsible for the enhanced sensitivity.

The purpose of the electrodes of the present invention is to permit detection and measurement of chemical concentrations that were unknown or less accurate prior to the present invention. However,

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° prior to measuring unknown concentrations, the newly fabricated microelectrodes should be conditioned. Conditioning is important and heretofore unknown. Conditioning is important because it increases sensitivity of the electrodes for the biogenic amines and other neurochemicals with a controlled and studied manner so as to be able to achieve the best sensitivity before the experimental assay begins. Thus conditioning achieves accurate pre- and post-calibration data for extrapolation to release and/or reuptake of extracellular concentration of neurotransmitter in the brain synapse, in vivo, and/or other in situ or in vitro measurements.

Conditioning requires determining the peaks of known concentrations of the targeted chemical or chemicals in vitro in solution. To ensure that the voltammogram peaks of these chemicals will not be contaminated by other chemicals, the solutions employed for conditioning typically include metabolites of the targeted chemicals and other biogenic chemicals that may either naturally be in specimens that are in vivo, in situ or in vitro and/or may have similar oxidation potentials as shown by previous redox reactions or the like.

Conditioning occurs by exposing the microelectrode in vitro to a phosphate buffer solution containing the targeted chemical(s) as well as metabolites of these targeted chemicals and other biogenic chemicals having oxidation potentials similar to those of the targeted chemicals. Typically the oxidation potentials of these biogenic chemicals are in the range ± 100 mV relative to the potentials of the targeted chemicals. The closeness of the oxidation potentials of the chemicals that should be in the conditioning solution depends on the electrochemical circuitry and/or the electrochemical

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- ° characteristics of the electrode being tested. For example, conditioning an electrode for use with a semidifferential circuit, one should test for one or more biogenic chemicals having a potential in the range ± 50 mV relative to the potential of the
5 targeted chemical(s).

The solution of chemicals is employed for conditioning because these metabolites and close chemicals could affect measuring the targeted chemicals.

- 10 The conditioning typically includes three to five in vitro exposures of the graphite indicator microelectrode (modified as described above) to the solution of chemicals in a phosphate buffer. In the first exposure, the concentration of each of the
15 chemicals is in the 25 nanomolar to 3 micromolar range and potential is applied and current is measured. In a subsequent exposure, the concentration of at least one of the chemicals in the mixture is raised to the 3-20 micromolar range and
20 potential is applied and current is measured. In a further subsequent exposure the concentration of at least one of the chemicals is raised to be in the 19-200 micromolar range and potential is applied and current is measured. This set of exposures occurs
25 over a 24 hour period. Then the set is repeated upon waiting at least 12 hours after the first set is completed. Optionally the set of exposures is repeated at least one more time, preferably one to four more times.

- 30 While exposing the graphite indicator microelectrode to this concentration, potential is applied to the graphite indicator microelectrode from -200 mV to +500 mV at a sensitivity of typically 2nA/V. The potential is applied with the
35 semidifferential circuitry shown in Example 15.

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° However, electrochemical techniques other than semidifferential voltammetry may be employed. No ultraviolet light sources should be in close proximity to the phosphate buffer solution or stock solutions of target and close chemicals.

5 Conditioning of microelectrodes to detect dopamine and serotonin is described here as a process preferably including a set of five repeated exposures (of each newly fabricated microelectrode) to a solution of phosphate buffer containing DA
10 (dopamine), DOPAC (3, 4- dihydroxyphenylacetic acid), AA (ascorbic acid), 5-HT (serotonin), 5-HIAA (5- hydroxyindoleacetic acid) and UA (uric acid) in 1-20 uM concentrations in a 1, 5, 10, 15 and 20 uM final aliquot step protocol, before insertion into any
15 discrete neuroanatomical or anatomical substrate.

 In the first exposure, the electrode is typically exposed to DA, DOPAC, AA, 5-HT, 5-HIAA and UA in a 1uM concentration. Other chemicals having oxidation potentials similar to dopamine or serotonin
20 may also be employed. The exposures and application of potential are then repeated at the above-mentioned higher concentrations to achieve the above-mentioned five exposures. The five exposures are preferably conducted in one 24 hour period. Then twelve or more
25 hours after the prior set of exposures is completed, the five exposures are repeated. Optionally the five exposures are further repeated at least one more time, preferably one to four more times. Each repetition occurs upon waiting at least twelve hours
30 after the prior set of exposures is completed. The electrodes are stored in physiological saline before brain insertion.

 Figure 31(a) shows the detection of dopamine during a first conditioning experiment, using the
35 graphite stearate electrode. Figure 31(b) shows the

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◦ detection of serotonin during a first conditioning experiment, using the graphite stearate electrode. Figure 31(c) shows the resulting voltammogram which occurs when only phosphate buffer and no other chemicals are present. The phosphate buffer for
5 these studies is calculated in great detail in Example 13. In these experiments, dopamine and serotonin were dissolved in phosphate buffer in 5uM amounts, simultaneously with 5uM amounts of AA, DOPAC, 5-HIAA and UA. Sigmoidal curves, resembling
10 plateaus rather than peaks, are commonly seen during the first conditioning experiment. The graphite stearate electrode should show no residual electrochemical response in phosphate buffer, even after the electrode is or has been previously exposed
15 to amines and acids. (Calibration unit $\text{nAs}^{-1/2}$, seen on Figures 31-43 and 1, 5 and 50, denotes current changes per inch of 4 axis of each voltammogram.)

Figure 32 is a semidifferential voltammogram
20 showing the detection of dopamine and serotonin with the graphite stearate electrode in phosphate buffer during a third conditioning experiment. The phosphate buffer contained 5uM amounts of DA, AA, DOPAC, 5-HT, 5-HIAA, and UA. The peak height of each
25 signal increased with increasing uM amounts of dopamine and serotonin, respectively. 3-4-Dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), ascorbic acid (AA), and uric acid (UA) did not
30 increase or alter the dopamine or serotonin signals. The graphite stearate electrode was unresponsive to 5-HIAA and DOPAC at any oxidation potential studied within the range of -0.2v and +0.5v. The graphite stearate electrode was not responsive to AA at the
35 same oxidation potential as that for dopamine or serotonin. However, there was a response to AA, at

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an oxidation potential of $+0.055\text{v} \pm 0.005\text{v}$, at high μM concentration (Figure 43). At low μM concentrations of AA, the response of the graphite stearate electrode to AA was insignificant or none at all (Figure 33). Additionally, the coulombic efficiency of the stearate electrode to detect AA was dramatically less than that coulombic efficiency seen for the detection of either dopamine or serotonin. When low μM amounts of ascorbate and dopamine were simultaneously tested in phosphate buffer, no electrocatalytic regeneration of dopamine by ascorbate occurred. HVA is detected within an oxidation potential range from $+0.4$ to $+0.5\text{v}$ and/or approximately $+0.42\text{v}$ to approximately $+0.49\text{v}$.

Figure 33 is a semidifferential voltammogram showing the detection of dopamine and serotonin with the graphite stearate electrode in phosphate buffer during the process of fifth conditioning. The phosphate buffer contained $5\mu\text{M}$ DA, AA, DOPAC, 5-HT, 5-HIAA and UA. The semidifferential voltammogram reflects a final conditioning experiment. Only dopamine and serotonin were detected. The oxidation potentials were within the expected range, i.e., $+0.140\text{v} \pm 0.015\text{v}$ and $+0.290\text{v} \pm 0.015\text{v}$ respectively. Additional amounts of DOPAC, 5-HIAA, AA and UA did not alter either signal. The caveat is noted that oxidation potentials for dopamine and serotonin can shift to the right or the left, dependent on differences in parameters such as temperature, pH, various resistance and capacitance characteristics, scan increments, frequencies, scan rates and time constants and other parameters.

Experiments in which final concentrations of $600\mu\text{M}$ DA, and AA were pipetted into the 3-electrode phosphate buffer system in a 3 step, $200\text{ }\mu\text{M}$ aliquot protocol, showed that at high μM concentrations of AA

and DA, 2 clearly singular peaks occurred for AA and DA. The AA and DA peaks were detected at oxidation potentials of $+0.055\text{v} \pm 0.005\text{v}$ and $+0.140\text{v} \pm 0.015\text{v}$ respectively. These data are seen in Figure 43. No electrocatalytic regeneration of dopamine by ascorbate was seen at equimolar 200 and 400uM concentrations of AA and DA. At 600uM AA and DA, the dopamine peak was enhanced by approximately 10%. Data from preliminary experiments showed that UA was detected by the graphite stearate electrode, but at high uM concentrations of UA and at oxidation potentials higher than those needed to detect dopamine and serotonin. High concentrations of DA (200uM) simultaneously tested with low uM concentrations of AA (20uM) produced two clearly singular peaks. The AA peak was a spiked peak. The DA peak was broad. The reverse condition showed an ascorbate peak at 0.090v. The peak resembled as an adsorptive prewave spike.

Thus, the present invention demonstrates that the graphite stearate electrode selectively detects dopamine at early positive oxidation potentials and selectively detects serotonin at later positive oxidation potentials, without detection of the possibly interfering chemicals, 3-4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), ascorbic acid (AA), uric acid (UA) and homovanillic acid (HVA) at the same oxidation potentials as those needed for the detection of dopamine or serotonin.

Conventional electrochemical wisdom dictates that DA, DOPAC and AA should oxidize at similar potentials. The ability to distinguish among these chemicals has been a major goal among electrochemists and neuroscientists for over a decade. The importance is felt in both in vitro and in in vivo

studies since biochemical catecholaminergic and ascorbate reactions are essential in neurophysiological processes. The present invention shows that separation of dopamine, ascorbate and DOPAC waves are complete with the small graphite stearate electrode in vitro. When DA, DOPAC and AA are simultaneously tested, the ascorbate wave is detected at a potential, which is more negative than that needed to detect dopamine. AA is detected with low coulombic efficiency and generally high concentrations are needed for the AA signal to be detected. DOPAC waves are not detected at early oxidation potentials even at high concentrations. Significant electrocatalytic regeneration of dopamine, which can occur probably because of the reducing properties of ascorbate, is not seen.

The present invention addressed the problems of electrocatalytic regeneration of dopamine by ascorbate during the simultaneous exposure of these chemicals to the stearate microelectrode and the results were that an insignificant electrocatalytic regeneration occurred at high μM concentrations. In this reaction, ascorbic acid acts as a reducing agent to reduce dopamine-quinone back to dopamine, thereby enhancing the dopamine current. The electrocatalytic regenerative capacity of an electrode is directly related to the size of the electrode, i.e., at smaller electrodes, very little enhanced current is obtained. It appears that the concentration of species in solution may be a factor in the phenomenon of signal enhancement due to electrocatalytic regeneration as described by Broderick et al. (op. cit.).

The stearate electrode responds to the detection of indoleamines at higher oxidation potentials than those oxidation potentials needed for the detection of the catecholamines. The coulombic efficiency of the electrode for serotonin, is two to three fold higher than that for dopamine. The better coulombic efficiency for serotonin is consistent with the adsorptive properties for serotonin. Although the exact mechanisms of the differences in the coulombic efficiency of the graphite stearate electrode for dopamine and serotonin need to be defined, other electrochemical processes, such as radial diffusion and slow scan rates, may be explanatory. The graphite stearate electrode of the present invention is not responsive to the detection of uric acid, at the same oxidation potential as that for dopamine or serotonin. The graphite stearate electrode will detect uric acid at high μM concentrations, at an oxidation potential of approximately +0.350 volts, which is beyond the oxidation potential needed for detection of either DA or 5-HT. Thus, the microelectrodes as described in the present application are important because they enable spatially resolved chemical measurements in vitro and in vivo.

Electrodes may be constructed as follows, but their construction is in no way limited to the method described herein. The graphite paste mixture constituting the microelectrode of the present invention can be packed in an acceptable encasement therefor comprised of stainless steel or other acceptable metal or an inert material such as, but not limited to, plastic, Teflon*, polypropylene and the like or glass. The stainless steel can be additionally encased by an inert material such as Teflon.

*Trade-mark

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Fig. 44 shows an embodiment of a stainless steel indicator (working) electrode 117. The Teflon-coat 121 of the stainless steel indicator electrode 117 (tip diameter size approximately 150-200 u) can be pulled over the stainless steel to a length of approximately 500-750 u and packed with graphite paste 123. Thus, the steel 119 forms the base of a cylindrical well and the teflon 121 forms the walls of that well (teflon wall comprising the well is approximately 500-750 u in length) into which well the graphite paste 123 is packed. The term microelectrode is a relative one since the size of the brain varies from species to species. Therefore, the size of the electrode should be proportional to the size of the discrete anatomical area being studied in a given species.

Total diameter size of the microelectrode (including inner and outer diameter) could range from about 0.001 u - 10 mm. Preferably, the diameter of the microelectrode is approximately 2 u - 500 u.

The length of the microelectrode should be sufficient to reach the desired neuroanatomical and other anatomical substrates and to prevent undue stress on such substrates.

The length of the microelectrode should be in such a proportion to the diameter of the microelectrode to prevent undue stress on the microelectrode itself.

An example of a graphite paste mixture that constitutes the microelectrode of the present invention and that can be packed into the cylindrical well described hereinabove is stearic acid (100 mg) mixed with 1.5 g graphite (carbon) powder in 1.24 cc extra heavy Nujol. Notable differences though may occur in the effects of oils by weight and/or by volume. Potentials were applied to the indicator

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- electrode between -1000 mv and +1000 mv.

The criteria for selection of appropriate indicator electrodes can be: (a) that the electrode showed at least 100 mv (current (nA)) deflection with about 10 μ M DAHCL (dopamine hydrochloride); (b) that
5 the paste composition presented homogeneously under a dissection microscope, 300 x magnification. The potentials were measured with respect to an Ag/AgCl (1 M NaCl) reference electrode.

The reference electrode can be fabricated by
10 coating with chloride the entire length of a forty mm silver wire, previously coiled around an 1/8 inch drill bit. The chloride can then be coated in a 1M-NaCl solution for one-half hour at a current of 2 mA per electrode. The electrode is then equilibrated
15 in fifty mL of a 1M-NaCl solution until the criterion for the reference electrode is satisfied, i.e. the potential difference between the fabricated reference electrode and a BAS reference electrode (not usable in brain) should not exceed 10 mv. More recently,
20 2.5 mv is a better criterion.

In addition, an auxiliary electrode, made of stainless steel, can be housed in combination with the reference Ag/AgCl electrode in a one ml Biotip brand pipette (Becton-Dickinson, Orangeburg, NY), or,
25 auxiliary and reference can be separately placed in contact with dura. A description of the reference electrode, as used in the brain of the freely moving animal is described as follows. The reference electrode is a Ag/AgCl reference electrode. The
30 Ag/AgCl reference electrode is constructed by plating 10mm silver wire (from Medwire Corp., Mt. Vernon, NY) with AgCl in a 1M NaCl solution for about a half hour with the voltmeter (from Micronta) set at a current of 2 milliamps per electrode. The wire is then
35 inserted into a 150 microliter capillary pipette

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° (from Cole-Palmer Instrument Co., Chicago, Ill.) with 5% agar in physiological saline. The opening of the pipette is then shut off with absorbent cotton. An amphenol pin is soldered to the coated silver electrode at the other end of the pipette.

5 Preferably, the electrodes are implanted in vivo using a stereotaxic surgery device such as the David Kopf device. A David Kopf device consists of pairs of microscaled bars which allow the surgeon to implant the electrodes at the precise organ site
10 desired.

The method of this invention may be used to measure biogenic chemical levels, release mechanisms or reuptake or reuptake inhibition mechanisms in synapses and extracellular fluid of both anesthetized
15 animals or humans and freely-moving (unanesthetized) animals or humans.

The method of this invention may also be used to elucidate behavioral determinants. By determining the levels of release or reuptake of
20 biogenic chemicals and the changes in those levels, release or reuptake, while observing certain animal and human behavior, one can correlate the behavior patterns with the biogenic chemical alterations. This observation can contribute to the possible
25 determinations of the causes of certain behavioral manifestations in all stages of life (neonatal, adult and aged), for example, brain reward/brain pain systems, euphoria, drug addiction, alcohol dependency, diabetes, self-administration studies, stereotypy, catalepsy, anti-anxiety or anxiety
30 paradigms, turning behavior paradigms, reactions to environmental stimuli throughout life stages, conflict/avoidance paradigms, muricide, and other behavioral studies such as memory loss or brain
35 injury, due to ischemia, stroke and other

cardiovascular consequences.

The method of this invention can be used in vivo in applications involving any warm-blooded or cold-blooded animal possessing a brain or a primitive brain or type of brain and other organs of the body, such as a human, a primate, a lower mammal, reptile or squid. It is particularly well-adapted to observing the levels of biogenic chemicals in mammals, including human beings, which has been heretofore unachievable.

Telemetric measuring devices known to those skilled in the art may also be used to monitor current via radio and TV signals such that external electrodes need not be attached to a stationary source which would hinder movement of the subject during measurement. For example, the telemetric dual antenna described in U.S. Patent No. 4,539,710 (Dinsmore) may be used in the telemetric embodiment of the process of this invention for detection of electrochemical signals from the brain or other suborgans of animals and humans.

The schematic diagram in Fig. 15 depicts the circuitry needed for the production of cathodic and anodic currents for semiintegral, semiderivative or semidifferential processing. An indicator electrode 17, reference electrode Ag/AgCl 16, and an auxiliary electrode 18 are connected to organ or sub-organ 19 and are connected to operational amplifiers, 14, 15 forming an electrochemical cell, 14-19. More specifically, operational amplifier 14 is connected between indicator electrode 17 and auxiliary electrode 18 and operational amplifier 15 is placed between reference electrode 16 and auxiliary electrode 18. The auxiliary electrode 18 and reference electrode 16 are placed in contact with the

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° cortex or outer layer 35, of the organ or sub-organ. The indicator electrode 17 is inserted into the interior of the specific part of the brain or other suborganism which is the object of study. Battery 32 which provides an equal and opposite potential to
5 that potential being measured is connected between the indicator electrode 17 and the reference electrode 16. A potentiostat (not shown) provides a known voltage against the electrochemical cell so that concentration changes at the electrode do not
10 occur and so that no residual current will flow, causing an IR drop across the cell and changing the electromotive force. The circuit arrangement of the electrochemical cell, 14 to 18 is connected to operational amplifiers 6 - 8. The basic design of an
15 operational amplifier is shown in Figure 29. Operational amplifier 6 produces an analog signal proportional to the current through the indicator electrode 17 which is processed through a semidifferential ladder network which can be and is
20 received by the y-axis external measuring instrument such as a recorder, 72. Operational amplifier 7 receives the analog voltage signal proportional to the current between electrodes 16 and 17 and the current is processed through a cathode follower, 71,
25 which may be plotted on the x-axis of any recording instrument or an oscilloscope 72. Operational amplifier 8 maintains a constant potential on auxiliary electrode 18 by means of a negative feedback circuit 76 (not shown in detail) which
30 automatically corrects for any drift of the potential difference between the reference and indicator electrodes. In accordance with the invention, the electrochemical cell 14-19 is connected in series with direct coupling amplifiers 10 and 13, which can
35 be triodes or pentodes examples of which are seen in

Fig. 30. Transistor amplifiers or integrated circuit amplifiers could, of course, also be used. These amplifiers respectively produce anodic and cathodic currents emitted from the organ being studied. RC filters 11 and 74 filter out unwanted and transient surges in electrical output. The resistance capacitance complex amplifier resists an exponential decay of signal. It is expressed as a time constant, in seconds. The time constant in seconds can be varied so that different components of the signal can be detected and interpreted. Differential operational amplifiers 1 and 2 are a ladder network of resistors and capacitors for semidifferentiating the current produced by the electrochemical cell. As stated previously, ladder network amplifiers such as 1 or 2 are described in the Oldham work (op. cit.). Ladder network amplifiers 1 and 2 are used to semidifferentiate cathodic (reduction) currents for the purposes of delineating irreversible and reversible biological chemicals and the like for medical diagnosis. Operational amplifier 1 transmits an input signal to operational amplifier 13 which like amplifier 10 may be a triode as shown in Figure 30. The signal is then transmitted to terminal output cables 22 for direct processing of semidifferential cathodic current. One aspect of the novel circuitry is the electrical connection 33 between amplifiers 1 and 13.

Input operational amplifier 15 is connected between auxiliary electrode 18 and reference electrode 16 and to the input of output operational amplifier 12 through RC filter 74. Amplifier 12, which has an output connected to an input of cathodic current amplifier 13, may also optionally be connected so as to output to anodic current amplifier 10. Since amplifiers 10 and 12 have a linear gain,

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° this arrangement will produce linear reproductions of the cathodic and anodic currents when a chart recorder such as 72 is connected to outputs of amplifiers 10 and 13. The linear scan is shown in Figure 16.

5 Optionally, an output of amplifier 12 can be connected to an input of semidifferential amplifier 1 and then the output obtained from amplifier 1 can be connected to the input of amplifier 13 to result in a signal having a steeper slope of current versus
10 oxidation potential as shown in figure 17. This signal has the advantage of more clearly defining the chemicals being analyzed and increasing the time for analysis as shown in Figure 10b. The output of
15 amplifier 2 can be directly connected to the y-axis of the recorder 72 by line 34.

 Power supply 73, which is shown providing plus fifteen and minus fifteen volts to semidifferential amplifier 2, may also be used as a power supply for linear operational amplifier 12.

20 Alternatively, in place of the electrical connection between 1 and 13, a potential divider circuit with a triode or transistor selecting switch (not shown) can be used to direct the anodic current for non-differentiated and nonsemidifferentiated
25 scanning techniques to triode operational amplifier 10 by connection line 90, in series with terminal output cables, 21. The potential divider circuit with an anode/cathode (triode) selecting switch can also be used to direct cathodic current for
30 differentiated and semidifferentiated scanning techniques to the reduction amplifier, 13, in series with the terminal output cables, 22. An electrical connection line between amplifier 1 and triode 10, will provide results that are not confusing for
35 currents that are non-differentiated. However,

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° confusing results for current production that is differentiated or semidifferentiated will occur.

Preferably the circuit employs isolated grounds. Optionally, a MiniguardTM surge suppressor (static-magnetic voltage regulating transformer) from Jefferson Electric is employed.

The method of this invention requires a cathodic current production. This current production is not a simple function of a change in polarity, which would produce the same signal, as shown by Figure 19; nor is it a simple function of the directionality of the applied potential, E_{app} , as shown by Figure 20. Figure 16 is a graphical representation of the use of anodic current used with non-differentiated current. Figure 17 is a graphical representation of the use of the method of this invention; cathodic current used with semidifferentiated current. The graph of Figure 17 shows sharp peaks produced by chemical species, compared to the curve generated by non-differentiated anodic current of Figure 16.

Figure 18 is a schematic diagram of a known three electrode system necessary to generate electrochemical signals from brain and other parts of living and non-living things. The figure is drawn in relationship to said operational amplifier circuitry needed for varying types of electrochemical procedures. Figures 21 through 25 are graphical representations of correct electrochemical signals vis-a-vis incorrect electrochemical signals; only the correct signals are useful as markers for diseased and healthy states and are thereby useful for diagnostic and therapeutic medical research.

Figure 26 shows the telemetric embodiment of the in vivo electrochemical semidifferential and linear detection of neurochemicals, biogenic amines

and peptides both in the mobile and non-mobile, human and non-human organism, for purposes of medical diagnostics and preclinical and clinical pharmacotherapeutic discovery. Shown at, 23, is a pictorial view illustrating a rat, a commonly used laboratory animal, bred for the expressed purposes of biomedical research. The three electrode electrochemical circuit, Figure 18, is implanted in the brain described in this invention. The electrode system is then cemented to the skull of the organism with acrylic and fitted with a snap on telemetric transmitter, 24, the principal elements of which comprise a Channel 8 or 9 TV Band, an oscillator, a silver oxide battery power supply, a contact switch assembly and a contactor for the three electrode snap on closure electrode system. Voltage is transmitted via electrolytes in the brain extracellular fluid, to a dual antenna network, 25-27, according to the invention of Dinsmore (op. cit.) which allows interference-free reception of physiological signals through telemetry. A plurality of RFFM receiving channels are each associated with an antenna. Each is comprised of one or more RF amplification stages 36, a mixer stage 37 and one or more intermediate frequency stages 38, 39 along with an analog circuit. The common local oscillator, 27, provides uniformity in the intermediate frequency. A current measurer operational amplifier changes the output voltage of the telemetric receiver to current by an analog circuit 30, and the signal is displayed on a computer printer or strip chart recorder, 31. The detector, 30, is preferably a 3089 IC from National Semiconductor Corporation in accordance with the method of Dinsmore (op. cit), in combination with the semi-differential circuit of Oldham (op. cit), in addition the novel circuitry of this invention described in

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° Fig. 15.

The automation of the method of this invention to aid in the rapid detection of healthy and diseased states is described. A computer monitor, 41, a disk drive, 47, a printer, 48 and a
5 computer keyboard, 49, are depicted in Fig. 27. Processor memory 82 and 83 are provided to move information from disk drive to memory. Memory disk drive 47 is provided as a means for bussing information. Chipboard 51 contains chips for the
10 central processing unit, a fast 8-bit microprocessor, controlling analog to digital converter function, 84, printer 48 and disk drive 47. The "transistor-transistor logic", or "TTL", produces the outputs to the remote control pins of the circuitry
15 depicted in Fig. 15. The output of organ 19 is connected to a detector (not shown) which contains all of the circuitry of Figure 15. The TTL outputs can be controlled by an internal clock 55 such as the clock in an Apple IIe. The principal concept
20 governing this automatic embodiment of the method of this invention is the application of the passage of a high voltage through a transistor in the computer TTL, 81, forming an on/off switch mechanism. The TTL logic is based on a switch which varies potential
25 from 0 to 5 Volts logic. The detector logic comprising the unamplified circuit of Figure 15 is TTL also, based on a 0-5 Volts switch off/on logic. In a practical embodiment, a strap cable can connect computer TTL switch 81 with detector TTL switch 80
30 through a terminal strip, with an interfacing device (not shown) which receives inputs and outputs from the TTL 80 and the TTL 81. In the present invention, this interfacing device has no electronic importance, but that does not by any means preclude additional
35 electronic importance in the future, e.g. operational

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° amplifiers, intended to amplify or dampen, are contained in this box which could amplify or dampen or otherwise attenuate or amplify signals from the brain and other living suborgans should the electrical interference from, say several computers, serve to dampen the signal. A strap cable from the TTL 80 connector, electrically connected to cell 14-19 of Fig. 15 comprises four or five single cables, designated to ground, scanning operation, cell and beginning and possibly ending applied potential in mv. TTL inputs and outputs on A/D converter 84 direct analog to digital converter TTL Logic in the interface (not shown) and switch 80. In addition, the attached programs e.g. shown in Figures 28(a) and 28(b) integrate the height and area under each analog signal which emanates from the brain and/or other suborgans, thus allowing for on-line analysis of neurotransmitter- and neurotransmitter-like substances in healthy and diseased states for the purposes of medical diagnosis.

Figures 29 and 30 set forth preferred embodiments for accomplishing specific functions of the circuit of this invention as shown schematically in Figure 15. In Figure 29(a), Z_1 and Z_2 are feed-in and feed-back impedances and, when the amplifier is equipped with high gain capacitance, the transfer function of the circuit can be delineated by Z_1 and Z_2 . The point B where Z_1 and Z_2 are connected together and to the amplifier input is the "summing junction," which provides a ground because the signal voltage is infinitesimal. The current through Z_1 is equal to the current through Z_2 . V, the potential drop across C is defined as: $V=$

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5 -1/C

$$\int i dt = \frac{-1}{CR} \int v dt.$$

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Figure 30 shows a type of direct coupling amplifier based on a triode 301, i.e., an electronic valve with a wire mesh grid 304 and a cathode 302. The triode is the basis of the amplifier. When the
 10 third electrode (anode) 303 is held at a small negative potential relative to the cathode, electrons flowing through the valve are not attracted to the grid 304 but can still pass through it to the anode. Any change of the grid potential will produce a far
 15 greater change in the electrical field close to the cathode than will a similar alteration of anode potential. The direct coupling of more than one amplifier circuit allows the output of the first amplifier to be applied between the cathode and the
 20 grid of the next. The grid of the next stage should be held at a small negative potential with respect to its own cathode, to be a cathodic amplifier. A battery can provide a potential difference between the anode and the succeeding grid.

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Therefore, one of the differences between the method of this invention and prior art methods can be seen by comparing a semiderivative voltammogram produced by using the method of this invention with graphs produced by prior art methods.
 30 In Figures 3A-3C, a "minus" notation denotes a reduction current and a "plus" notation denotes an oxidation current. Figure 3A shows a graph obtained by a linear scan of biogenic chemicals. It is a typical curve representing an anodic current in
 35 conventional electrochemical form [Kissinger et

al. (op. cit.)). In Figure 3A, the peaks for biogenic amines, such as serotonin, are masked and undifferentiated from each other such as the dopamine peak. Figure 3B is a semiderivative voltammogram which also presents an anodic current but is presented in

5 non-conventional electrochemical form. [Lane, Hubbard and Blaha, J. Electrochemistry, Vol. 95, (1979), p. 117.] In Figure 3B, the dopamine and serotonin signals are contaminated by many other amines and many metabolites in addition to ascorbic

10 acid and possible uric acid. Figure 3B shows differentiated serotonin and dopamine peaks. Figure 3C is a representation of a semiderivative voltammogram using nonconventional electrochemical notation, derived from using the method of this

15 invention. It shows sharp, differentiated uncontaminated serotonin and dopamine peaks using a cathodic current and selective electrodes that are either brain treated or electrodes that have undergone the electrode conditioning process

20 described herein, before implantation of the electrode in discrete brain regions. Unexpectedly, these and other chemicals heretofore undescribed can be reproducibly detected, such as in Fig. 17, by using a cathodic current according to the method of

25 this invention.

Thus, the reproducibility and purity of signals generated by the process of this invention allow the automation of the cathodic current as demonstrated in Figures 27 and 28, as well as the

30 telemetric application of the process of this invention to aid in the diagnosis of diseased states as shown in Figure 26.

The process of this invention may also be used in humans as a neurochemical mapping device in

35 order to show electrode placement in the human

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° brain. By knowing the typical signal pattern produced in each part of the brain, a physician or researcher would be aware of the exact location of the electrode in a living patient without the necessity of dissecting the brain.

5 The method of this invention can be used to measure biogenic chemicals in the organs of the body as well as in different parts of the brain, e.g. the striatum, tuberculum olfactorium, nucleus accumbens, median raphe, periaqueductal gray, hippocampus, locus
10 coeruleus, the frontal cortex, amygdala, hypothalamus, thalamus, substantia nigra, globus pallidus and other areas. The methods of this invention can also be used to measure biogenic chemicals in such organs of the body as the heart,
15 the retina, the gut, the cervix, kidney, liver, gall bladder, vagina and the like so as to diagnose, e.g., cervical cancer and the like. Subsequent neurochemical profiles are useful in diagnosis. For example, the method can also be used as a mapping
20 device to determine electrode placement in the human, as shown in Figs. 5 and 6.

 Moreover, it can be used as a diagnostic tool to measure dynamic levels of biogenic chemicals and compare them to established normal dynamic
25 values. The method of this invention can be used to measure levels of biogenic chemicals such as amines, amine metabolites, ascorbic acid, amino acids, particularly dopamine, homovanillic acid, tryptophan (a chemically irreversible substance), serotonin,
30 enkephalins and enkephalinamides, neurotransmitters, putative neurotransmitters, and neuromodulators. It is believed that neuropeptides can also be measured using the method of this invention.

 It is also believed that non-electroactive
35 substances such as acetylcholine and electroactive

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substances that have small or low diffusion coefficients can be measured using the method of this invention. Non-electroactive substances and electroactive substances with small or low diffusion coefficients as well as electroactive substances having more than one electroactive moiety can be detected by immunoassay in association with or combination with the instant invention. An antigen is bound to an antibody and injected iontophoretically or by other means into brain or other organs in vivo and/or in vitro. Free and bound antigen differ in their electrochemical characteristics and the distinction can provide the detection of new substances. Free or unbound antigen can be more electroactive than bound antigen. United States Patent No. 4,395,312, issued July 16, 1983 to McCreery discusses means by which spectroelectrochemical means, associated with immunoassay means, are used to effect detection of some biochemical species in vitro. The instant invention can be used in combination with spectroelectrochemical means and/or immunoassay means and/or radioimmunoassay means.

These chemicals may be present at certain dynamic levels in normal persons without the administration of any stimuli. However, in persons having abnormal psychological characteristics, and, therefore, forms of mental illness, different dynamic levels of biogenic chemicals may be present. Thus, the in vivo electrochemical method of this invention may be used to diagnose mental illnesses, such as obesity, depression, manic depression, drug addiction, and others, such as neurochemical disorders in AIDS, e.g., AIDS related depression, etc., according to the dynamic levels of certain chemicals in the brain. The method of this invention

° can also be used for predicting the advent of neurological and other diseases such as diabetes, Alzheimer's, Parkinson's and Huntington's diseases based on the comparison between known normal levels and abnormal levels of particular substances, e.g. dopamine. The method can be used in combination with and to complement electrophysiological investigations and spectrophotofluorometric devices for studying calcium and other depolarizing agents in lymphocytes and fibroblasts as well as skin biopsies of patients with diseases, e.g. Alzheimer's and schizophrenia. It can also be used in studies of neuroimmunomodulation.

Moreover, certain biogenic chemicals measurable by the method of this invention are produced and/or altered in reaction to central or peripheral administration of chemical stimuli, such as drugs. These chemicals which may produce such reactions are psychopharmacological and neuropsychopharmacological agents such as neuroleptics, neuropeptides, amino acids, analgesics, gut and brain hormones, calcium blockers, addictive agents, antidepressants, antianxiety agents, anti-panic agents, amphetamines, endorphins, particularly beta and gamma-endorphins, enkephalins, enkephalinamides such as D-Met²-Pro⁵-enkephalinamide, cholecystokinin, dynorphin, marijuana, morphine, cocaine and other drugs and agents known to affect the human condition. Non-electroactive moieties and electroactive moieties which are slow to diffuse may be detected by attaching antigens to antibodies, the antigens having electroactive moieties, such as McCreery has done in vitro. However, in vitro applications are difficult to transfer to in vivo situations. For example, the central nervous

system's effect on the release and reuptake of various neurotransmitters after peripherally-administered enkephalinamides have been detected, surprisingly, for the first time using the method of this invention.

5 The method of this invention can, therefore, be used for studying the mechanisms of action in the brain of particular agents. This would aid in the development of new psychotherapeutic agents by the study of structure-activity relationships of
10 administered drugs. Analogs of the studied drugs could be evaluated for their effect on dynamic biogenic chemical levels in order to develop more effective therapeutic agents which have fewer and less severe side effects.

15 The following examples further illustrate certain embodiments of the method of this invention. Of course, they do not serve to limit the scope of this invention in any way. It should be noted that Figures 4, 5, 6, 9 and 10 illustrate representations
20 of cathodic currents generated by using the method of this invention presented in the upright, nonconventional electrochemical notation. They should not be confused with conventional notation used throughout the descriptive example.

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EXAMPLE 1

Teflon covered, stainless steel wire, 150-200u diameter (Medwire, Mt. Vernon, NY) was cut with a sterile #10 scalpel blade (Becton, Dickinson &
30 Co., Rutherford, NJ) into a 30 mm section. The cut was examined under a dissecting microscope (Nikon SMZ-1* with Nikon Transformer Illuminator XN) to determine the smoothness of the Teflon cut surface. Several blades should be used to achieve a flawless
35 cut. The Teflon covering was then very gently pulled

*Trade-mark

500u past the stainless steel. This exposed (2-5mm) wire without Teflon in the middle of the electrode, while leaving the Teflon intact at the upper end of the wire. The exposed stainless steel was covered with spherical shaped acrylic dental cement. The electrode was then refined to a 10mm length and a copper amphenol pin was soldered at the upper end, from which 2mm of Teflon had been removed.

When the Teflon covering was gently pulled over the edge of the stainless steel, a cylindrical holder was formed. It was into this holder that the graphite stearate paste was packed. The graphite stearate paste was actually tapped into the Teflon holder by gentle prods, easing the grey claylike paste into the cylindrical holder from the surface of a clean glass plate, which was previously washed three times and separately with 95% ethanol and doubly distilled water. Slow, small tapping motions are used while coaxing the paste into the cylindrical holder. The paste should be homogeneously tapped into the cylindrical holder; the homogeneity of the paste should be examined under a dissecting microscope at 30x magnification. Excess graphite stearate paste was removed from around the tip of the electrode by gently applying the tip of the electrode, held at a 45 degree angle, to taut Teflon Tape (Fisher Scientific, Springfield, N.J.). The Teflon tape was previously washed three times and separately with 95% ethanol and doubly distilled water.

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EXAMPLE 2

The fabrication of the graphite fatty acid paste was as follows: 1.24 ml Nujol (extra heavy), containing DL- α -tocopherol as a stabilizer, (Plough Inc., Memphis TN) was admixed with 100 mg of stearic

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acid (99+%, Sigma, St. Louis, MO). This was done by stirring the extra heavy Nujol-stearic acid mixture manually with a glass stirring rod, while heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental temperatures should begin at 25°C and end at 55°C over the 10-min period. The Nujol-stearic acid was completely admixed before 1.5 g of Ultra Carbon (Ultra Superior Purity, Ultra Carbon, Bay City, MI) was added to the heated Nujol-stearic acid mixture. All stearic acid crystals should be melted before the mixture is removed from the heating device. The synthesized paste was allowed to cool. Then the paste was placed into a glass container. The glass container was rinsed with 100% ethanol and dried in nitrogen prior to placing the paste into it. The synthesized paste was aged and stored in the dark at room temperature. The vials were covered with parafilm. A great deal of care was taken not to allow dust and other extraneous particles near the paste mixture.

Combustion analysis and gas chromatographical analysis were performed on 5 mg synthetic graphite stearate paste mixtures to determine the accuracy of the content of graphite and stearic acid. The graphite content was determined by combustion analysis and the stearic acid content was determined by gas chromatographical analysis. The level of Nujol can be calculated by the difference. These methods are important in the verification of paste composition. These methods have never been suggested for verification of any paste mixture heretofore.

EXAMPLE 3

The fabrication of the graphite fatty acid paste was as follows: 1.24 ml Nujol (extra heavy),

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° containing DL- α -tocopherol as a stabilizer, (Plough Inc., Memphis TN) was admixed with 100 mg of arachidic acid (Sigma, St. Louis, MO). This was done by stirring the extra heavy Nujol-arachidic acid mixture manually with a glass stirring rod, while
5 heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental temperatures should begin at 25°C and end at 55°C over the 10-min period. The Nujol-arachidic acid was completely admixed before 1.5 g of Ultra Carbon
10 (Ultra Superior Purity, Ultra Carbon, Bay City, MI) was added to the heated Nujol-arachidic acid mixture. All arachidic acid crystals should be melted before the mixture is removed from the heating device. The synthesized paste was allowed to cool.
15 Then the paste was placed into a glass container. The glass container was rinsed with 100% ethanol and dried in nitrogen prior to placing the paste into it. The synthesized paste was aged and stored in the dark at room temperature. The vials were covered
20 with parafilm. A great deal of care was taken not to allow dust and other extraneous particles near the paste mixture.

Combustion analysis and gas chromatographical analysis were performed on 5 mg
25 synthetic graphite arachidic paste mixtures to determine the accuracy of the content of graphite and arachidic acid. The graphite content was determined by combustion analysis and the arachidic acid content was determined by gas chromatographical analysis.
30 The level of Nujol can be calculated by the difference. These methods are important in the verification of paste composition and have never been suggested for paste composition verification heretofore.

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EXAMPLE 4

The fabrication of the graphite fatty acid paste was as follows: 1.00 ml Nujol (extra heavy), containing DL-a-tocopherol as a stabilizer, (Plough Inc., Memphis TN) was admixed with 100 mg of
5 arachidic acid (Sigma, St. Louis, MO). This was done by stirring the extra heavy Nujol-arachidic acid mixture manually with a glass stirring rod, while heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental
10 temperatures began at 25°C and ended at 55°C over the 10-min period. The Nujol-arachidic acid was completely admixed before 1.5 g of Ultra Carbon (Ultra Superior Purity, Ultra Carbon, Bay City, MI) was added to the heated Nujol-arachidic acid
15 mixture. All arachidic acid crystals were melted before the mixture was removed from the heating device. The synthesized paste was allowed to cool. Then the paste was placed into a glass container. The glass container was rinsed with 100% ethanol and
20 dried in nitrogen prior to placing the paste into it. The synthesized paste was aged and stored in the dark at room temperature. The vials were covered with parafilm. A great deal of care was taken not to allow dust and other extraneous particles near the
25 paste mixture.

Combustion analysis and gas chromatographical analysis was performed on approximately a 5 mg synthetic graphite arachidic paste mixture to determine the accuracy of the
30 content of graphite and arachidic acid. The graphite content was determined by combustion analysis and the arachidic acid content was determined by gas chromatographical analysis. The level of Nujol was calculated by the difference. These methods were
35 important in the verification of paste composition

- ° and have never been suggested for paste composition verification heretofore.

EXAMPLE 5

The fabrication of the graphite fatty acid
5 paste is as follows: 1.24 ml Nujol (mineral oil),
containing DL- α -tocopherol as a stabilizer, is
admixed with 100 mg of arachidic acid (Sigma, St.
Louis, MO). This is done by stirring the
Nujol-arachidic acid mixture manually with a glass
10 stirring rod, while heating the Nujol mixture at
incremental temperatures over a 10-min time period.
The incremental temperatures should begin at 25°C and
end at 55°C over the 10-min period. The
Nujol-arachidic acid is completely admixed before 1.5
15 g of Ultra Carbon (Ultra Superior Purity, Ultra
Carbon, Bay City, MI) is added to the heated
Nujol-arachidic acid mixture. All arachidic acid
crystals should be melted before the mixture is
removed from the heating device. The synthesized
20 paste is allowed to cool. Then the paste was placed
into a glass container. The glass container was
rinsed with 100% ethanol and dried in nitrogen prior
to placing the paste into it. The synthesized paste
is aged and stored in the dark at room temperature.
25 The vials are covered with parafilm. A great deal of
care was taken not to allow dust and other extraneous
particles near the paste mixture.

Combustion analysis and gas
chromatographical analysis are performed on
30 approximately a 5 mg synthetic graphite arachidic
paste mixture to determine the accuracy of the
content of graphite and arachidic acid. The graphite
content is determined by combustion analysis and the
arachidic acid content is determined by gas
35 chromatographical analysis. The level of Nujol can

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be calculated by the difference. These methods are important in the verification of paste composition and have never been suggested for paste composition verification heretofore.

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EXAMPLE 6

The fabrication of the graphite fatty acid paste is as follows: 1.24 ml Nujol (mineral oil), containing DL- α -tocopherol as a stabilizer, admixed with 100 mg of stearic acid (Sigma, St. Louis, MO). This is done by stirring the Nujol-stearic acid mixture manually with a glass stirring rod, while heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental temperatures should begin at 25°C and end at 55°C over the 10-min period. The Nujol-stearic acid is completely admixed before 1.5 g of Ultra Carbon (Ultra Superior Purity, Ultra Carbon, Bay City, MI) is added to the heated Nujol-stearic acid mixture. All stearic acid crystals should be melted before the mixture is removed from the heating device. The synthesized paste is allowed to cool. Then the paste is placed into a glass container. The glass container is rinsed with 100% ethanol and dried in nitrogen prior to placing the paste into it. The synthesized paste is aged and stored in the dark at room temperature. The vials are covered with parafilm. A great deal of care should be taken not to allow dust and other extraneous particles near the paste mixture.

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Combustion analysis and gas chromatographical analysis are performed on 5 mg synthetic graphite stearic acid paste mixtures to determine the accuracy of the content of graphite and stearic acid. The graphite content is determined by combustion analysis and the stearic acid content is

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- ° determined by gas chromatographical analysis. The level of Nujol can be calculated by the difference. These methods are important in the verification of paste composition and have never been suggested for paste composition verification heretofore.

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EXAMPLE 7

The fabrication of the graphite fatty acid paste was as follows: 1.00 ml Nujol (extra heavy), containing DL-a-tocopherol as a stabilizer, (Plough
10 Inc., Memphis TN) was admixed with 50 mg of stearic acid (99+%, Sigma, St. Louis, MO) and 50 mg of arachidic acid (Sigma, St. Louis, MO). This was done by stirring the 1.00cc of Nujol and 50 mg stearic acid and 50 mg arachidic acid manually with a glass
15 stirring rod, while heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental temperatures began at 25°C and ended at 55°C over the 10-min period. The Nujol-stearic-arachidic acid was completely admixed
20 before 1.5 g of Ultra Carbon (Ultra Superior Purity, Ultra Carbon, Bay City, MI) was added to the heated Nujol-stearic-arachidic acid mixture. All stearic-arachidic acid crystals were melted before the mixture was removed from the heating device. The
25 synthesized paste was allowed to cool. Then the paste was placed into a glass container. The glass container was rinsed with 100% ethanol and dried in nitrogen prior to placing the paste into it. The synthesized paste was aged and stored in the dark at
30 room temperature. The vials were covered with parafilm. A great deal of care was taken not to allow dust and other extraneous particles near the paste mixture.

Combustion analysis and gas
35 chromatographical analysis were performed on

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approximately a 5 mg synthetic graphite stearic-arachidic paste mixture to determine the accuracy of the content of graphite, stearic and arachidic acid. The graphite content was determined by combustion analysis and the stearic acid and arachidic acid content were determined by gas chromatographical analysis. The level of Nujol was calculated by the difference. These methods were important in the verification of paste composition and have never been suggested for paste composition verification heretofore.

EXAMPLE 8

The fabrication of the graphite fatty acid paste was as follows: 0.10 ml Nujol (extra heavy), containing DL-a-tocopherol as a stabilizer, (Plough Inc., Memphis TN) was admixed with 1 mg N-stearoyl-DL-dihydrosphingosine (10% of the Palmitine Analog) (Sigma, St. Louis, MO). This was done by stirring the 0.10 cc Nujol (extra heavy)-N-stearoyl-DL-dihydrosphingosine (stearoyl-sphingosine) mixture manually with a glass stirring rod, while heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental temperatures began at 25°C and ended at 55°C over the 10-min period. The Nujol-stearoyl-sphingosine mixture was completely admixed before 0.15 g of Ultra Carbon (Ultra Superior Purity, Ultra Carbon, Bay City, MI) was added to the heated Nujol-stearoyl-sphingosine mixture. All stearoyl-sphingosine crystals were melted before the mixture was removed from the heating device. The synthesized paste was allowed to cool. Then the paste was placed into a glass container. The glass container was rinsed with 100% ethanol and dried in nitrogen prior to placing the paste into it. The

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synthesized paste was aged and stored in the dark at room temperature. The vials were covered with parafilm. A great deal of care was taken not to allow dust and other extraneous particles near the paste mixture.

5 Combustion analysis and gas chromatographical analysis is performed on approximately a 5 mg synthetic graphite stearoyl-sphingosine paste mixture to determine the accuracy of the content of graphite, stearoyl and sphingosine. The graphite content is determined by
10 combustion analysis and the stearoyl - sphingosine content is determined by gas chromatographical analysis. The level of Nujol is calculated by the difference. These methods are important in the verification of paste composition and have never been
15 suggested for paste verification heretofore.

EXAMPLE 9

The fabrication of the graphite fatty acid
20 paste was as follows: 0.10 ml Nujol (extra heavy), containing DL-a-tocopherol as a stabilizer, (Plough Inc., Memphis TN) was admixed with 1 mg arachidric acid stearyl ester (Sigma, St. Louis, MO). This was done by stirring the 0.10 cc Nujol (extra
25 heavy)-arachidric-stearyl ester mixture manually with a glass stirring rod, while heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental temperatures began at 25°C and ended at 55°C over the 10-min period. The
30 Nujol-arachidric-stearyl compound was completely admixed before 0.15 g of Ultra Carbon (Ultra Superior Purity, Ultra Carbon, Bay City, MI) was added to the heated Nujol-stearic acid mixture. All of the arachidric-stearyl compound was melted before the
35 mixture was removed from the heating device. The

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° synthesized paste was allowed to cool. Then the paste was placed into a glass container. The glass container was rinsed with 100% ethanol and dried in nitrogen prior to placing the paste into it. The synthesized paste was aged and stored in the dark at room temperature. The vials were covered with parafilm. A great deal of care was taken not to allow dust and other extraneous particles near the paste mixture.

Combustion analysis and gas chromatographical analysis is performed on approximately a 5 mg synthetic graphite arachidric-stearyl paste mixture to determine the accuracy of the content of graphite and arachidric acid stearyl ester. The graphite content is determined by combustion analysis and the arachidric acid - stearyl ester content is determined by gas chromatographical analysis. The level of Nujol can be calculated by the difference. These methods are important in the verification of paste composition. These methods have never been suggested or used for paste composition verification heretofore.

EXAMPLE 10

The fabrication of the graphite fatty acid paste was as follows: 0.10 ml Nujol (extra heavy), containing DL- α -tocopherol as a stabilizer, (Plough Inc., Memphis TN) was admixed with 1 mg stearic acid arachidyl ester (Sigma, St. Louis, MO). This was done by stirring the 0.10 cc Nujol (extra heavy or regular)-stearic acid-arachidyl ester mixture manually with a glass stirring rod, while heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental temperatures began at 25°C and ended at 55°C over the 10-min period. The Nujol-stearic acid-arachidyl ester was

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completely admixed before 0.15 g of Ultra Carbon (Ultra Superior Purity, Ultra Carbon, Bay City, MI) was added to the heated Nujol-stearic acid-arachidyl ester mixture. All stearic acid-arachidyl ester crystals was melted before the mixture is removed from the heating device. The synthesized paste was allowed to cool. Then the paste was placed into a glass container. The glass container was rinsed with 100% ethanol and dried in nitrogen prior to placing the paste into it. The synthesized paste was aged and stored in the dark at room temperature. The vials were covered with parafilm. A great deal of care was taken not to allow dust and other extraneous particles near the paste mixture.

Combustion analysis and gas chromatographical analysis are performed on approximately a 5 mg synthetic graphite stearic acid-arachidyl ester paste mixture to determine the accuracy of the content of graphite, and stearic acid - arachidyl ester. The graphite content was determined by combustion analysis and the stearic acid and arachidyl ester content are determined by gas chromatographical analysis. The level of Nujol is calculated by the difference. These methods are important in the verification of paste composition and have never been suggested for paste composition verification heretofore.

EXAMPLE 11

The fabrication of the graphite fatty acid paste was as follows: 0.05 ml Nujol (extra heavy), containing DL- α -tocopherol as a stabilizer, (Plough Inc., Memphis TN) was admixed with 0.5 mg arachidric acid stearyl ester (Sigma, St. Louis, MO). This was done by stirring the 0.05 cc Nujol (extra heavy) -arachidric-stearyl mixture manually with a glass

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stirring rod, while heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental temperatures began at 25°C and ended at 55°C over the 10-min period. The Nujol-arachidric-stearyl compound was completely admixed before 0.075 g of Ultra Carbon (Ultra Superior Purity, Ultra Carbon, Bay City, MI) was added to the heated Nujol-arachidric-stearyl mixture. All arachidric-stearyl compound was melted before the mixture was removed from the heating device. The synthesized paste was allowed to cool. Then the paste was placed into a glass container. The glass container was rinsed with 100% ethanol and dried in nitrogen prior to placing the paste into it. The synthesized paste was aged and stored in the dark at room temperature. The vials were covered with parafilm. A great deal of care was taken not to allow dust and other extraneous particles near the paste mixture.

Combustion analysis and gas chromatographical analysis are performed on approximately a 5 mg synthetic graphite arachidric-stearyl paste mixture to determine the accuracy of the content of graphite, the arachidric acid - stearyl ester. The graphite content is determined by combustion analysis and the arachidric acid - stearyl ester content is determined by gas chromatographical analysis. The level of Nujol is calculated by the difference. These methods are important in the verification of paste composition and have never been suggested for paste composition verification heretofore.

EXAMPLE 12

The fabrication of the graphite fatty acid paste was as follows: 0.05 ml Nujol (extra heavy),

° containing DL- α -tocopherol as a stabilizer, (Plough Inc., Memphis TN) was admixed with 0.5 mg stearyl cerebroside (bovine brain cerebroside) (Sigma, St. Louis, MO). This was done by stirring the 0.05 cc of Nujol (extra heavy or regular)-stearyl cerebroside mixture manually with a glass stirring rod, while heating the Nujol mixture at incremental temperatures over a 10-min time period. The incremental temperatures began at 25°C and ended at 55°C over the 10-min period. The Nujol-stearyl cerebroside compound was completely admixed before 0.075 g of Ultra Carbon (Ultra Superior Purity, Ultra Carbon, Bay City, MI) was added to the heated Nujol-stearyl cerebroside mixture. All compound was melted before the mixture was removed from the heating device. The synthesized paste was allowed to cool. Then the paste was placed into a glass container. The glass container was rinsed with 100% ethanol and dried in nitrogen prior to placing the paste into it. The synthesized paste was aged and stored in the dark at room temperature. The vials are covered with parafilm. A great deal of care was taken not to allow dust and other extraneous particles near the paste mixture.

Combustion analysis and gas chromatographical analysis are performed on approximately a 5 mg synthetic graphite stearyl cerebroside paste mixture to determine the accuracy of the content of graphite and stearyl cerebroside. The graphite content is determined by combustion analysis and the stearyl cerebroside content at least in part, is determined by gas chromatographical analysis. The level of Nujol is calculated by the difference. These methods are important in the verification of paste composition and have never even been suggested for verification of paste composition

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° for in vivo studies, heretofore.

EXAMPLE 13

The phosphate buffer (Na_2HPO_4 :
 NaH_2PO_4 ph 7.4) was made by mixing 0.02 M
5 Na_2HPO_4 (dibasic) with 0.02M NaH_2PO_4
(monobasic) in approximately a 4:1 ratio. Quite
specifically, 405 ml dibasic was mixed with 95 mls
monobasic for in vitro electrode conditioning for in
vivo brain implants. Physiological saline (500mls)
10 was added. The final Molarity was 0.01M. The buffer
was daerated with prepurified Nitrogen gas (N_2 ,
extra dry grade, 5 psi, T.W. Smith Co., Bklyn,
N.Y.). N_2 gas was perfused through the phosphate
buffer and regulated with a Variable Area Flowmeter
15 (Purgemaster, Fisher Sci., Fadem, NJ). The Variable
Area Flowmeter was calibrated at about 100 cc/min.
Deaeration of the phosphate buffer with N_2 took
place for one minute before each voltammetric scan
began.

20 Dopamine (DA), ascorbic acid (AA),
3-4-dihydroxyphenylacetic acid (DOPAC), serotonin
(5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and uric
acid (UA) were each pipetted into the phosphate
buffer. The protocol consisted of separately testing
25 a 1uM amount of each compound in the phosphate buffer
in which the graphite stearate electrode, Ag/AgCl
reference electrode and auxiliary electrode were
immersed. This was followed by pipetting cumulative
5uM, 10uM, 15uM and 20uM amounts of each compound
30 separately into the same phosphate buffer, in 10
controlled studies. Each study began with an
electrode test in 30mls phosphate buffer with no
added chemicals. The final volume of buffer with
added chemicals was 30.12mls. The electrodes were
35 not exposed to brain extracellular fluid at anytime

° before or during the studies. In further experiments, homovanillic acid (HVA) (1-20uM solutions) was pipetted into the phosphate buffer after the addition of DA, AA, DOPAC, 5-HT, 5-HIAA and UA.

5 In further experiments, final concentrations of 600uM DA and ascorbate were measured in the 3-electrode phosphate buffer system in a 3-step, 200uM aliquot protocol. Additionally, in preliminary experiments, final concentrations of 200uM dopamine
10 with 20uM ascorbate were measured. Also concentrations of 20uM dopamine and 200 uM ascorbate were measured. The detection of uric acid with the graphite stearate electrode was also tested at high concentrations (up to 600uM).

15 Detectable amounts of DA and 5-HT, which are less than 50nM and 25nM respectively, will be studied in the future.

EXAMPLE 14

20 The electroanalytical technique of linear potential sweep voltammetry (linear scan voltammetry) with a semidifferential output of current, which was modified according to the method of this invention with novel circuitry for the production of a cathodic
25 (reduction) current, was used throughout the following examples.

The components of a DCV-5 detector made by Bioanalytical Systems (BAS-DCV5) were modified according to the method of this invention to produce
30 cathodic circuitry for reproducible detection of biogenic characteristics in diseased and healthy states. The circuitry was plugged into an isolated ground. Also, the circuitry included a miniguard surge suppressor (from Jefferson Electric) having an
35 input of 60 Hz and 95-130 volts. The output of the

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° suppressor was 250 VA and 120 volts at 2.08 amps maximum. The modified DCV-5 was connected to a working electrode and a combined reference/auxiliary electrode. The teflon-coated working microelectrode (150-200 microns) was coated with a material
5 consisting of graphite paste and nujol modified with stearate, which allows the measurement of changes in dopamine concentration and/or release and/or reuptake without interference from ascorbic acid or the dopamine metabolite, 3,4-dihydroxyphenylacetic acid
10 (DOPAC) and the detection of serotonin without interference from uric acid or the metabolite of serotonin, 5-hydroxyindoleacetic acid.

Adult, male, Sprague-Dawley rats were group housed and fed Purina rat food and water daily.
15 Behavioral and biochemical studies were routinely carried out on these rats in the afternoons for better reproducibility. Surgical experiments were carried out in the mornings.

Prior to testing, a reproducible stable
20 baseline measurement of the test rats' dopamine level was achieved in a period of 1.25 hours. The microelectrodes were first tested in vitro in phosphate buffer solution pH 7.4 (0.16 M NaCl). Potentials were applied within a range of -0.001 or
25 -0.100 to + 0.5 v or higher or any combination thereof. The potentials were measured with respect to a reference Ag/AgCl electrode. Both the reference electrode and a platinum auxiliary electrode were placed in contact with the cortex of the brain. A
30 stainless steel auxiliary electrode may also be used. The working electrode was stereotaxically implanted, using a David Kopf stereotoxic device, in the tuberculum olfactorium, according to the atlas of Pellegrino and Cushman, 1967 (coordinates: 2.6 mm
35 anterior to Bregma, 2.5 mm lateral to midline and 9.5

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° mm below the dura mater, or skull surface).

On each experimental day, the animals were injected intraperitoneally with D-Ala²-D-Pro⁵-enkephalinamide monoacetate (DAP) dissolved in distilled water (5 mg/kg/ml), along with other animals injected with appropriate vehicle or saline control injections. Semiderivative voltammograms from rats anesthetized with chloral hydrate (450 mg/kg) were recorded every ten minutes for up to two hours at scan rate of 10 mv sec⁻¹ and a sensitivity of 0.2 nA sec^{-1/2}cm⁻¹.

Changes in the dopamine signal in the tuberculum olfactorium after injection of DAP enkephalinamide were measured by comparing the mean of the pre-injection values with both the mean and the maximum of the post-injection values. The effects of the administration of the DAP enkephalinamide, in terms of basal dopamine release, is shown in Figure 4, which shows two semiderivative voltammograms, one pre-injection and one post-injection. The dopamine signal (left-hand peak) is clearly unchanged from pre- to post-injection. The serotonin signal (right-hand peak) is, however, measurably higher after the injection.

It was observed that DAP did not inhibit the rats' locomotor activity. This observation is consistent with conventional theory which places control of locomotor activity in this part of the brain. It shows that dopamine levels in the tuberculum olfactorium are not affected by doses of DAP enkephalinamide. That locomotor activity was not affected is consistent with the placement of locomotor control in the tuberculum olfactorium because behaviorally DAP does not affect locomotor activity.

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EXAMPLE 15

Rats were prepared for testing as in Example 14. The rats were injected with d-amphetamine sulfate (2.5 mg/kg) dissolved in saline, or DAP (5 mg/kg) followed in one-half hour by d-amphetamine sulfate. The amphetamine induced stereotypy in the treated rats, i.e. sniffing, head movement, rearing, licking, chewing, grooming, forepaw pacing and locomotor activity were observed.

D-Ala²-D-Pro⁵-enkephalinamide monoacetate was administered intraperitoneally. Changes in central nervous system dopamine concentrations in rat striatum and rat nucleus accumbens (coordinates adjusted to 4.0 mm and 7.5 mm below dura mater respectively), after administering the enkephalinamide were measured by comparing the mean of the pre-injection values with both the mean and the maximum of post-injection values. Figure 5 shows semiderivative voltammograms from rat striatum (Fig. 5A) and rat nucleus accumbens (Figure 5B). The semiderivative voltammograms show a significantly decreased dopamine signal from the striatum and no change in the dopamine signal from the nucleus accumbens. It was observed that the DAP inhibited head-bobbing, sniffing and frequency of rearing. It did not significantly inhibit the amphetamine-induced effect on locomotor activity. This is consistent with behavioral theory that associates stereotyped behaviors with nigrostriatal dopamine activation and locomotor activity with mesolimbic areas of the brain such as nucleus accumbens.

EXAMPLE 16

Rats were prepared for intraperitoneal administration of drug as in Example 14. D-morphine sulfate in distilled water solution was injected

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intraperitoneally (about 5mg/kg rat weight) one hour after reproducible basal dopamine and serotonin signals were recorded from rat anterior striatum. The effect of morphine on striatal dopamine and serotonin signals was studied for a period of three hours. Alterations in the dopamine and serotonin signals, after morphine was injected, were measured by comparing the mean of pre-morphine injection values with both the mean and the maximum of post-morphine injection values. Figure 6 shows the resultant semiderivative voltammograms taken 1, 2 and 3 hours after administration of morphine and shows changes in release and/or reuptake of brain chemicals in response to the drug. The results can be directly related to brain somatosensory systems involving tolerance to pain, addiction mechanisms and mechanisms of brain reward.

EXAMPLE 17

The method of this invention was used to diagnose diabetes mellitus as follows: male Sprague-Dawley rats were made diabetic with a single intraperitoneal injection of streptozotocin, 60 mg/kg. The diabetic state was assessed by positive glucose concentrations via urine analysis and by measurement of unfasting plasma glucose with the glucose oxidase 2 method (Beckman Glucose Analyzer). Blood was taken from the animals in capillary tubes via the intraocular method. Plasma glucose levels were in excess of 300 mg/100 mL. Neurotransmitters from these animals were studied along with age-matched controls at 3-day-and-a-month intervals following the induction of diabetes by measuring rate of release and reuptake using the circuit set forth in Figure 15. The method of this invention was used to measure percent change from endogenously released

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dopamine and serotonin signals in a non-diabetic rat. The results of these measurements are diagrammed in Fig. 7. The method of this invention can therefore be applied to diagnose diseased states. The results show that serotonin is particularly vulnerable to the diabetic state and may indeed cause endogenous depression.

EXAMPLE 18

The method of Example 17 was used to study the dopamine signals from the brains of rats partially depleted of oxygen such as may be observed in brain injuries. This condition is known as "hypoxic hypoxia".

The left femoral artery of male Wistar rats (260-360 g) was cannulated for measurements of arterial partial pressures of oxygen (PaO_2) and carbon dioxide (PaCO_2) to determine the degree of hypoxia produced by a 15% O_2 inspiratory gas mixture. Bloods were drawn into 150 uL sodium heparin capillary tubes. Blood gases were measured by the ABL 30 Blood Gas Analyzer (Radiometer Amer. Inc., Westlake, Ohio). Gases were administered at a flow rate of six cubic feet per hour via a glove apparatus fitted over the animals' nose and mouth. Compressed air was administered to the animal. After a reproducible and stable baseline of extracellular dopamine and serotonin was evident, the animal was treated with 15% O_2 (See Fig. 8). The 15% O_2 was followed by compressed air treatment and repeated. The first recording in each treatment group was taken two minutes after the respective treatment was begun.

The administration of 15% O_2 treatment produced a significant increase on rat striatal extracellular dopamine, whereas administration of 20% O_2 returned the dopamine signal to control values.

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° A second 15% O₂ treatment caused an irreversible increase in rat striatal extracellular dopamine. Data from femoral artery cannulations showed that PaO₂ values decreased as expected during the administration of 15% O₂ and returned to normoxic levels during the administration of each 20% O₂ treatment. Administration of 15% O₂ produced no significant effects on rat striatal extracellular serotonin.

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EXAMPLE 19

The method of Example 17 was used to study pain or "analgetic" mechanisms. These mechanisms reflect cerebral blood flow related abnormalities related to somatosensory function and mechanisms of brain reward and drug addiction. These studies of brain analgetic mechanisms, use the opioid peptide, dynorphin. Dynorphin is a non-addicting opioid. Figure 9a shows a typical voltammogram, of dopamine and serotonin release from rat striatum. Dopamine peaks at +130 - +150 mv and serotonin peaks at +270 - +290 mv when stearate is added to the graphite in vivo microelectrode. The effect of dynorphin administration on rat striatal dopamine and serotonin release is shown in Figure 9b. Dopamine release decreased significantly, (30% below control value) and serotonin release increased significantly (40% above control values). The opposing effects of dynorphin occurred over a three-hour period after the administration of dynorphin. These results show that dynorphin does interact with analgetic brain mechanisms.

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EXAMPLE 20

The effect of cocaine on dopamine release from striata of male, Sprague-Dawley rats was studied

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using the method of Example 17. The rats were injected with 20 mg/kg of cocaine subcutaneously. Chloral hydrate anesthetized rats underwent stereotaxic surgery for positioning in the anterior striatum of a teflon-coated working graphite electrode modified by stearate. A Ag/AgCl reference electrode and a stainless steel auxiliary electrode were placed in contact with the rat cortex. Semiderivative voltammograms were recorded every ten minutes. Potentials were applied between -200 and +500 mv, at a scan rate of 10 mv/sec. The results of these scans are illustrated in figure 10a as the percentage of basal dopamine release over a period of time, i.e. a decreased dopamine release was observed, emphasizing the control of dopaminergic function across membranes in brain reward and addiction processes.

A rat died of respiratory arrest after being administered a cocaine dose and was subsequently measured for dopamine release. The results of this measurement were compared to a similar measurement in a rat which had not received cocaine. These measurements are set forth in Figures 10(b) showing that cocaine has a direct effect on the membrane.

25 EXAMPLE 21 (Comparative Example)

The terming of this example as a comparative example is not an admission that it is prior art. The in vitro detection of dopamine and serotonin is measured by the semidifferential (semiderivative) voltammetry technique of Example 13, employing the phosphate buffer of Example 14, at a pH of 7.4, and or stearic acid (stearate) working electrode. The stearic acid electrode is packed with 1 mg of a mixture made from 1.5 gms carbon (graphite), 1.00 cc Nujol (extra heavy) and 100 mg stearic acid.

- ° Electrodes are fabricated as in Example 1.
Electrodes are then conditioned.

Conditioning includes exposing the electrode to a phosphate buffer solution including DA, DOPAC, AA, 5-HT, 5-HIAA and UA in 1-20 uM concentrations in a 1,5,10,15,20uM five aliquot step protocol and applying - 200 to +500 mV potential at a sensitivity of 2nA/V. The potential was applied by a procedure similar to that of Example 14. In the first exposure, the electrode was exposed to DA DOPAC, AA, 5-HT, 5-HIAA and UA in a 1 uM concentration. The exposures were then repeated at the higher concentrations mentioned above to achieve five exposures. The five exposures were conducted in a 24 hour period.

Figure 45 shows a semiderivative (semidifferential) voltammogram showing the in vitro detection of dopamine (20 uM) and serotonin (20 uM) in phosphate buffer, at a pH of 7.4 with the above-described stearic acid electrode.

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EXAMPLE 22

The steps of Example 21 were repeated. However, the electrode was packed with 1.5 gms graphite, 1.24 cc Nujol (extra heavy), and 100 mg arachidic acid.

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Fig. 46 shows a semidifferential (semiderivative) voltammogram showing the in vitro detection of dopamine (20 uM) and serotonin (20 uM) in phosphate buffer at a pH of 7.4 with the above-described arachidic acid electrode.

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A comparison of Figs. 45 and 46 shows the arachidic acid electrode has greater sensitivity than the stearic acid electrode

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EXAMPLE 23

The steps of Example 21 were repeated. However, the electrode was packed with 1 mg of paste made from a mixture of 0.075 gms graphite, 0.05 cc Nujol (extra heavy), and 0.5 mg stearyl cerebroside.

5 Fig. 47 shows a semidifferential (semiderivative) voltammogram showing the in vitro detection of dopamine (5 uM) and serotonin (5 uM) in a PO_4 buffer containing dopamine and serotonin with the above-described stearyl cerebroside electrode.

10 A comparison of Figs. 45 and 47 shows that the stearyl cerebroside is more sensitive than the stearate (stearic acid) electrode for detecting dopamine and serotonin. The stearyl cerebroside electrode was employed to detect dopamine and
15 serotonin at one-fourth the concentration employed with the stearate electrode. However, the sensitivity of the stearyl cerebroside electrode for serotonin is about fifteen to twenty fold that of the stearate electrode. The sensitivity of the stearyl
20 cerebroside electrode for dopamine is about three to six times that of the stearate electrode. Thus, in spite of the lower concentrations, Fig. 47 shows that the stearyl cerebroside electrode results in significant peaks for serotonin and dopamine.

25 Also, on other days, this stearyl cerebroside electrode was employed for detecting dopamine (DA) and serotonin (5-HT) in a PO_4 buffer containing the chemicals DA, 5-HT, DOPAC, 5-HIAA, AA, UA and HVA at a pH of 7.4. 1, 5, 10, 15 and 20uM
30 concentrations of each chemical were employed in five respective tests. All the chemicals are at the same concentration in a given test.

EXAMPLE 24 (Comparative Example)

35 The stearic acid electrode of Example 21 was

reconditioned by repeating the conditioning steps of Example 21 at six and nine months after Example 21 was conducted. Then the electrode was employed for in vitro detection of dopamine (20 uM) and serotonin (20 uM) in phosphate buffer, at a pH of 7.4 by the steps described in Example 21. Fig. 48 shows a semiderivative (semidifferential) voltammogram of this in vitro detection of dopamine (20 uM) and serotonin (20 uM). The terming of this example as a comparative example is not an admission that is is prior art.

EXAMPLE 25

The arachidic acid electrode of Example 22 was reconditioned by repeating the conditioning steps of Example 22 at six and nine months after Example 22 was conducted. Then the electrode was employed for in vitro detection of dopamine (20 uM) and serotonin (20 uM) in phosphate buffer, at a pH of 7.4 by the steps described in Example 22. Fig. 49 shows a semiderivative (semidifferential) voltammogram showing this in vitro detection of dopamine (20 uM) and serotonin (20 uM).

Comparison of Figs. 48 and 49 shows the arachidic acid electrode is more sensitive to serotonin and dopamine even when months separate the conditioning repetitions.

Additional examples of the results of the use of the method and apparatus of this invention are set forth in Patricia A. Broderick, "Rat Striatal Dopamine Release Mechanisms of Cocaine" National Institute On Drug Abuse Research Monographs, 1986; Patricia A. Broderick, "Delineation of Striatal Dopaminergic Autoreceptor Agonist Properties of -(-) Apomorphine with In Vivo Electrochemistry", Annals of the New York Acad. Sc., Vol. No. 473, 1986; Patricia

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- ° A. Broderick, "In Vivo Electrochemical Studies of Rat Striatal Dopamine and Serotonin Release after Morphine," Life Sciences, Vol. 36, p. 2269, 1985.
- 5 Patricia A. Broderick, "Opiate Regulation of Mesolimbic Serotonin Release ...", Neuropeptides Vol. 5, p. 587, 1985; Patricia A. Broderick et al. "Decreased Release of Striatal Serotonin and Dopamine in the Diabetic Rat As Measured By In Vivo Voltammetry;" The Endocrine Society, 1985; Patricia A. Broderick, et al., "In Vivo Electrochemical and Behavioral Evidence for Specific Neural Substrates Modulated Differentially by Enkephalin in Rat Stimulant Stereotypy and Locomotion;" Biological Psychiatry, Vol. 19, No. 1, p. 45, 1984; Patricia A. Broderick, et. al., "Similar Effects of an Enkephalin Analog on Mesolimbic Dopamine Release and Hyperactivity in Rats;" Life Sci. Vol. 33, p. 635, 1983; Patricia A. Broderick et al., "In Vivo Electrochemical Evidence for an Enkephalinergic Modulation Underlying Stereotyped Behaviour: Reversibility by Naloxone;" Brain Res. Vol. 269, p. 378, 1983. Patricia A. Broderick, "Striatal Neurochemistry of Dynorphin (1-13); In Vivo Electrochemical Semidifferential Analysis", Neuropeptides, Vol. 10/No.4, p. 369, 1987; Patricia A. Broderick et al., "Serotonergic Function in Diabetic Rats", Biol. Psychiat., Vol. 24, No. 2, p. 234, 1988; Patricia A. Broderick, "Diabetes-Related Changes in L-Tryptophan-Induced Release of Striatal Biogenic Amines, Diabetes, Vol. 37, No. 7, p. 956, 1988; Patricia A. Broderick et al. "Dopamine and Serotonin in Rat Striatum During In Vivo Hypoxic Hypoxia, Metab. Br. Diseases, Vol. 4, No. 2, p. 143, 1989; Patricia A. Broderick, Distinguishing In Vitro Electrochemical Signatures for Norepinephrine and Dopamine, Neurosci. Lett.,
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- ° Vol. 95, p. 275, 1988; Patricia A. Broderick et al, An In Vivo Voltammetric Analysis of Dopamine Release in Nucleus Accumbens: I. Morphine Effects. II. Cocaine Effects, Adv. in the Biosciences, Vol. 75, p. 209, 1989; Patricia A. Broderick, Characterizing Stearate Probes In Vitro for the Electrochemical Detection of Dopamine and Serotonin, Brain Res., Vol. 495, No. 1 p. 115, 1989. Broderick, State-of-the Art Microelectrodes for In Vivo Voltammetry, 2 Electroanalysis, 241-251 (1990).

10 While specific embodiments of the invention have been shown and described, it should be apparent that many modifications can be made thereto without departing from the spirit and scope of the invention. Accordingly, the invention is not limited by the foregoing description, but is only limited by the claims appended hereto.

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CLAIMS:

1. A microelectrode for inserting in vivo, in situ or in vitro into a warm-blooded or cold-blooded animal brain or body, or extra-corporeally of said animal brain or body and measuring, in vivo, in situ, or in vitro, intracellular and/or extracellular concentrations and/or release and/or reuptake inhibition of one or more biogenic chemicals, comprising, a mixture of graphite, oil and at least one compound selected from the group consisting of complex lipids and simple lipids and salts thereof and wherein the complex lipid is selected from the group consisting of acylglycerols, phosphoglycerides, sphingolipids, glycolipids, lipoproteins and waxes and the simple lipid is selected from the group consisting of terpenes, steroids and prostaglandins, said graphite, said oil, and the compound being present in said microelectrode in a ratio of graphite, in weight in grams, oil, in volume, in cubic centimeters, and compound, in weight in grams, of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5, respectively, the diameter of the microelectrode ranging from about 0.001 μm to about 10 mm, and said oil has a specific gravity from 0.5 to 2.
2. The microelectrode according to claim 1, wherein the biogenic chemical is selected from the group consisting of amines, amine metabolites, ascorbic acid, amino acids, neurotransmitters, putative neurotransmitters, neuromodulators, and neuropeptides.
3. The microelectrode according to claim 2, wherein the biogenic chemical is selected from the group consisting of dopamine, norepinephrine, serotonin and ascorbic acid.
4. The microelectrode according to claim 1, wherein the graphite, oil and compound is present in a ratio of graphite, in weight in grams, oil, in volume in cubic centimeters, and compound, in weight in grams of 1.0 to 2.0:0.1 to 2.0:0.01 to 0.5.

5. The microelectrode according to claim 1, wherein the compound is an acylglycerol, phosphoglyceride, sphingolipid, wax, or salt thereof.
6. The microelectrode according to claim 5, wherein the compound is an acylglycerol or salt thereof.
7. The microelectrode according to claim 6, wherein the compound is arachidic acid stearyl ester.
8. The microelectrode according to claim 5, wherein the compound is a phosphoglyceride or salt thereof.
9. The microelectrode according to claim 5, wherein the compound is a sphingolipid or salt thereof.
10. The microelectrode according to claim 9, wherein the compound is a sphingosine, cerebroside, ceramide, dihexoside, trihexoside, tetrahexoside or salt thereof.
11. The microelectrode according to claim 10, wherein the compound is a sphingosine or salt thereof.
12. The microelectrode according to claim 11, wherein the compound is N-stearoyl-DL-dihydrosphingosine.
13. The microelectrode according to claim 10, wherein the compound is a ceramide or salt thereof.
14. The microelectrode according to claim 10, wherein the compound is a cerebroside or salt thereof.
15. The microelectrode according to claim 14, wherein the compound is a galactocerebroside or salt thereof.
16. The microelectrode according to claim 15, wherein the compound is N-stearoylcerebroside.

17. The microelectrode according to claim 15, wherein the compound is a sulfatide or salt thereof.
18. The microelectrode according to claim 14, wherein the compound is a psychosine or salt thereof.
19. The microelectrode according to claim 10, wherein the compound is a dihexoside, trihexoside, tetrahexoside or salt thereof.
20. The microelectrode according to claim 5, wherein the compound is a wax or salt thereof.
21. The microelectrode according to claim 11, having a diameter ranging from about 2 μm to about 500 μm .
22. The microelectrode according to claim 21, having a diameter ranging from about 150 μm to about 200 μm .
23. The microelectrode according to claim 11, having a diameter ranging from about 50 μm to about 1 mm.
24. The microelectrode according to claim 1, wherein the graphite, oil and compound is present in a ratio of graphite, in weight in grams, oil, in volume in cubic centimeters, and compound, in weight in grams, of 1.0 to 1.5:1.14 to 1.34:0.01 to 0.5.
25. The microelectrode according to claim 1, wherein the compound is a prostaglandin or salt thereof.
26. The microelectrode according to claim 1, wherein the compound is a steroid or salt thereof.
27. The microelectrode according to claim 1, wherein the compound is a terpene or salt thereof.

28. A microelectrode for inserting in vivo, in situ and in vitro into a warm-blooded or cold-blooded animal brain or body, or extra-corporeally of said animal brain or body and measuring, in vivo, in situ, or in vitro intracellular and/or extracellular concentrations and/or release and/or reuptake inhibition of one or more biogenic chemicals, comprising, a mixture of graphite, oil and at least two compounds, the first being arachidic acid and the second being stearic acid, said at least two compounds being employed in a 1:1 weight ratio with respect to each other, said mixture having a ratio of graphite, in weight in grams, oil, in volume in cubic centimeters, and combined compound weight in grams of 1.0 to 2.0:0.1 to 5.0:0.01 to 0.5, respectively, and the diameter of said microelectrode ranging from about 2 μm to about 1 mm.

29. The microelectrode according to claim 28, having a diameter ranging from about 2 μm to about 500 μm .

30. The microelectrode according to claim 28 having a diameter ranging from about 50 μm to about 1 mm.

31. A device capable of analyzing the concentration of one or more biogenic chemicals in vivo, in vitro, or in situ comprising:

an auxiliary microelectrode for placing in contact with an outer layer of an in vivo organ, in vivo sub-organ, in situ specimen or in vitro specimen to be analyzed;

a reference microelectrode for placing in contact with the outer layer of the organ, sub-organ, in situ specimen or in vitro specimen to be analyzed;

an indicator microelectrode comprising, a mixture of graphite, oil and at least one compound selected from the group consisting of complex lipids and simple lipids and salts thereof and wherein the complex lipid is selected from the group consisting of acylglycerols, phosphoglycerides, sphingolipids, glycolipids, lipoproteins and waxes and the simple lipid is selected from the group consisting of

terpenes, steroids and prostaglandins, for inserting into the organ or sub-organ interior, said graphite, said oil and the compound being in a ratio of graphite in weight in grams, oil, in volume, in cubic centimeters, and compound, in weight in grams, of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5, respectively, said indicator microelectrode having a diameter ranging from about 0.001 μm to about 10 mm and said oil has a specific gravity from 0.5 to 2; and means for electrochemically measuring said concentration of said one or more biogenic chemicals by a technique selected from the group consisting of square wave measurement, amperometric measurement, semidifferential voltammetry measurement, linear scanning measurement, differential pulse measurement, chronoamperometry measurement and double differential pulse measurement, and wherein said auxiliary microelectrode, said reference microelectrode, and said indicator microelectrode are functionally connected to said means for electrochemically measuring.

32. A device capable of analyzing the concentration of one or more biogenic chemicals in vivo, in vitro, or in situ comprising:

- an auxiliary microelectrode for placing in contact with an outer layer of an in vivo organ, in vivo sub-organ, in situ specimen or in vitro specimen to be analyzed;

- a reference microelectrode for placing in contact with an outer layer of an in vivo organ, in vivo sub-organ, in situ specimen or in vitro specimen to be analyzed;

- an indicator microelectrode comprising, a mixture of graphite, oil and at least one compound selected from the group consisting of unsaturated fatty acids, derivatives of unsaturated fatty acids and salts thereof, for inserting into the organ or sub-organ interior, said graphite, said oil and the compound being in a ratio of graphite, in weight in grams, oil, in volume, in cubic centimeters, and compound, in weight in grams, of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5, respectively, said indicator microelectrode having a diameter ranging from

about 0.001 μm to about 10 mm; said oil having a specific gravity from 0.5 to 2; and

means for electrochemically measuring said concentration of said one or more biogenic chemicals by a technique selected from the group consisting of square wave measurement, amperometric measurement, semidifferential voltammetry measurement, linear scanning measurement, differential pulse measurement, chronoamperometry measurement and double differential pulse measurement, and wherein said auxiliary microelectrode, said reference microelectrode, and said indicator microelectrode are functionally connected to said means for electrochemically measuring.

33. A microelectrode for inserting in vivo, in vitro or in situ into a warm-blooded or cold-blooded animal brain or body, or extra-corporeally and measuring intracellular and/or extracellular concentration and/or release and/or reuptake of one or more biogenic chemicals while measuring said chemical in situ, or in vivo or in vitro and said microelectrode comprises a mixture of graphite, oil and at least one compound selected from the group consisting of palmitoleic acid, oleic acid, linoleic acid, arachidonic acid, elaidic acid, and transhexadecenoic acid and salts thereof and in which the graphite, oil and compound are present in a ratio of graphite, weight in grams, oil volume in cubic centimeters, compound, weight in grams, of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5 and said oil has a specific gravity from 0.5 to 2, and the diameter of said micro-electrode ranges from 0.001 μm to about 10 mm.

34. A microelectrode according to claim 33, wherein the biogenic chemical is selected from the group consisting of amines, amine metabolites, ascorbic acid, uric acid, amino acids, neuro-transmitters, putative neurotransmitters, neuromodulators, and neuropeptides.

35. A microelectrode according to claim 34, wherein a biogenic chemical is selected from the group consisting of dopamine, norepinephrine, serotonin, gamma aminobutyric acid (GABA), glutamate, glycine, aspartate and tryptophan.

36. A microelectrode according to claim 33, having a diameter which ranges from about 50 μm to about 1 mm.

37. A microelectrode according to claim 33 having a diameter which ranges from about 2 μm to about 500 μm .

38. A microelectrode according to claim 33, in which the graphite, oil and compound are present in a ratio of graphite, weight in grams, oil, volume in cubic centimeters, compound, weight in grams, of 1.0 to 2.0:0.1 to 2.0 to 0.01 to 0.5.

39. A microelectrode according to claim 33 in which the compound is oleic acid.

40. A microelectrode according to claim 33 in which the compound is elaidic acid.

41. A microelectrode for inserting in vivo, in vitro or in situ into a warm-blooded or cold-blooded animal brain or body and measuring intracellular and/or extracellular concentrations and/or release and/or reuptake of one or more biogenic chemicals while measuring said chemicals in situ, or in vivo or in vitro and said microelectrode comprising a mixture of graphite, oil and at least one compound selected from the group consisting of acetic acid, propionic acid, butyric acid, pentanoic acid, caproic acid, caprylic acid, capric acid, tridecanoic acid, pentadecanoic acid, heptadecanoic acid, nonadecanoic acid, lauric acid, myristic acid, arachidic acid, lignoceric acid, lignoceric acid, cerebronic acid, lactobacillic acid and salts thereof, the graphite, oil and compound being in a ratio of graphite, weight in grams, oil, volume in cubic centimeters, compound,

weight in grams, of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5, respectively, said oil having a specific gravity from 0.5 to 2, and the diameter of said microelectrode ranges from about 0.001 μm to about 10 mm.

42. A microelectrode according to claim 41, wherein the biogenic chemical is selected from the group consisting of amines, amine metabolites, ascorbic acid, uric acid, amino acids, neurotransmitters, putative neurotransmitters, neuromodulators, and neuropeptides.

43. A microelectrode according to claim 41, wherein the biogenic chemical is selected from the group consisting of dopamine, norepinephrine, serotonin, gamma aminobutyric acid (GABA), glutamate, glycine, aspartate and tryptophan.

44. A microelectrode according to claim 41, having a diameter which ranges from about 50 μm to about 1 mm.

45. A microelectrode according to claim 41, having a diameter which ranges from about 2 μm to about 500 μm .

46. A microelectrode according to claim 45, having a diameter which ranges from about 150 μm to about 200 μm .

47. A microelectrode according to claim 41 in which the graphite, oil and compound are present in a ratio of graphite, weight in grams, oil, volume in cubic centimeters, compound, weight in grams, of 1.0 to 2.0:0.1 to 2.0:0.01 to 0.5.

48. A microelectrode according to claim 41, wherein the compound is arachidic acid.

49. A microelectrode for inserting in vivo, in vitro or in situ and measuring intracellular and/or extracellular concentrations and/or reuptake of one or more biogenic chemicals while measuring said chemicals in situ or in vivo or

in vitro and said microelectrode comprising a mixture of graphite, oil and stearic acid, said graphite, said oil and said stearic acid being present in said microelectrode in a ratio of graphite, weight in grams, oil, volume in cubic centimeters, stearic acid, weight in grams, of 1.0 to 1.5:1.19 to 5.0:0.01 to 0.5, respectively, and in which the oil has a specific gravity from 0.5 to 2, and the diameter of said microelectrode ranges from about 2 microns to about 1 mm.

50. A microelectrode according to claim 49, wherein the biogenic chemical is selected from the group consisting of amines, amine metabolites, ascorbic acid, uric acid, amino acids, neurotransmitters, putative neurotransmitters, neuromodulators, and neuropeptides.

51. A microelectrode according to claim 50, wherein the biogenic chemical is selected from the group consisting of dopamine, norepinephrine, serotonin, gamma aminobutyric acid (GABA), glutamate, glucine, aspartate and tryptophan.

52. A microelectrode according to claim 51, having a diameter which ranges from about 150 μm to about 200 μm .

53. A microelectrode according to claim 49, having a diameter which ranges from about 2 μm to about 500 μm .

54. A microelectrode according to claim 49, having a diameter which ranges from about 50 μm to about 1 mm.

55. A microelectrode according to claim 49, in which the graphite, oil and compound are present in a ratio of graphite, weight in grams, oil, volume in cubic meters, and stearic acids weight in grams, of 1.0 to 1.5:1.19 to 1.34:0.01 to 0.10.

56. A device capable of analyzing the concentration of one or more biogenic chemicals in vivo, in vitro, in situ or extra-corporeally comprising:

an auxiliary microelectrode for placing in contact with an outer layer of an in vivo organ, in vivo sub-organ, in situ specimen or in vitro specimen to be analyzed;

a reference microelectrode for placing in contact with the outer layer of said organ, said sub-organ, in situ specimen or in vitro specimen to be analyzed;

an indicator microelectrode comprising, a mixture of graphite, oil and at least one compound selected from the group consisting of acetic acid, propionic acid, butyric acid, pentanoic acid, caproic acid, caprylic acid, tridecanoic acid, pentadecanoic acid, heptadecanoic acid, nonadecanoic acid, lauric acid, myristic acid, arachidic acid, lignoceric acid, cerebronic acid, lactobacillic acid, and salts thereof, for inserting into said organ, or said sub-organ's interior, said graphite, said oil and said compound being present in said microelectrode in a ratio of graphite, weight in grams, oil, volume in cubic centimeters, and compound, weight in grams of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5, respectively, and said microelectrode having a diameter ranging from about 0.001 μm to about 10 mm; and

means for electrochemically measuring by a technique selected from the group consisting of square wave measuring, amperometric measuring, semidifferential voltammetric measuring, linear scanning measuring, differential pulse measuring, chronoamperometric measuring and double differential pulse measuring, wherein said auxiliary microelectrode, said reference microelectrode, and said indicator microelectrode are functionally connected to said means for measuring.

57. A method for making a microelectrode for measuring in vivo, in vitro or in situ electrochemical measurements on a warm-blooded or cold-blooded animal brain or body, or extra-corporeally and measuring intracellular and/or

extracellular concentrations and/or release and/or reuptake of one or more biogenic chemicals comprising the steps of:

(a) providing a hollow shell for said microelectrode; and
(b) mixing graphite, oil and at least one additional compound selected from the group consisting of fatty acids and salts thereof selected from the group consisting of palmitoleic acid, oleic acid, linoleic acid, arachidonic acid, elaidic acid and transhexadecenoic acid, acetic acid, propionic acid, butyric acid, pentanoic acid, caproic acid, caprylic acid, capric acid, tridecanoic acid, pentadecanoic acid, heptadecanoic acid, tuberculostearic acid, nonadecanoic acid, lauric acid, myristic acid, palmitic acid, arachidic acid, lignoceric acid, cerebronic acid, lactobacillic acid and in which said graphite, said oil and said at least one compound being present in said microelectrodes in a weight ratio of 0.15 to 7.5:0.1 to 5.0:0.01 to 0.5, respectively, and forming a mixture, inserting said mixture into said hollow shell and forming said microelectrode.

58. A method according to claim 57, further comprising aging the mixture.

59. A method according to claim 57, further comprising conditioning the microelectrode by exposing said microelectrode to solutions comprising concentrations of each biogenic chemical in the femtomolar to molar range, while increasing the concentration of said biogenic chemicals and metabolites in progressive fashion and applying potentials and currents to said microelectrode and electrochemically denoting and detecting said biogenic chemicals and metabolites.

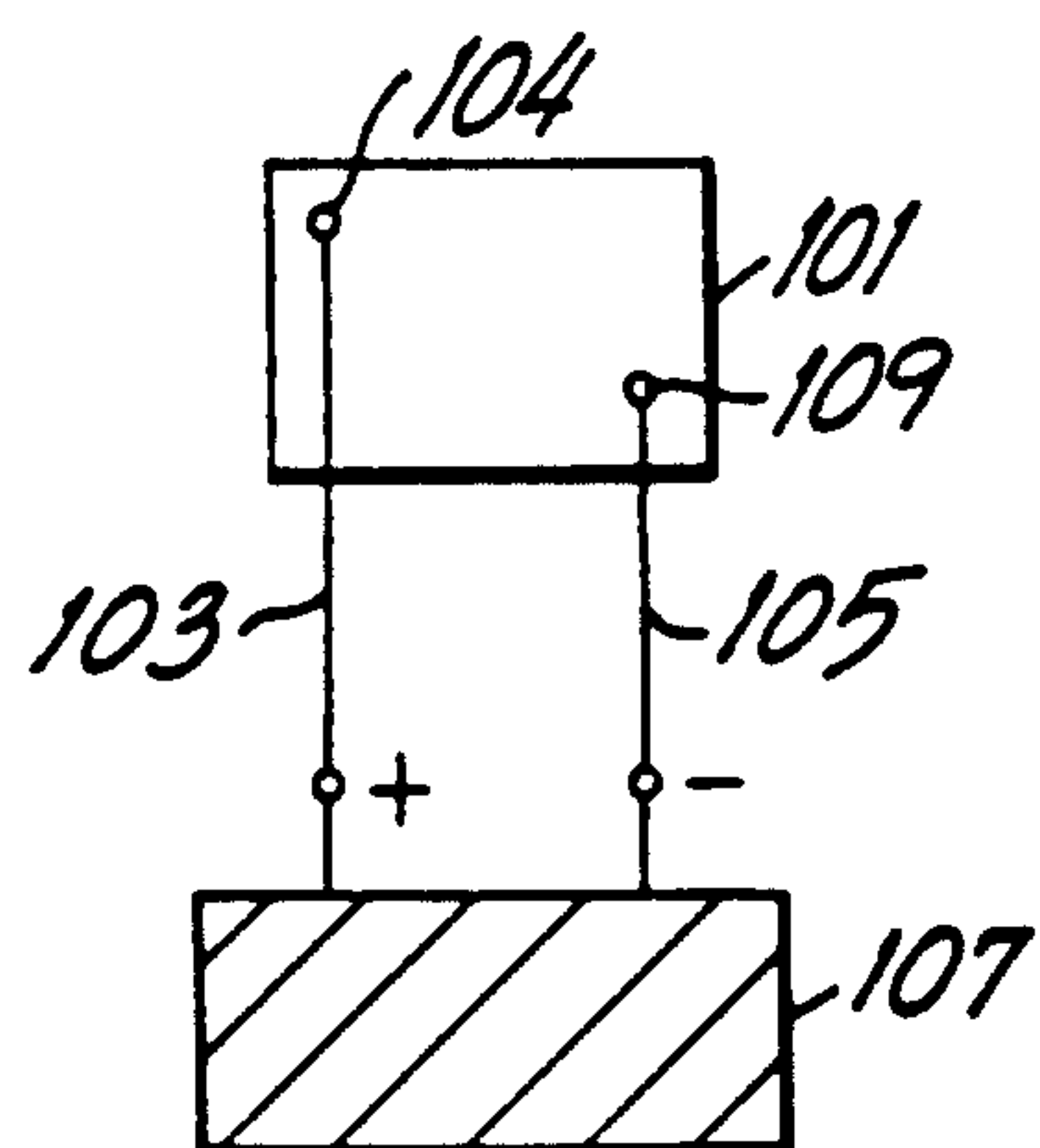


FIG. 1

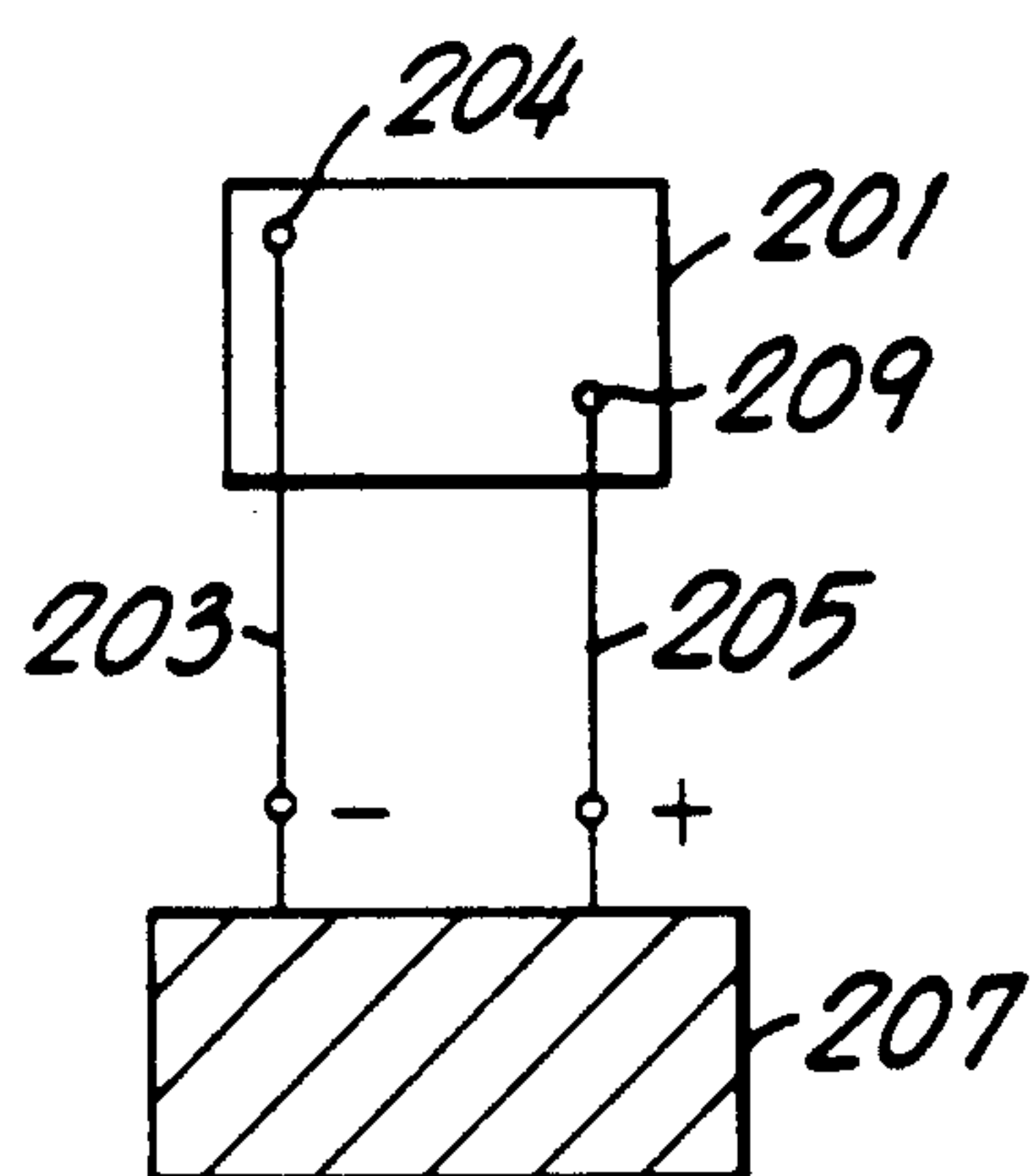


FIG. 2

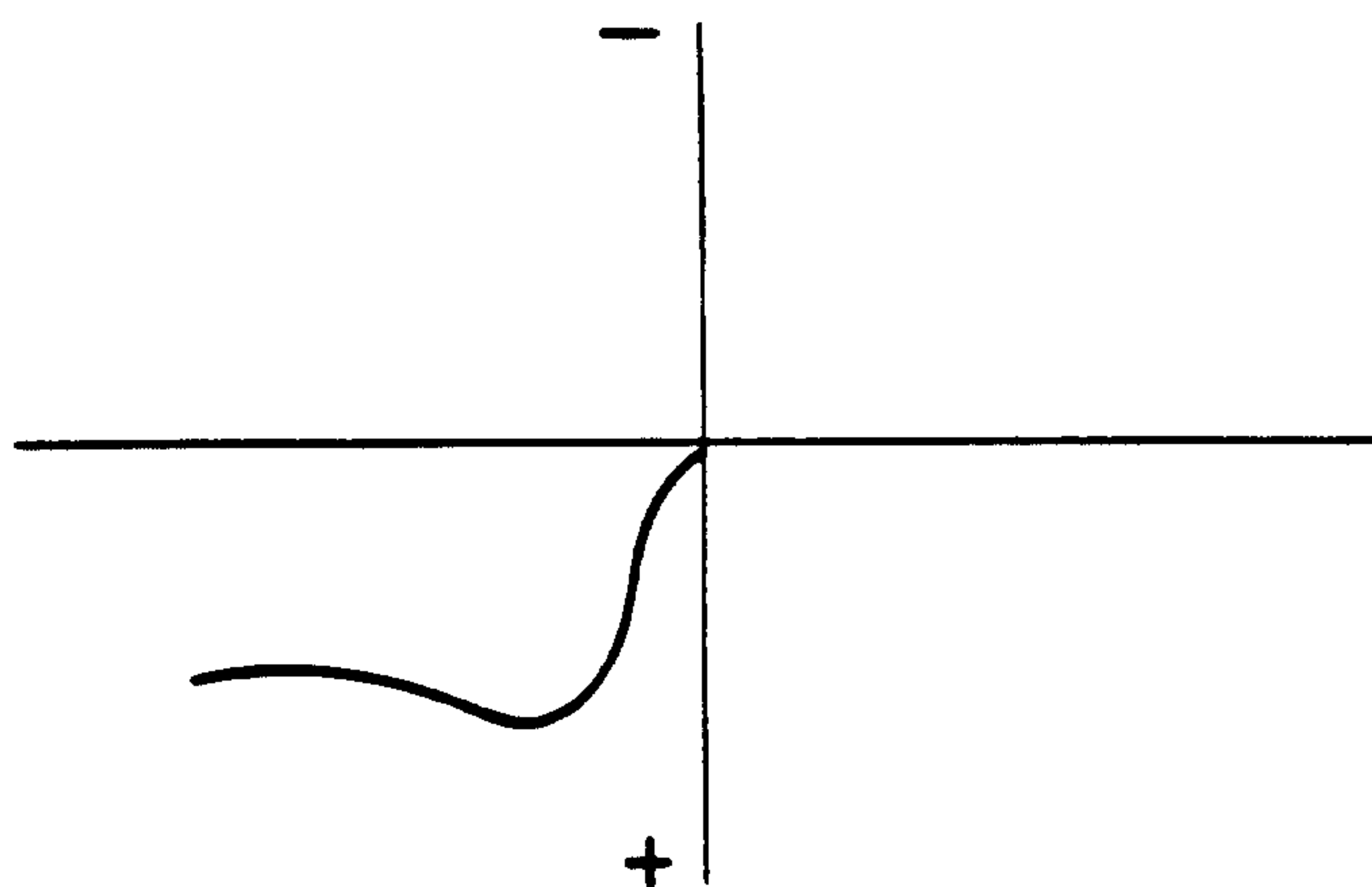


FIG. 3A

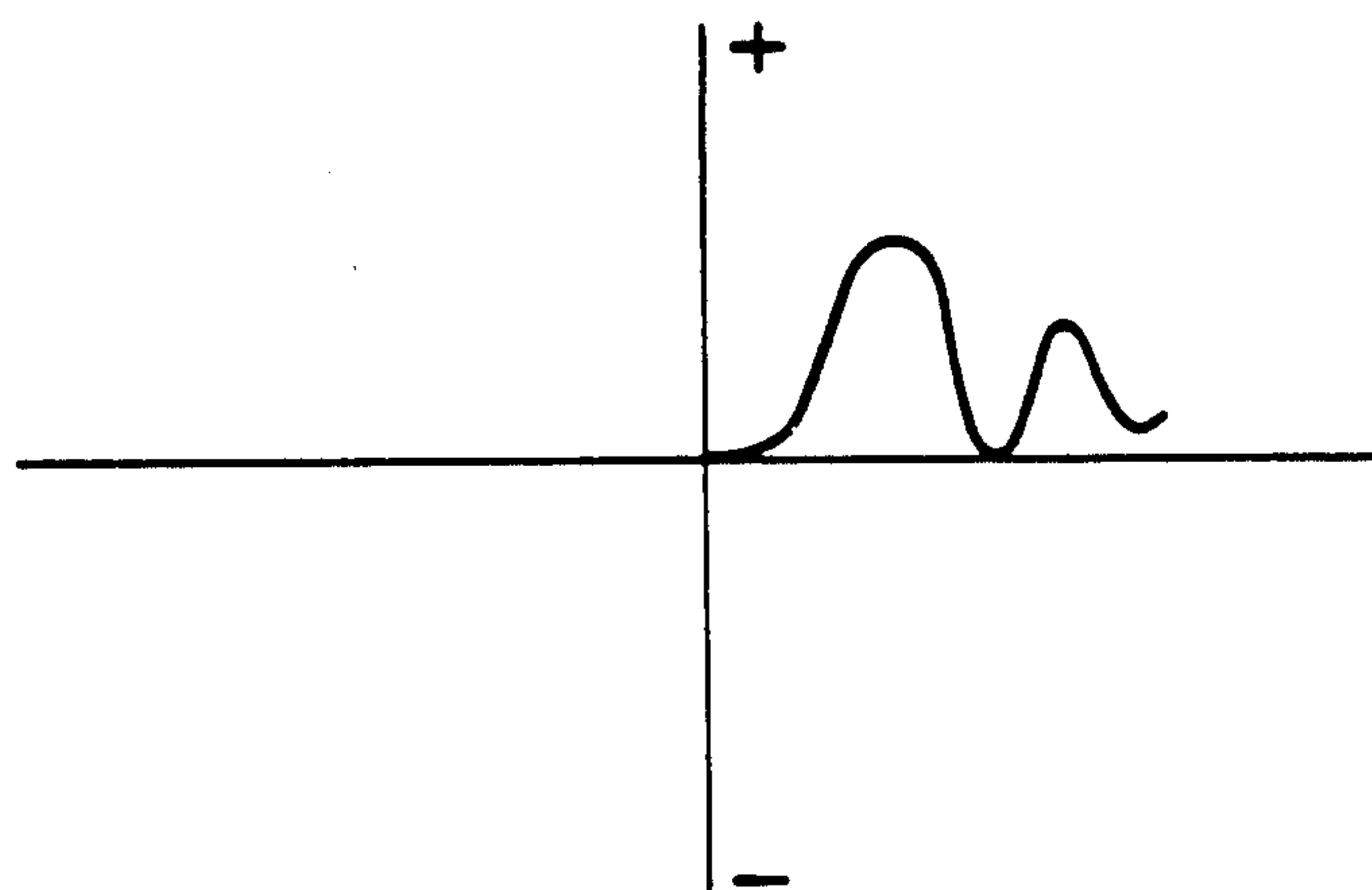


FIG. 3B

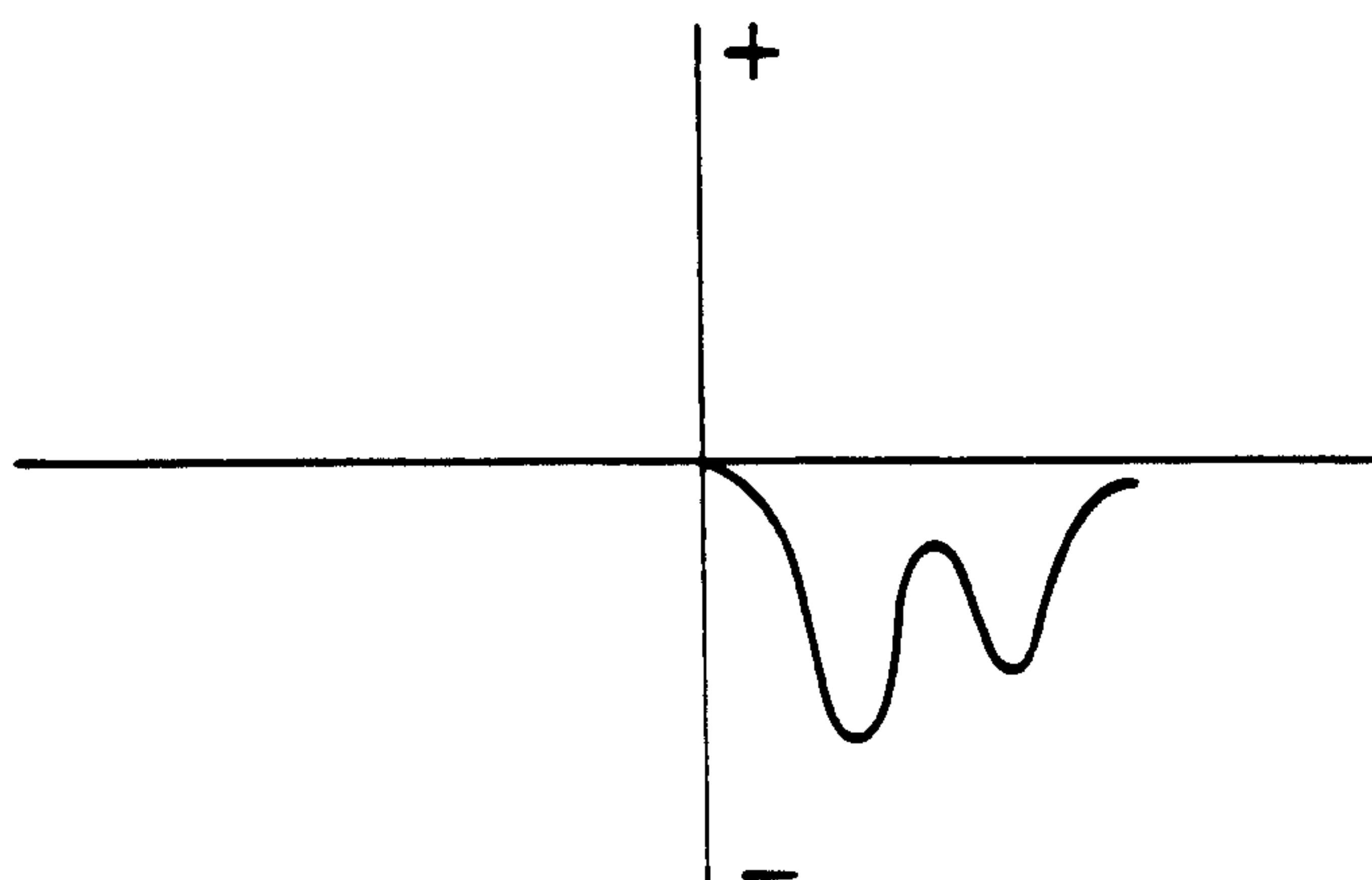


FIG. 3C

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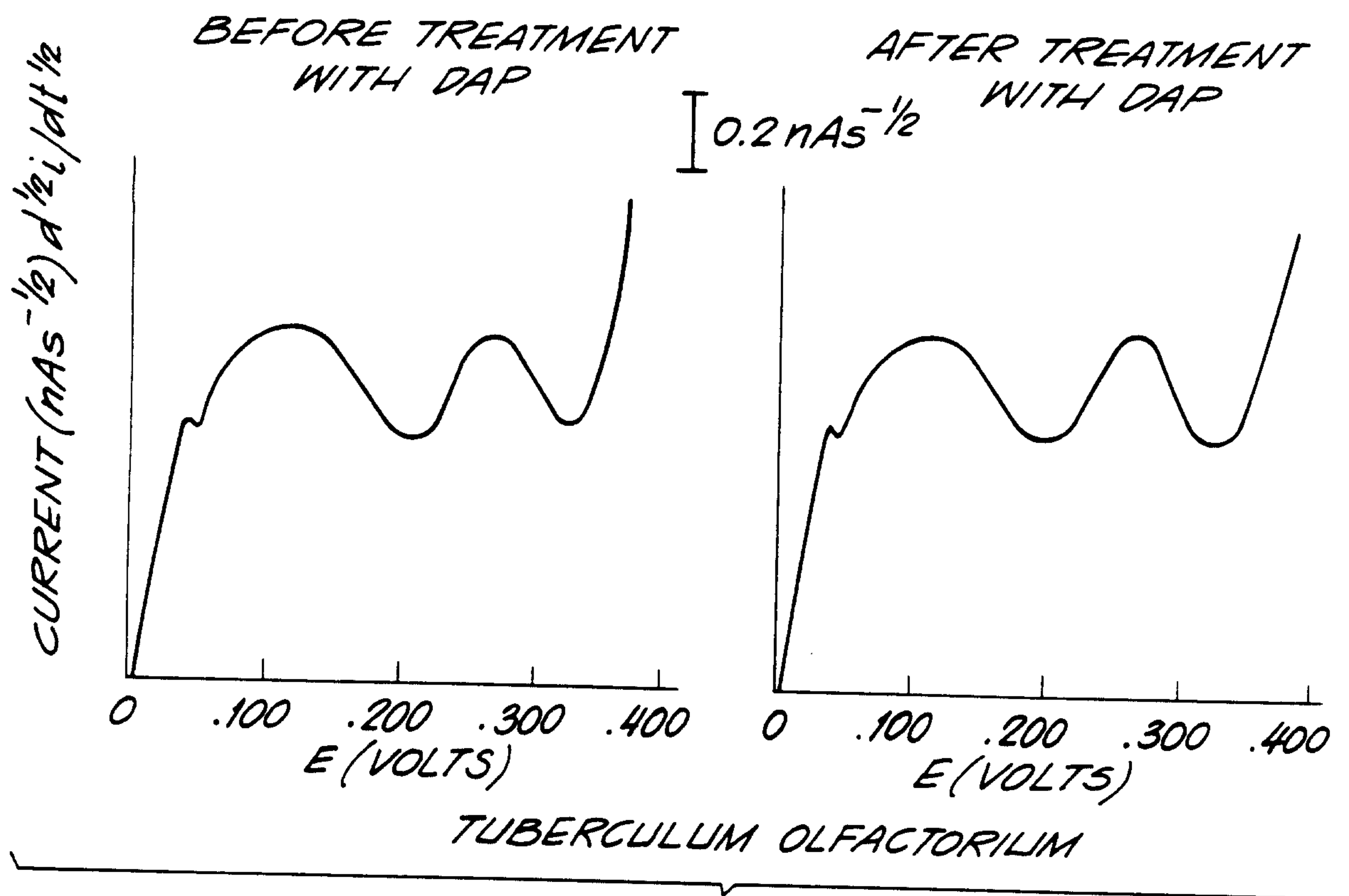
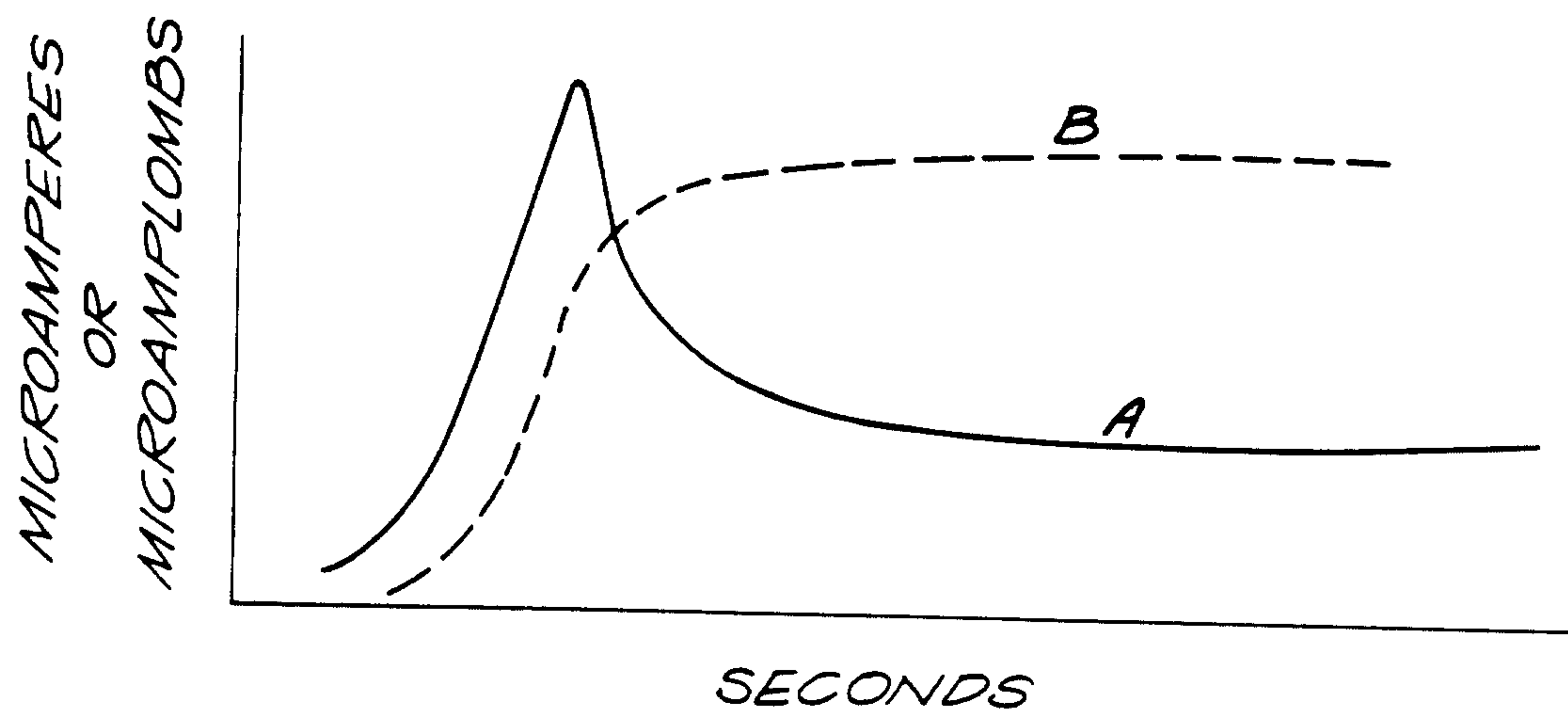


FIG. 4

FIG. 14
PRIOR ART

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FIG. 5A

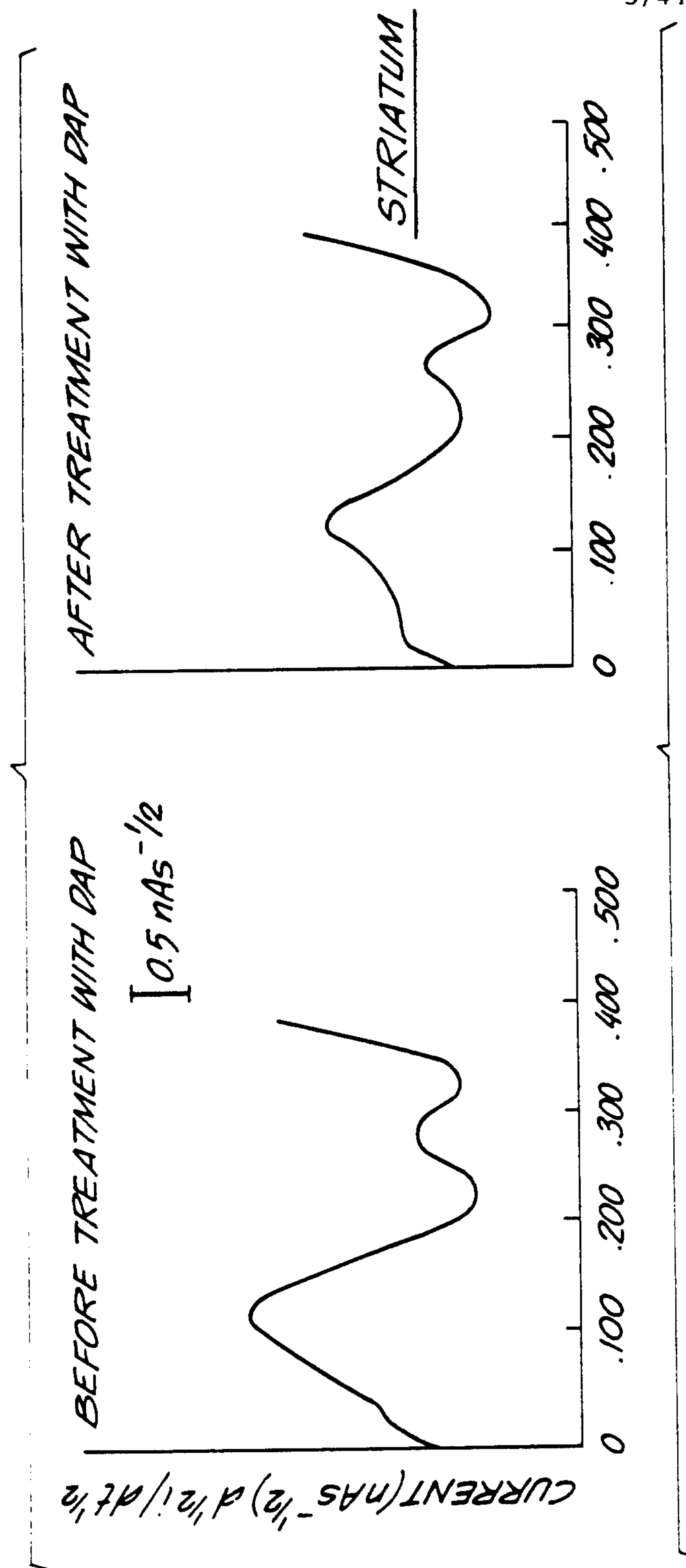
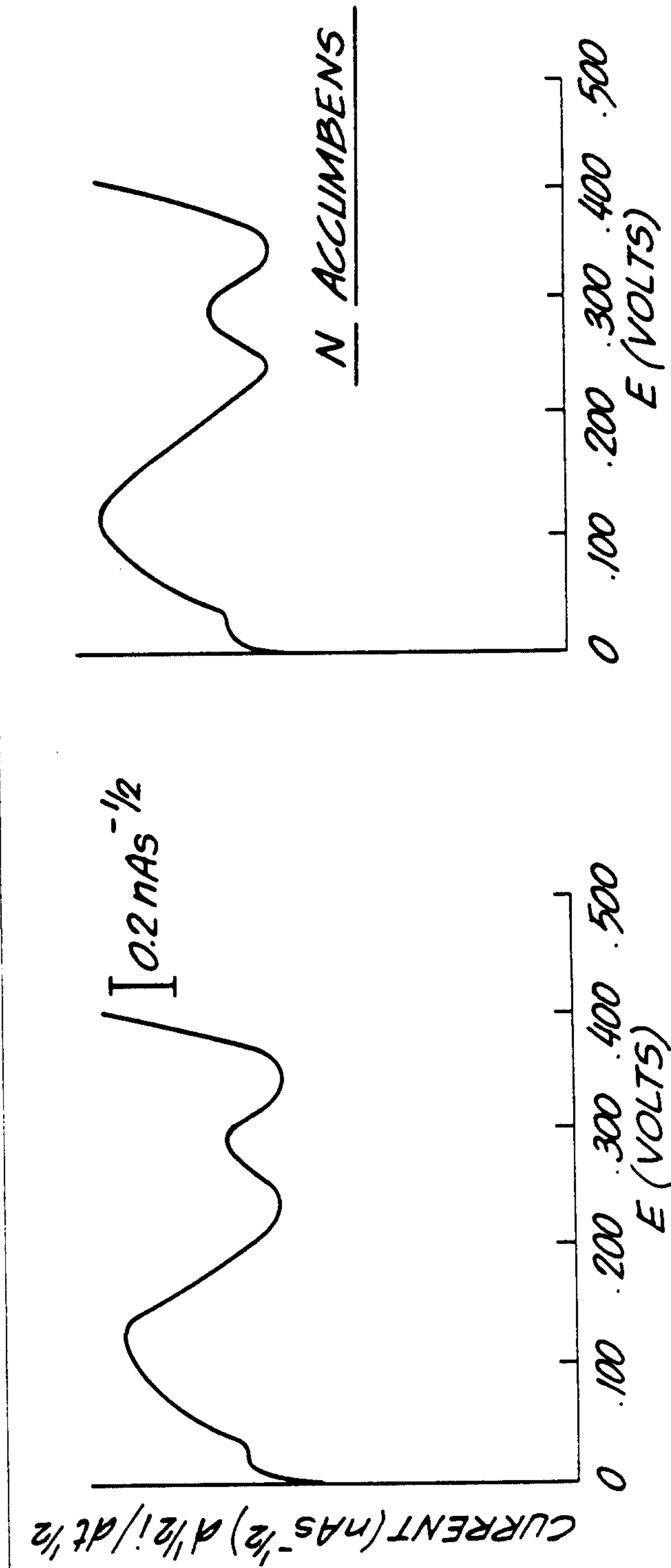


FIG. 5B



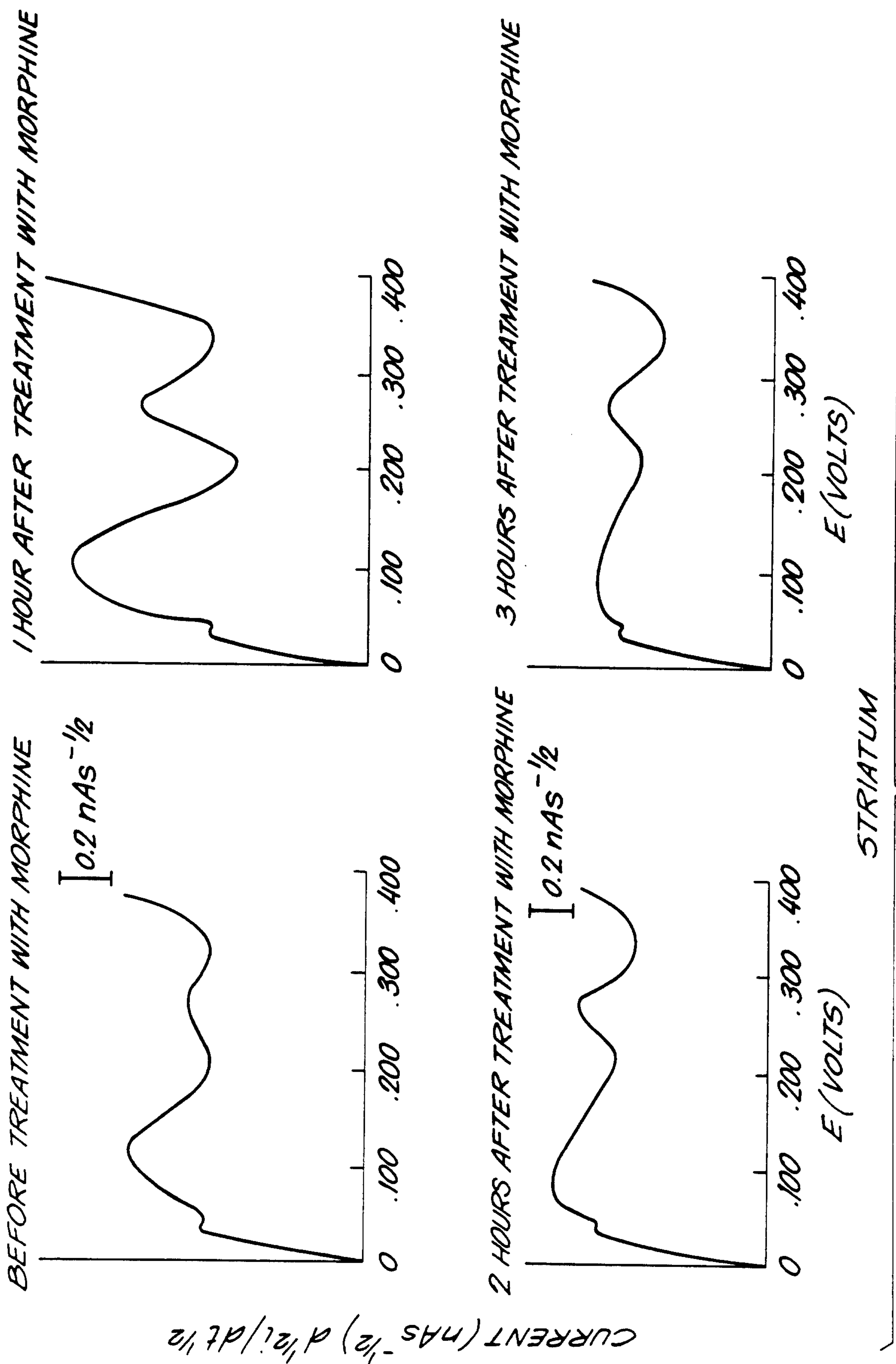


FIG. 6

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DIABETES MELLITUS EXAMPLE

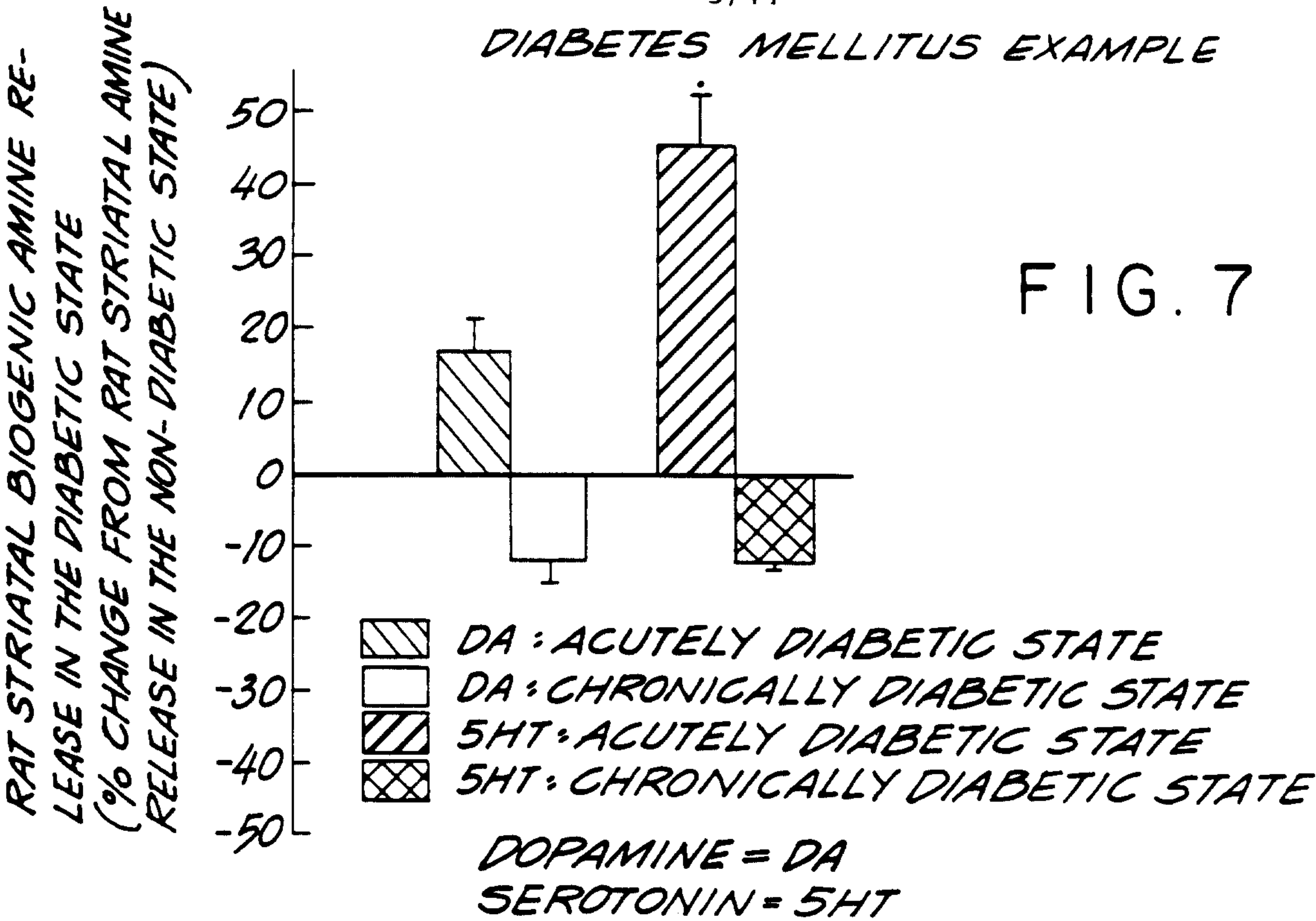


FIG. 7

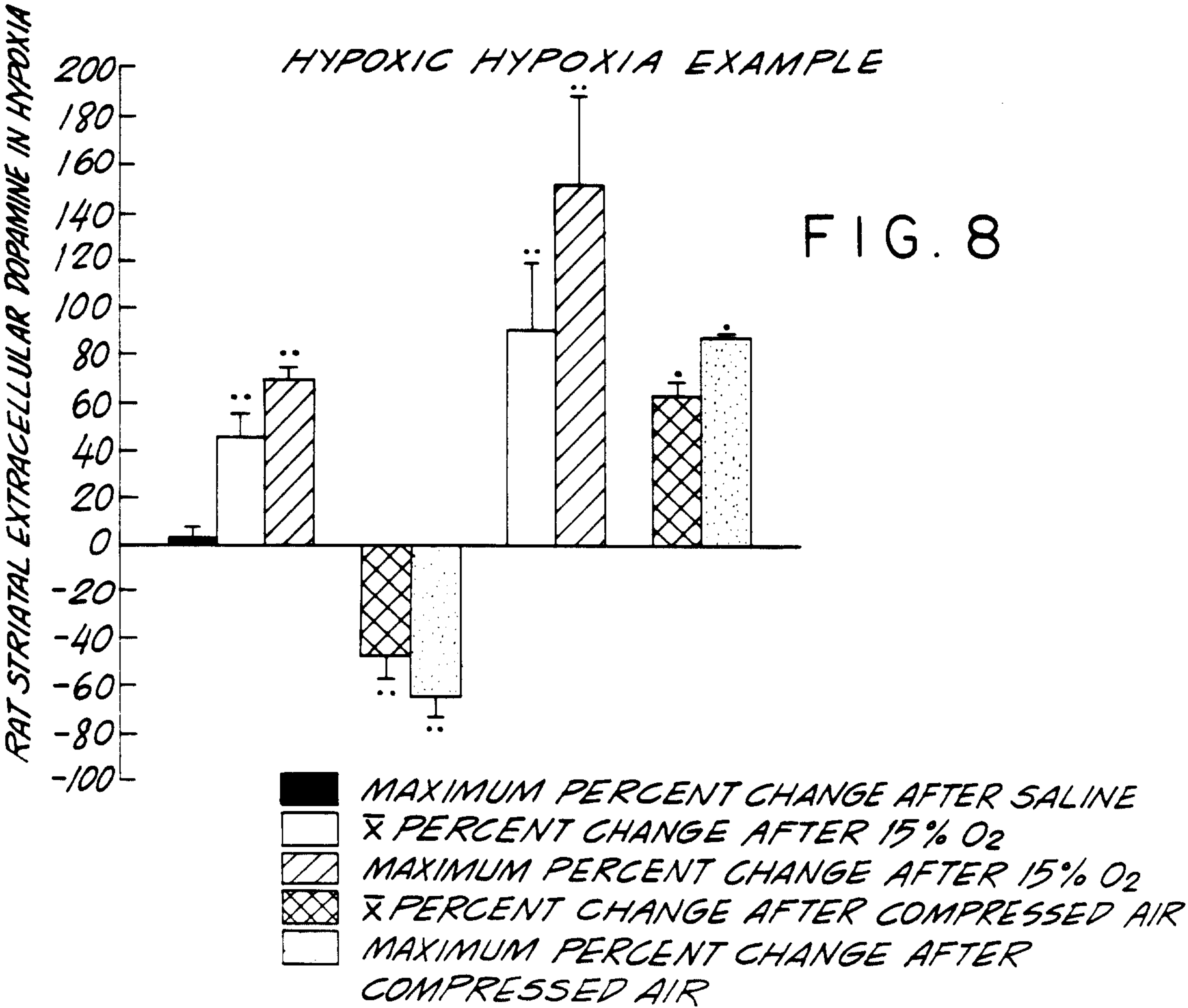
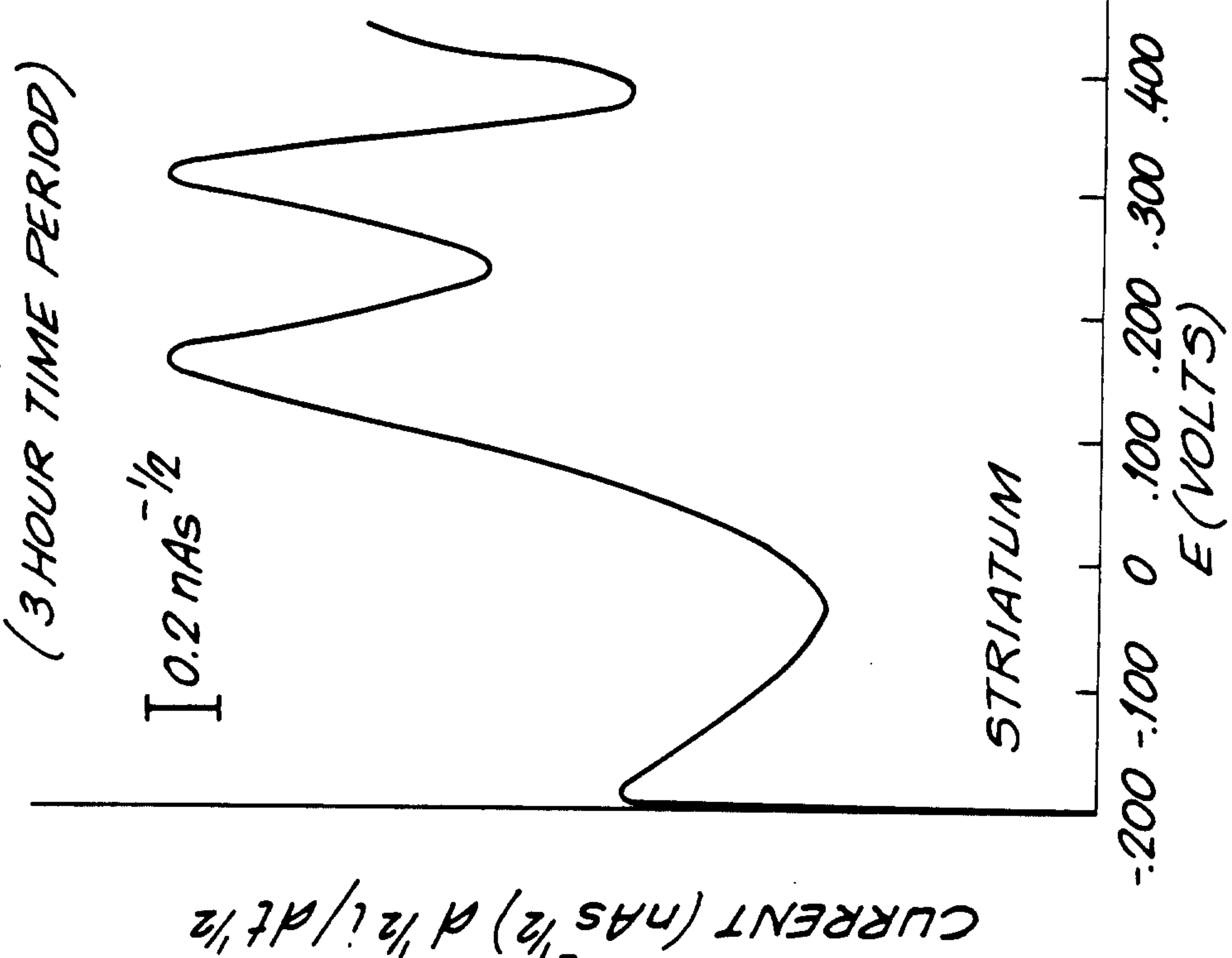


FIG. 8

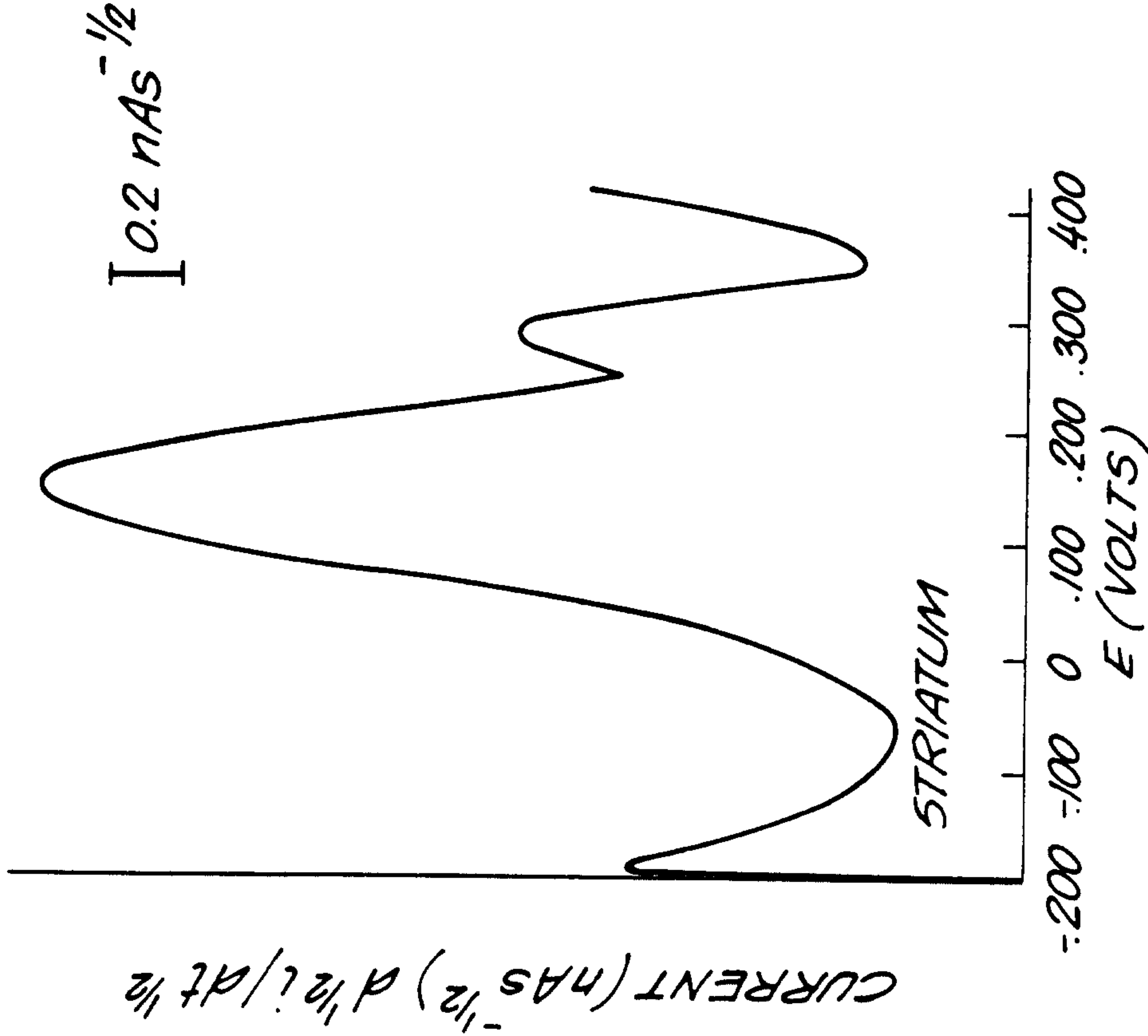
AFTER TREATMENT WITH DYNORPHIN
(1-13)



ANALGESIC MECHANISMS
WITH DYNORPHIN

FIG. 9b

BEFORE TREATMENT WITH DYNORPHIN (1-13)



ANALGESIC MECHANISMS
WITH DYNORPHIN

FIG. 9a

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FIG. 10(a)

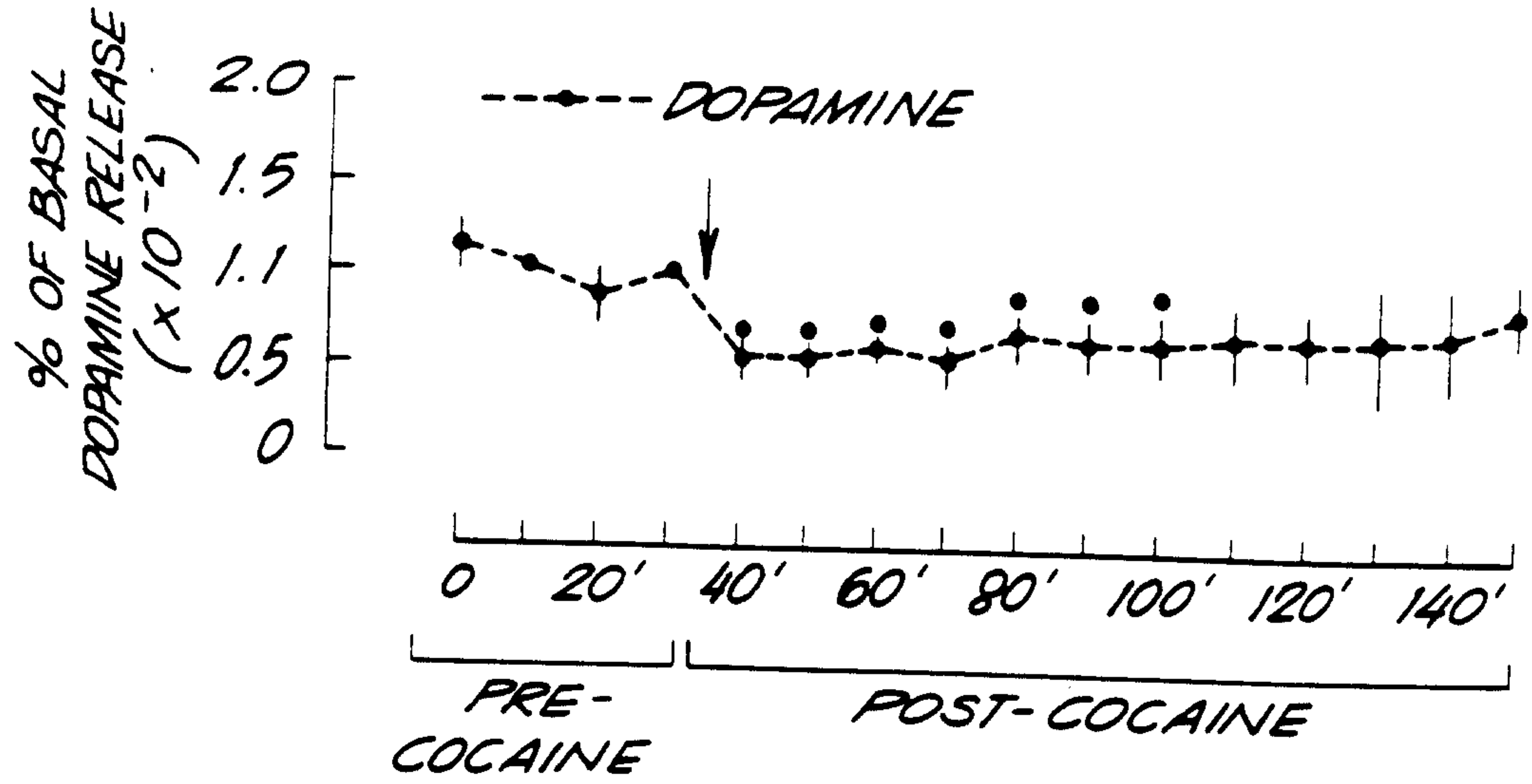
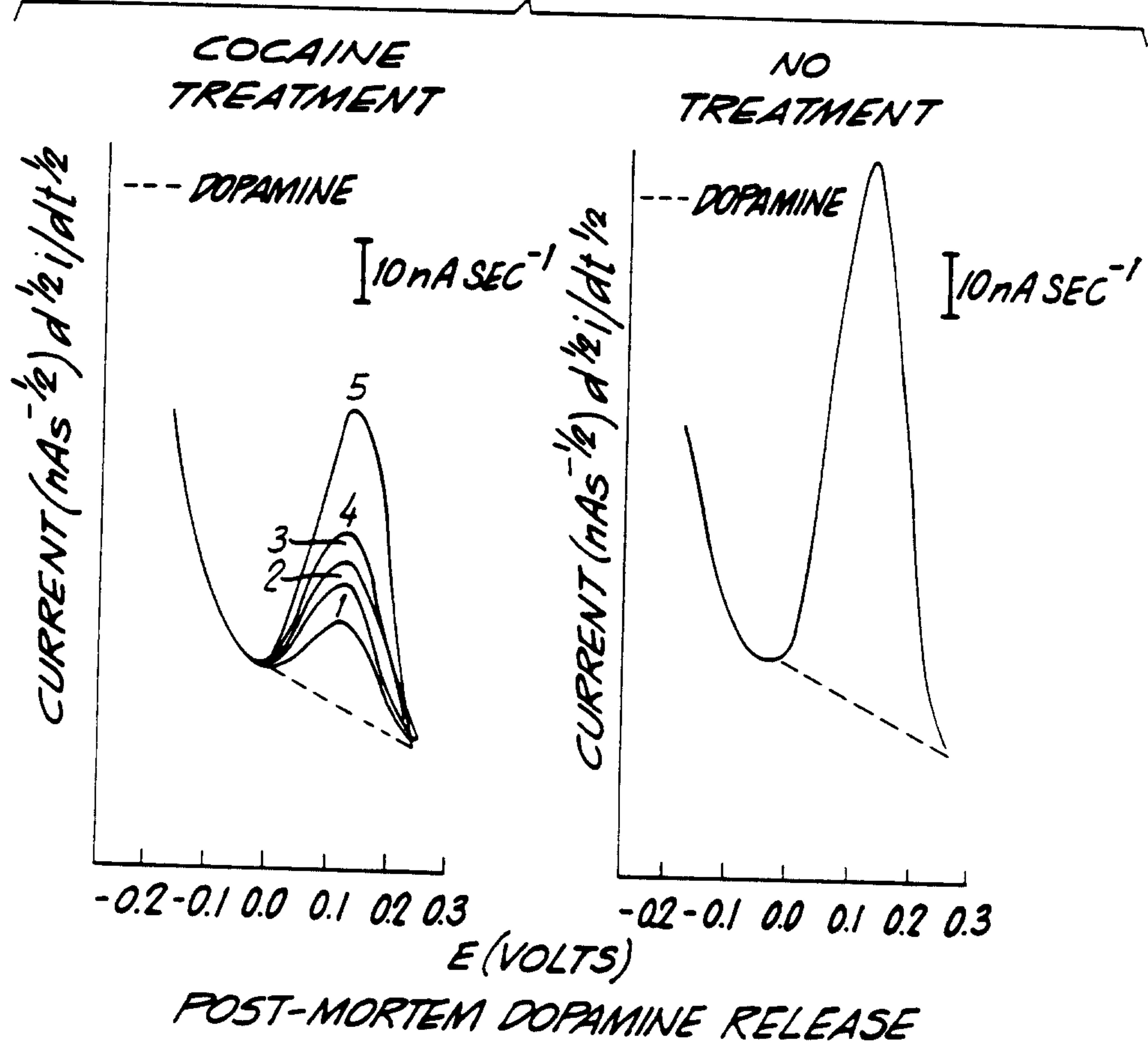


FIG. 10(b)



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FIG. 11 PRIOR ART

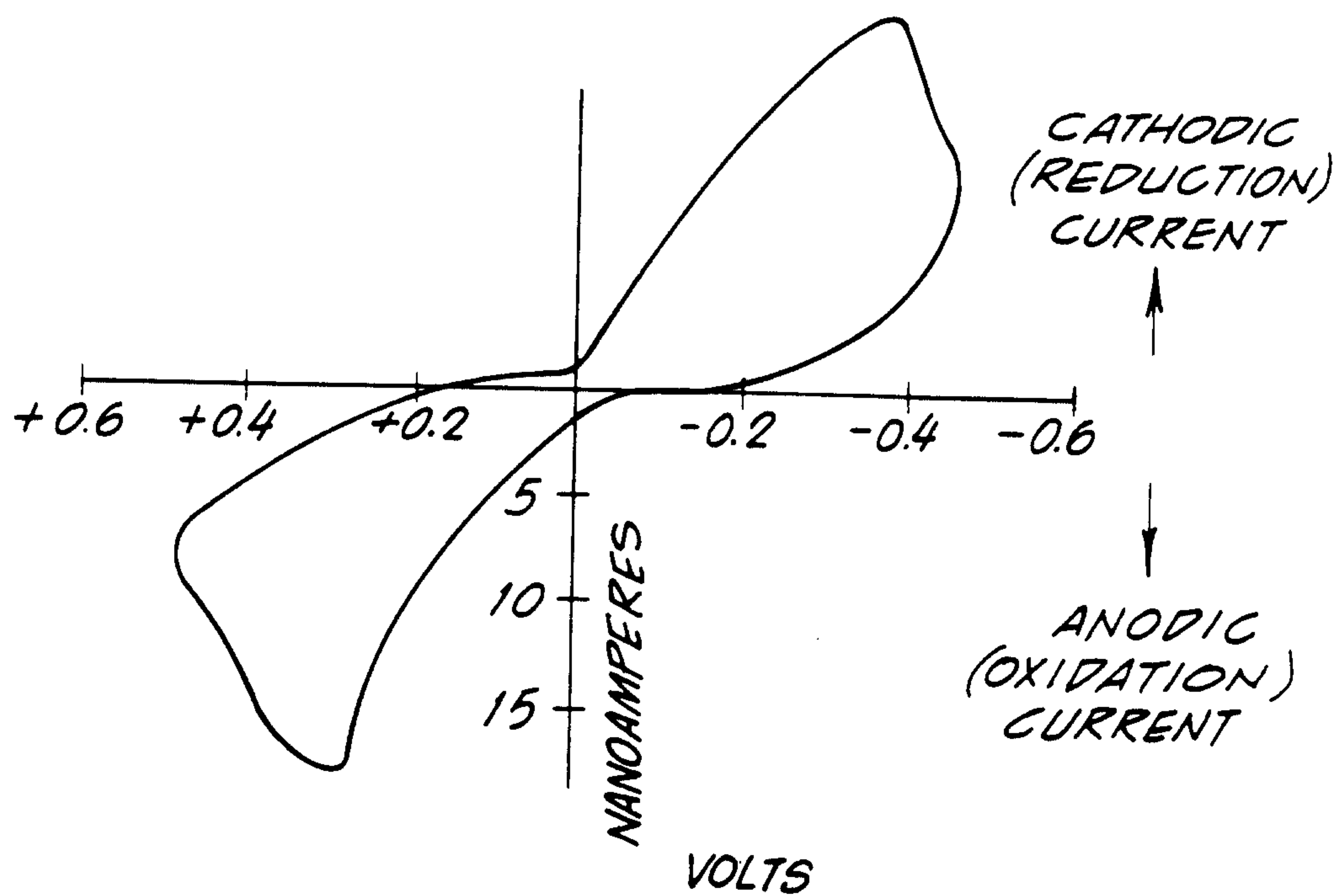


FIG. 12 PRIOR ART

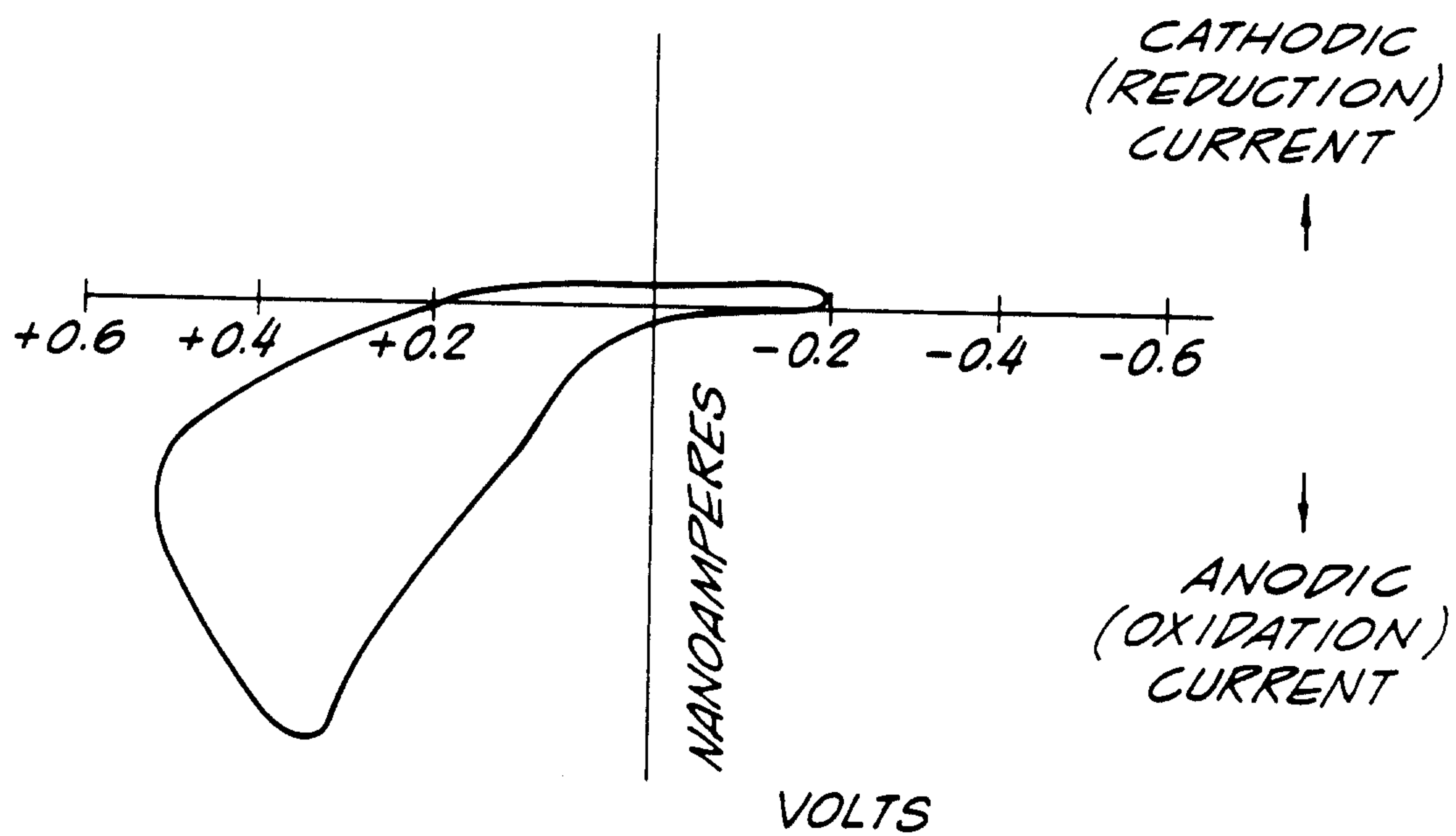
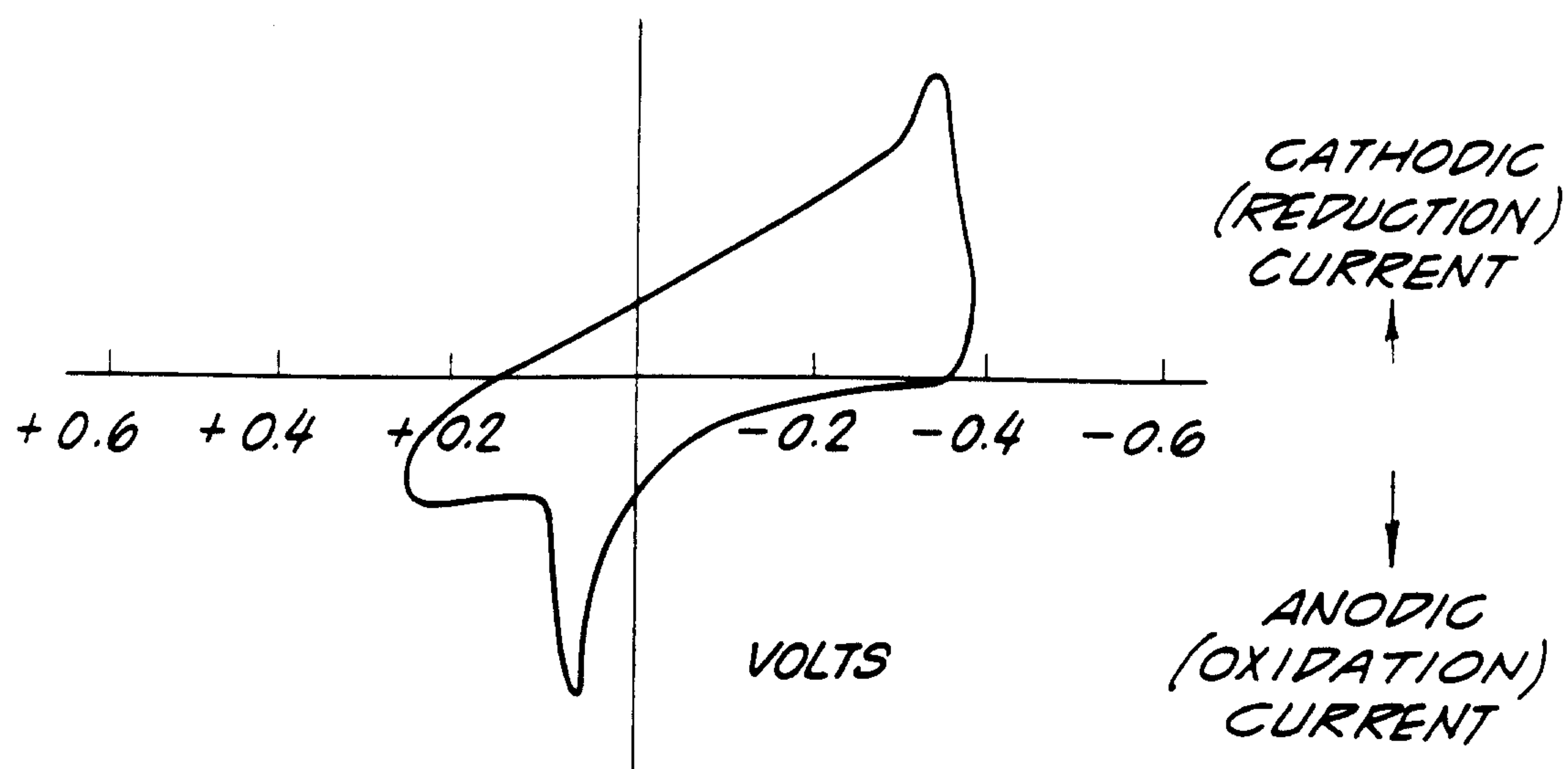
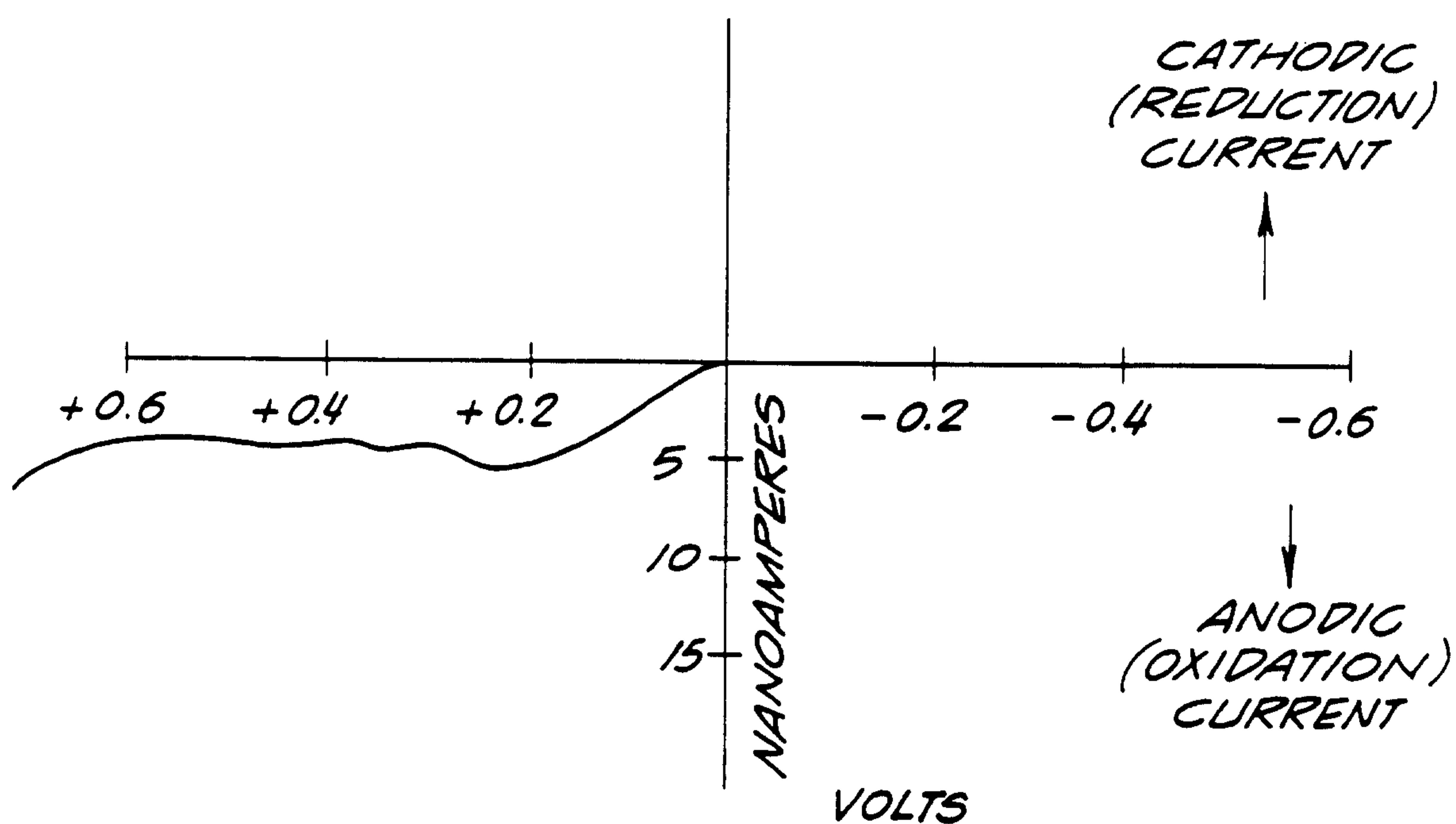


FIG. 13 PRIOR ART



WHERE +0.08 V REPRESENTS THE
OXIDIZED SPECIES OF 6OHDA AND
-0.32 V REPRESENTS THE
REDUCED SPECIES OF 6OHQ.

FIG. 16 PRIOR ART



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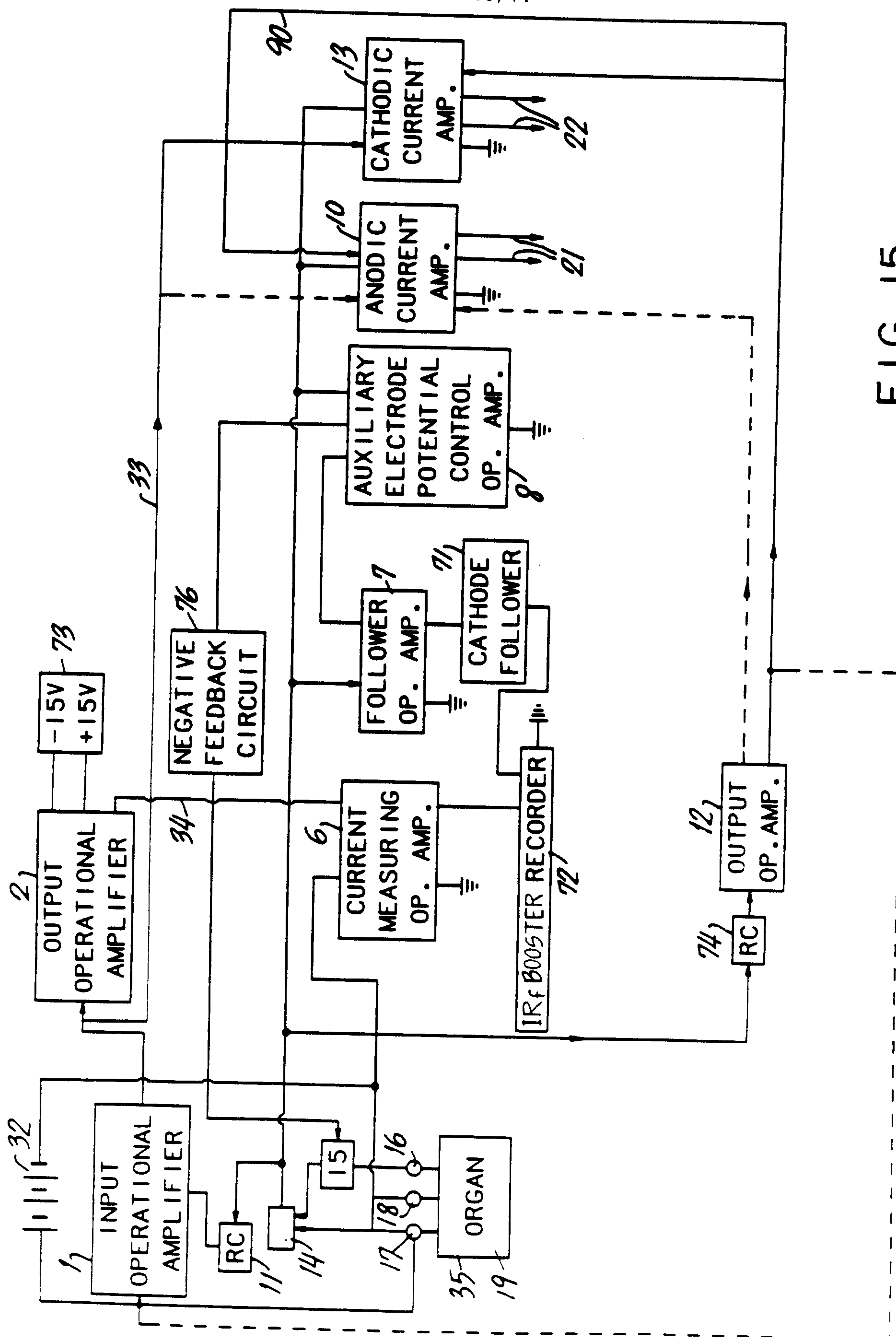
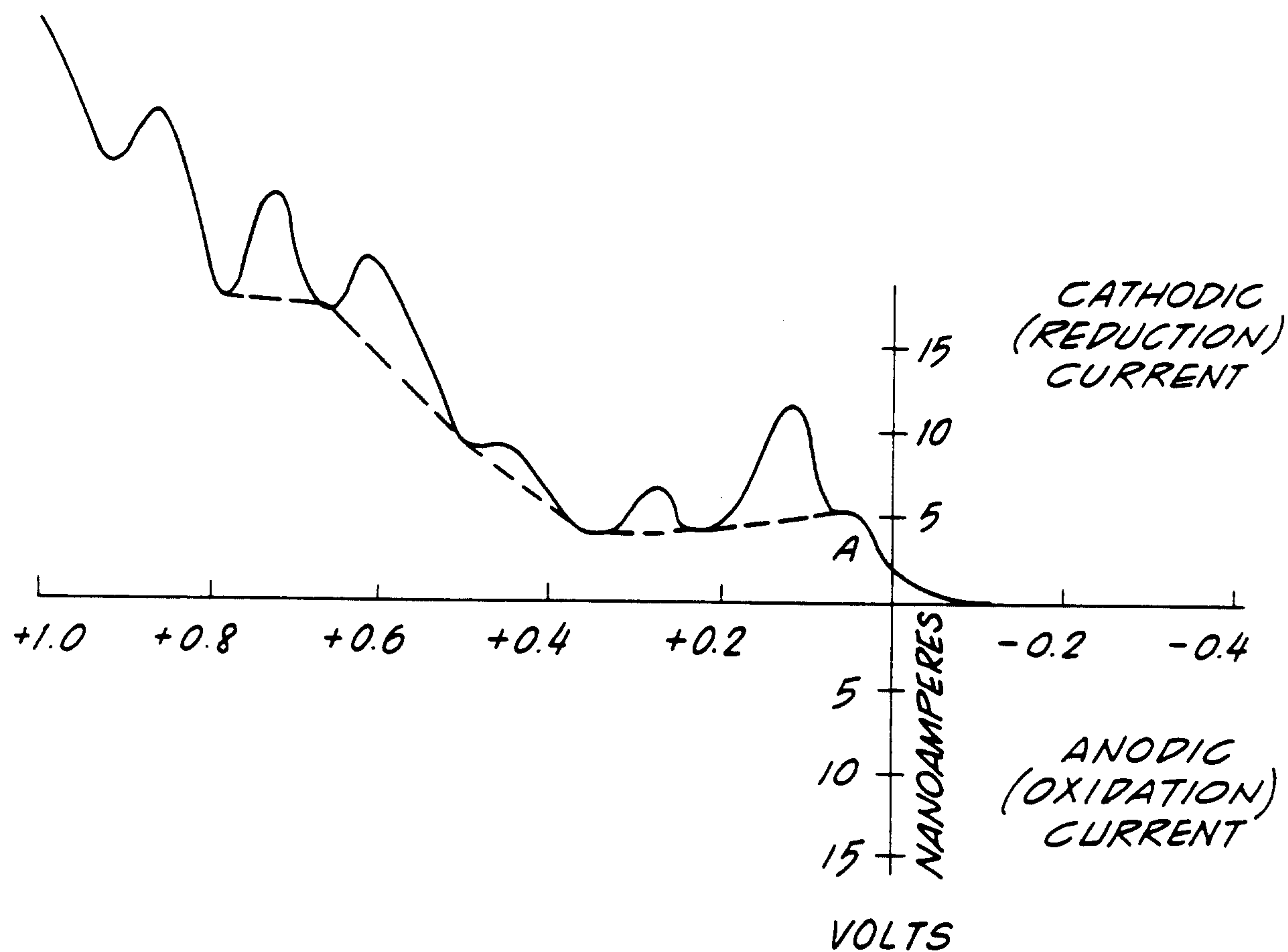


FIG. 15



*A = CHARGING CURRENT AND IS NON-FARADAIC
ie., NON-FARADAIC
MEANING NON-PHYSIOLOGICAL*

FIG. 17

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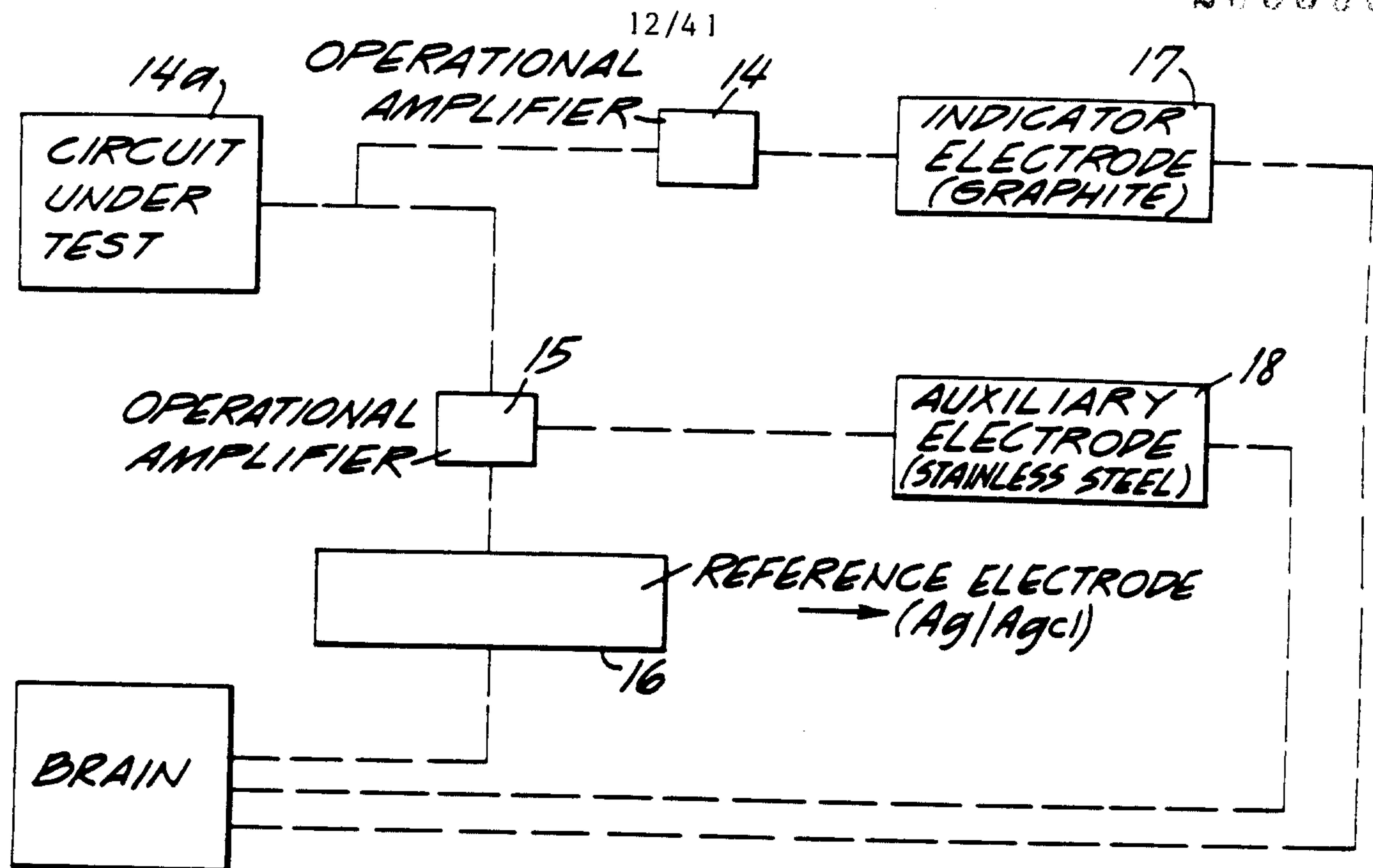


FIG. 18
PRIOR ART

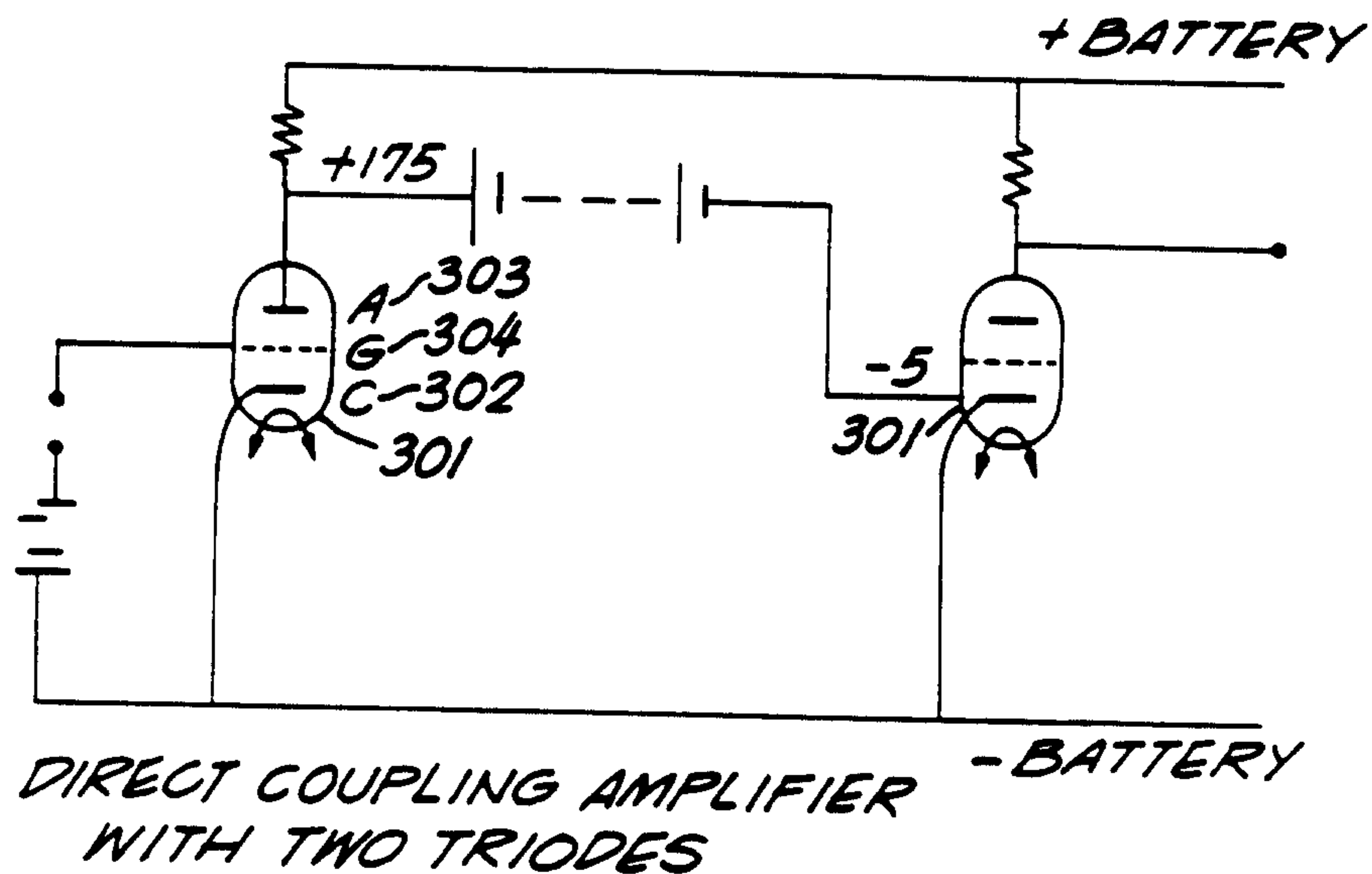


FIG. 30
PRIOR ART

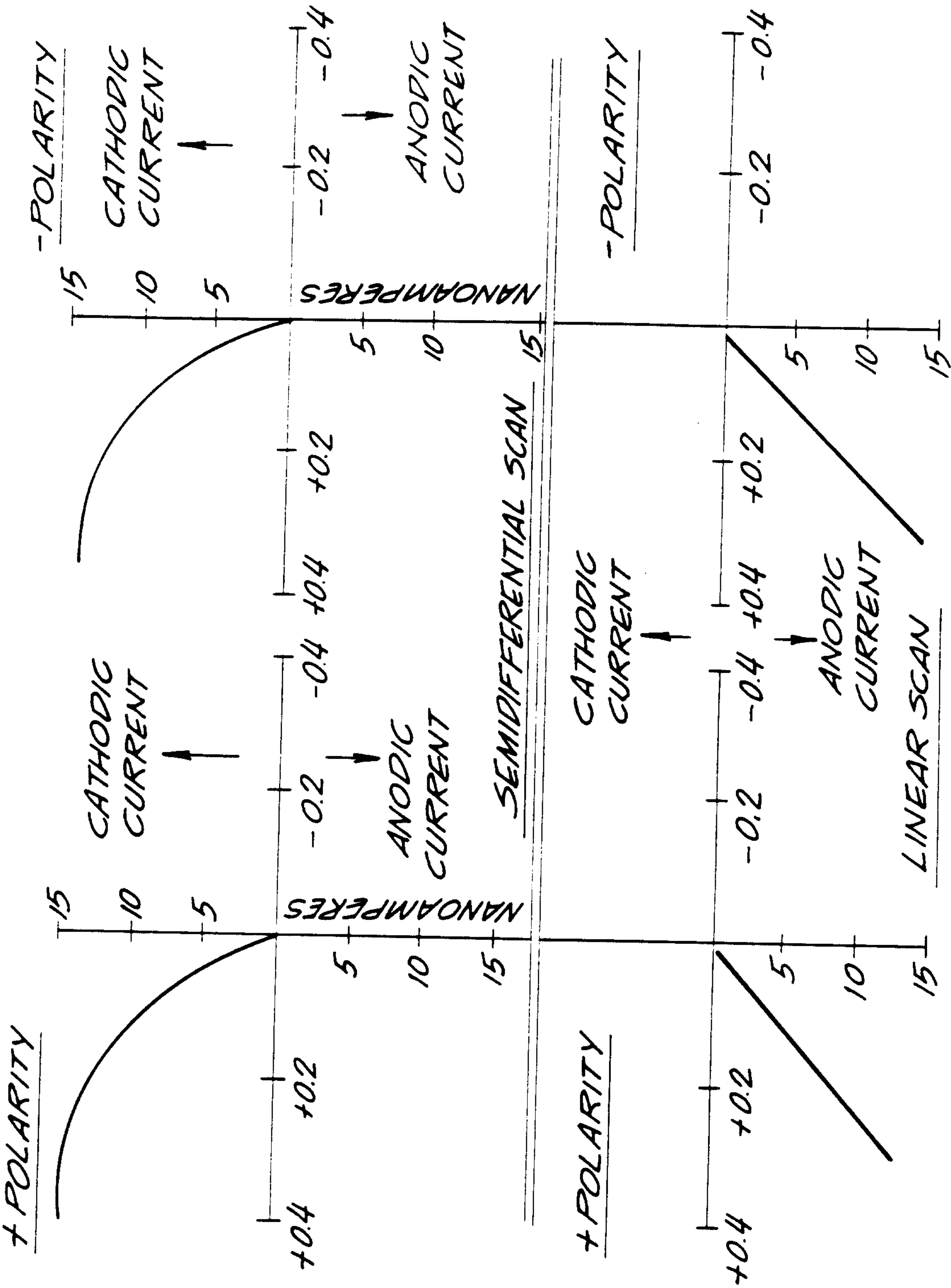


FIG. 19

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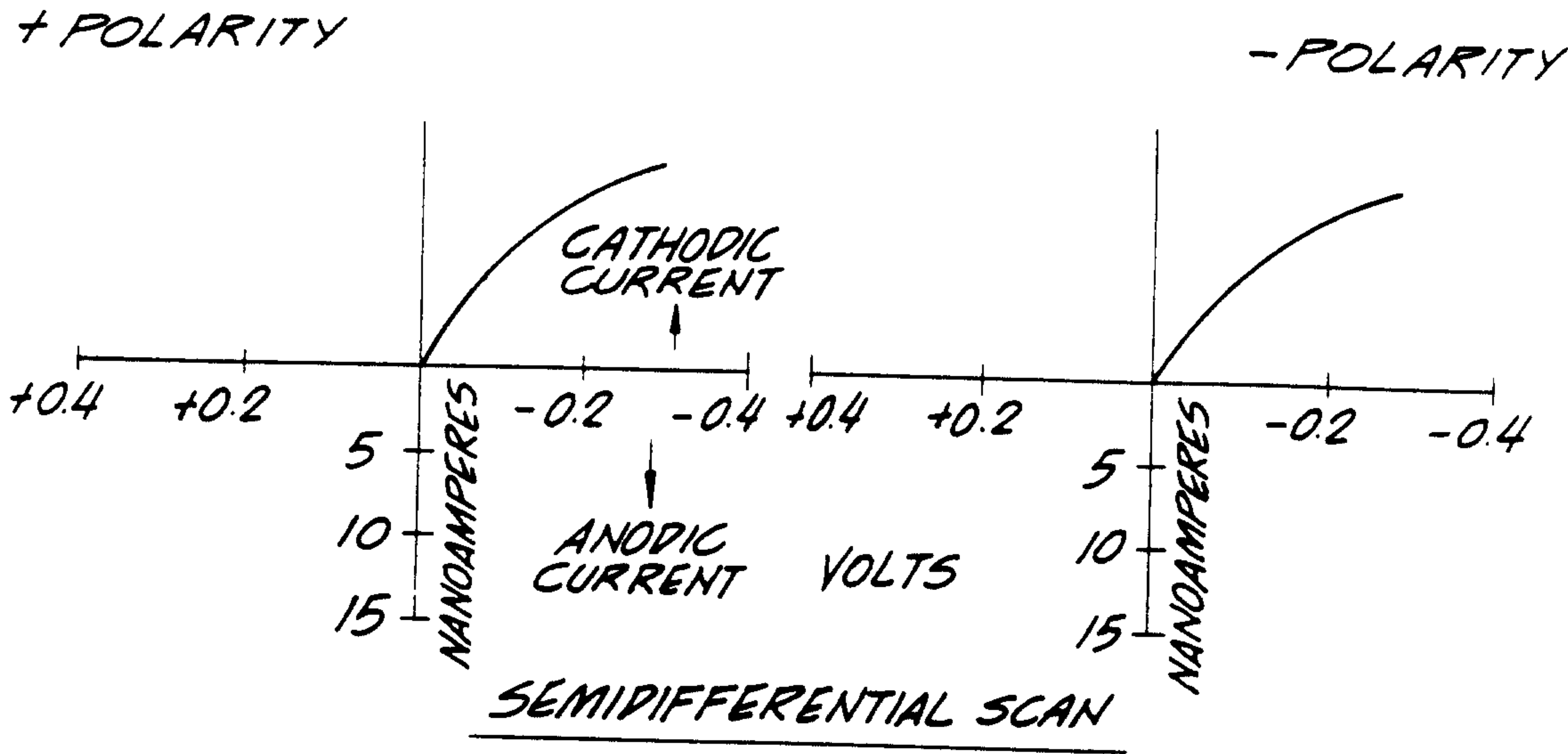


FIG. 20(a)

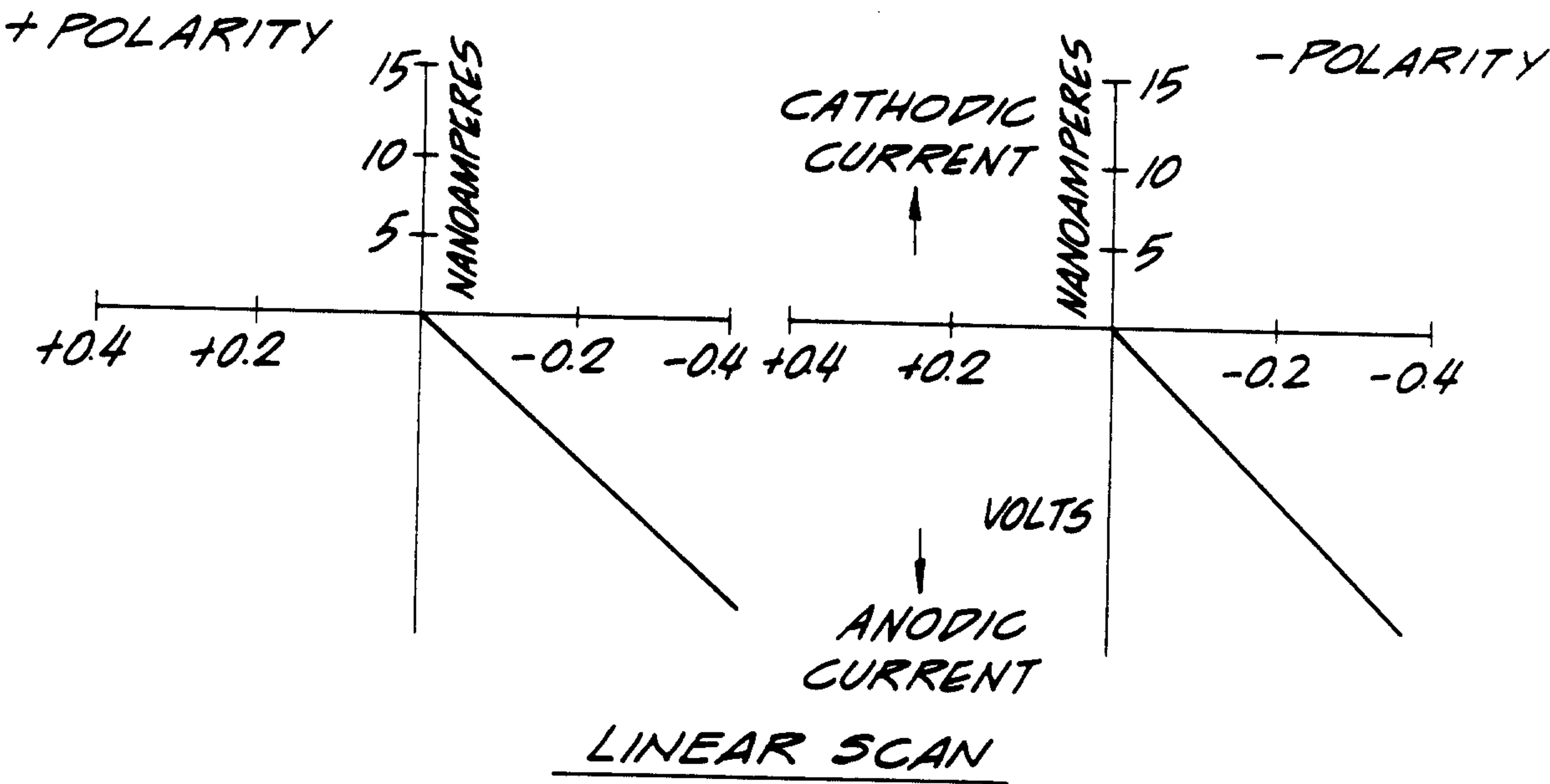


FIG. 20(b)

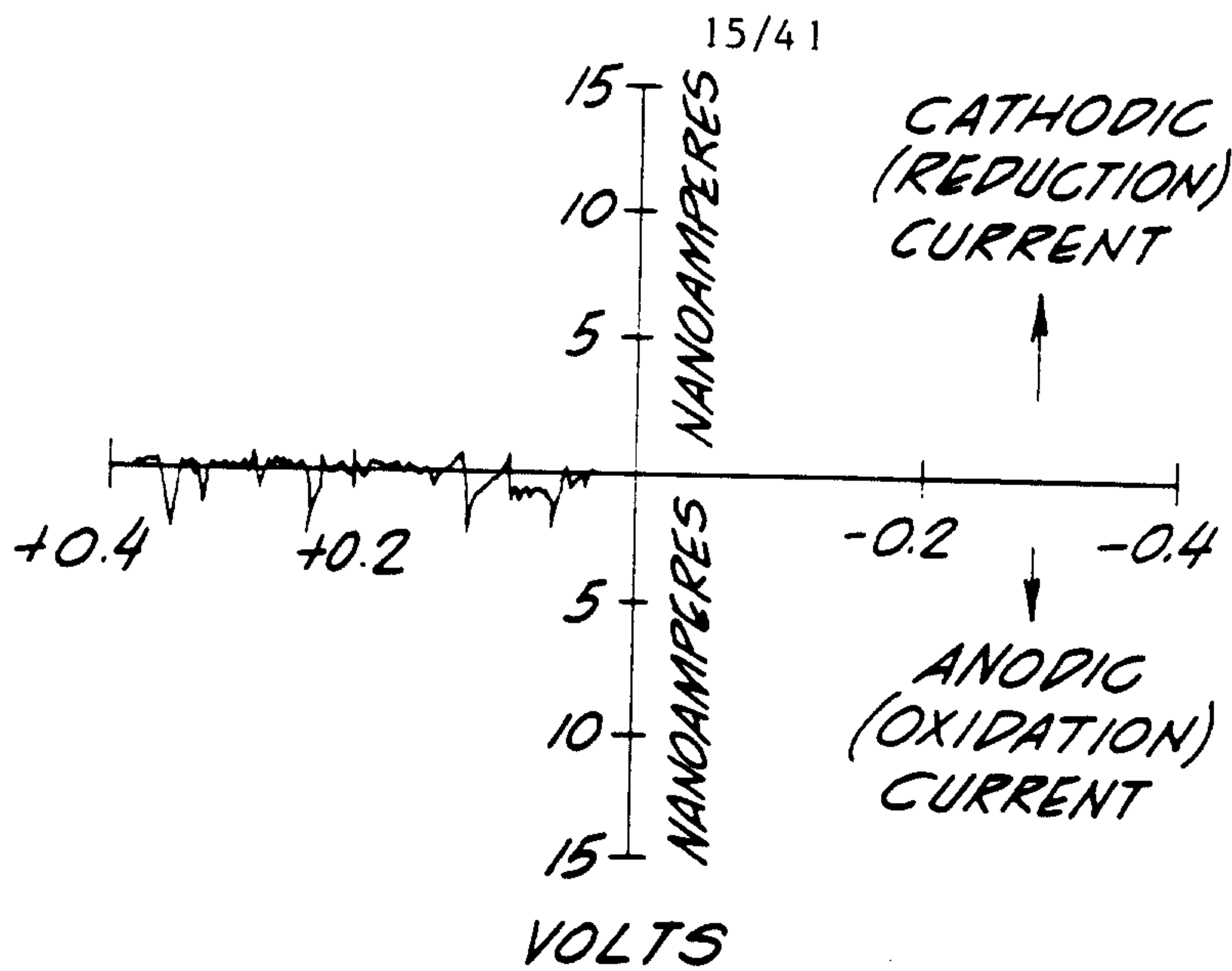


FIG. 21

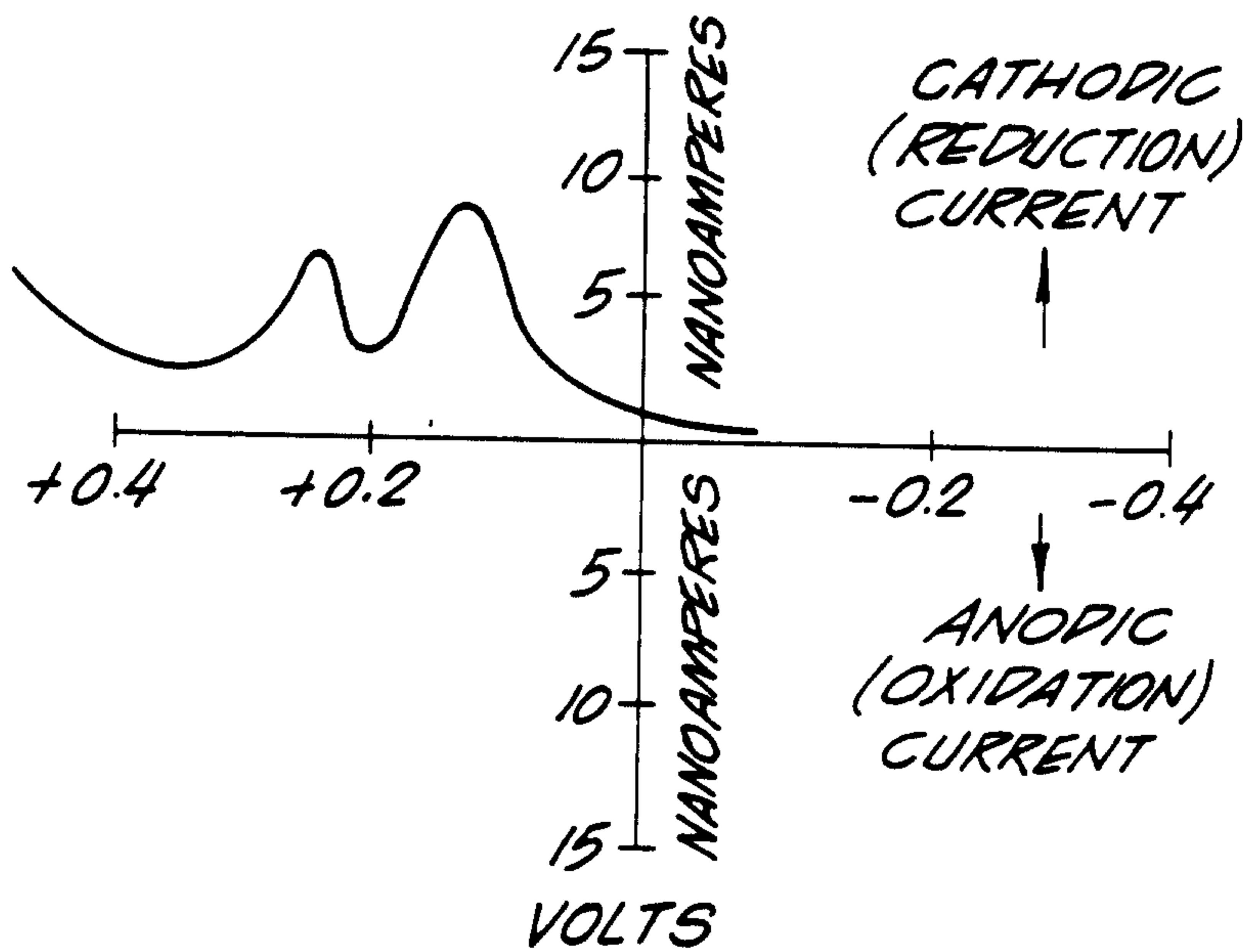


FIG. 22

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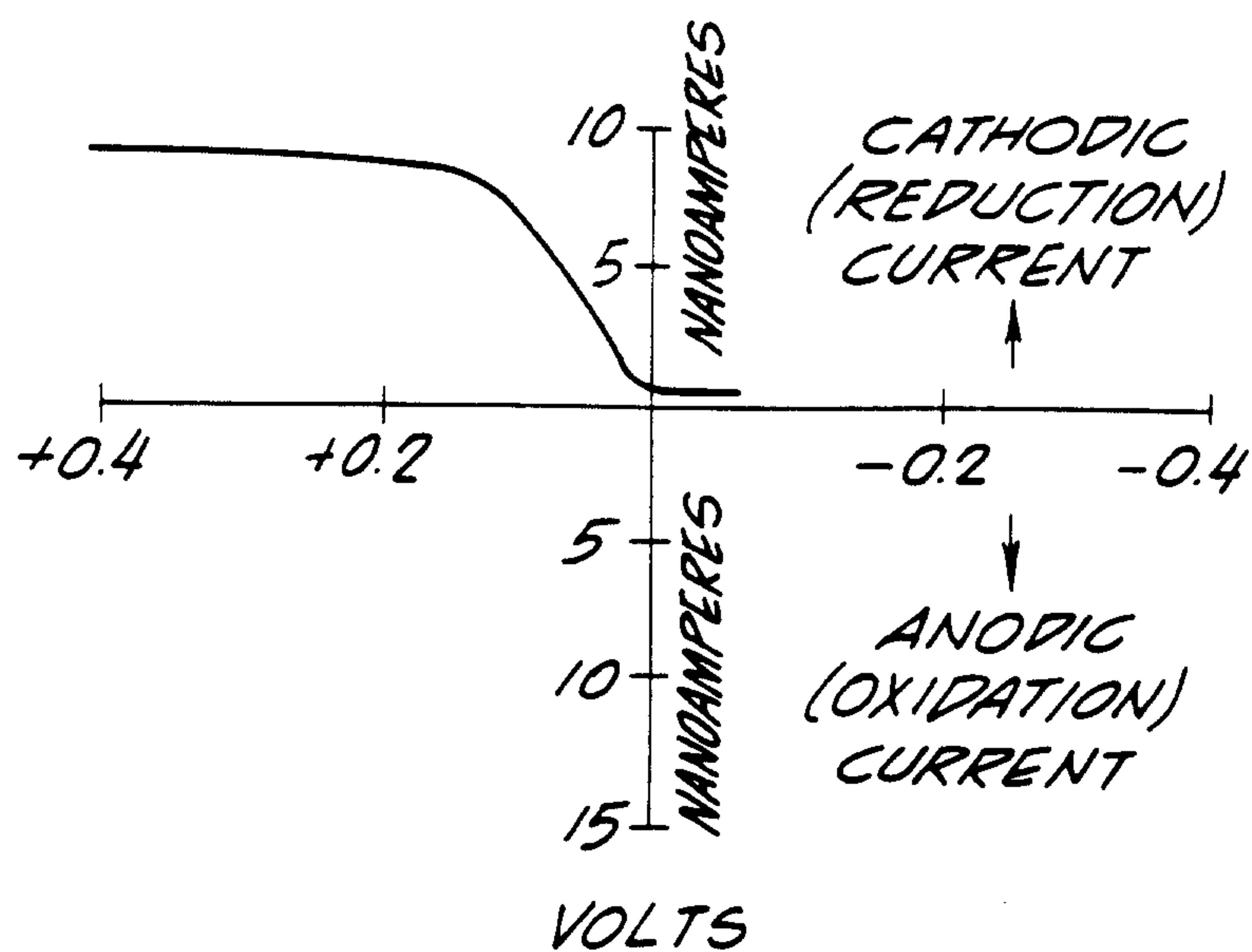


FIG. 23

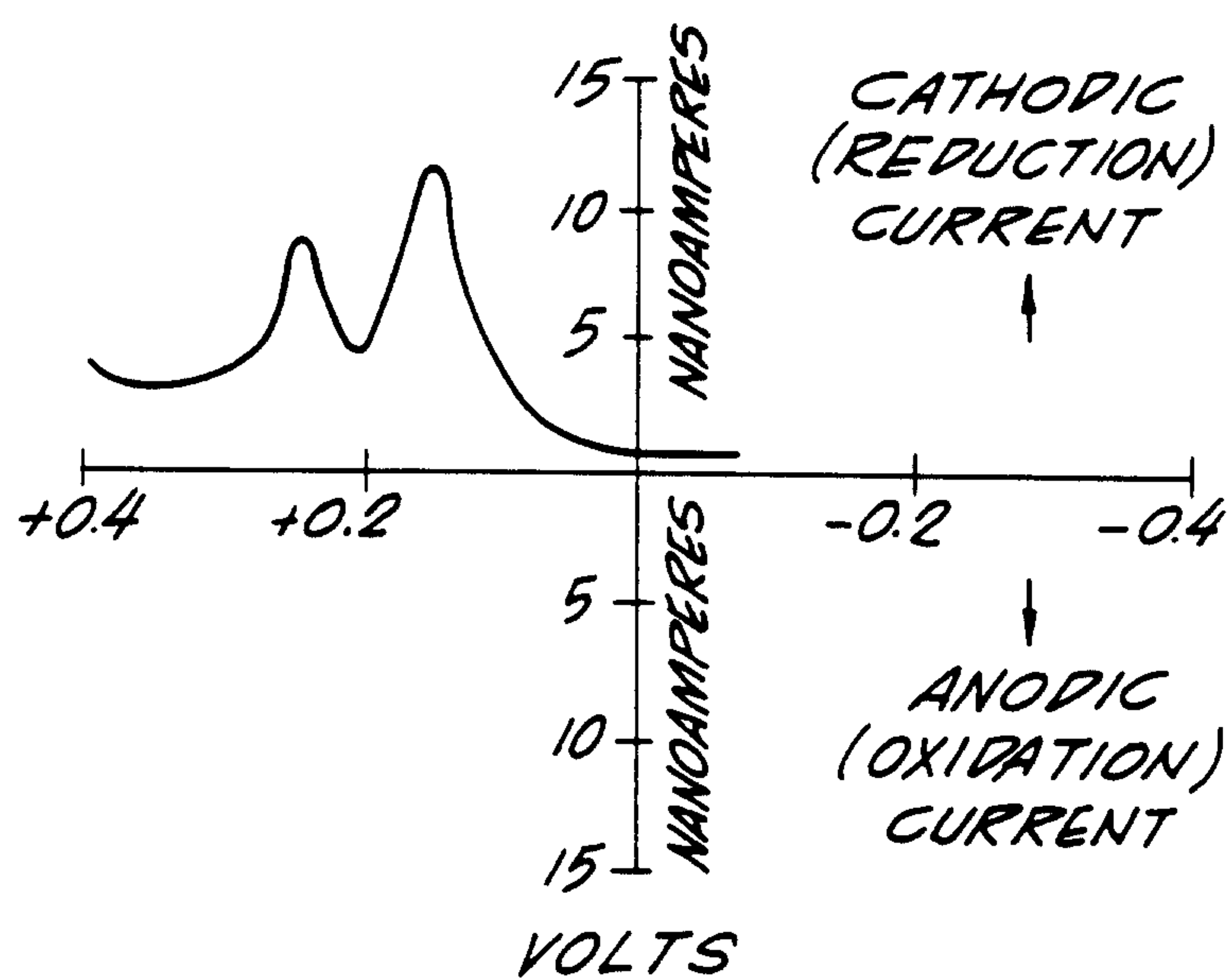


FIG. 24

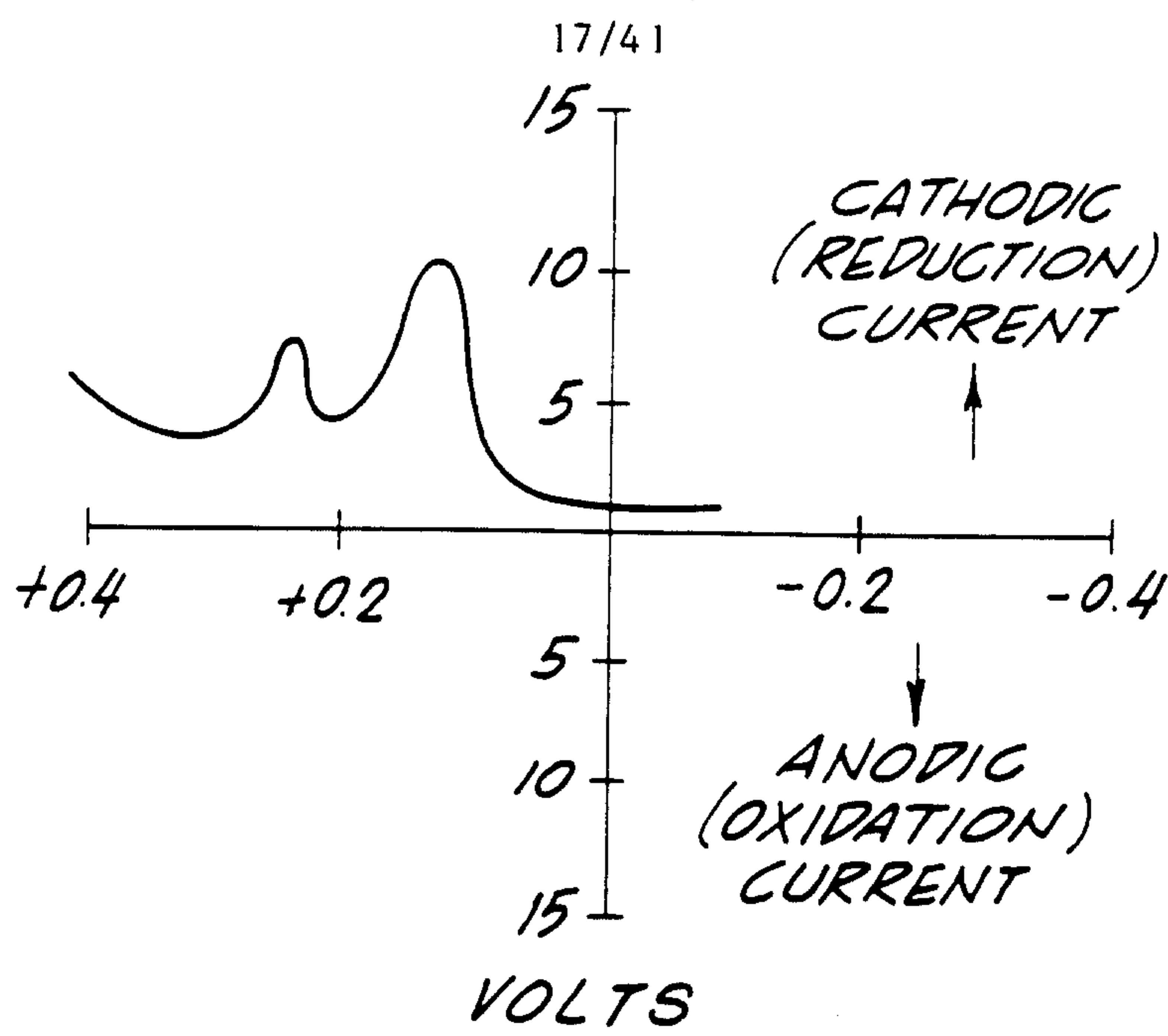


FIG. 25

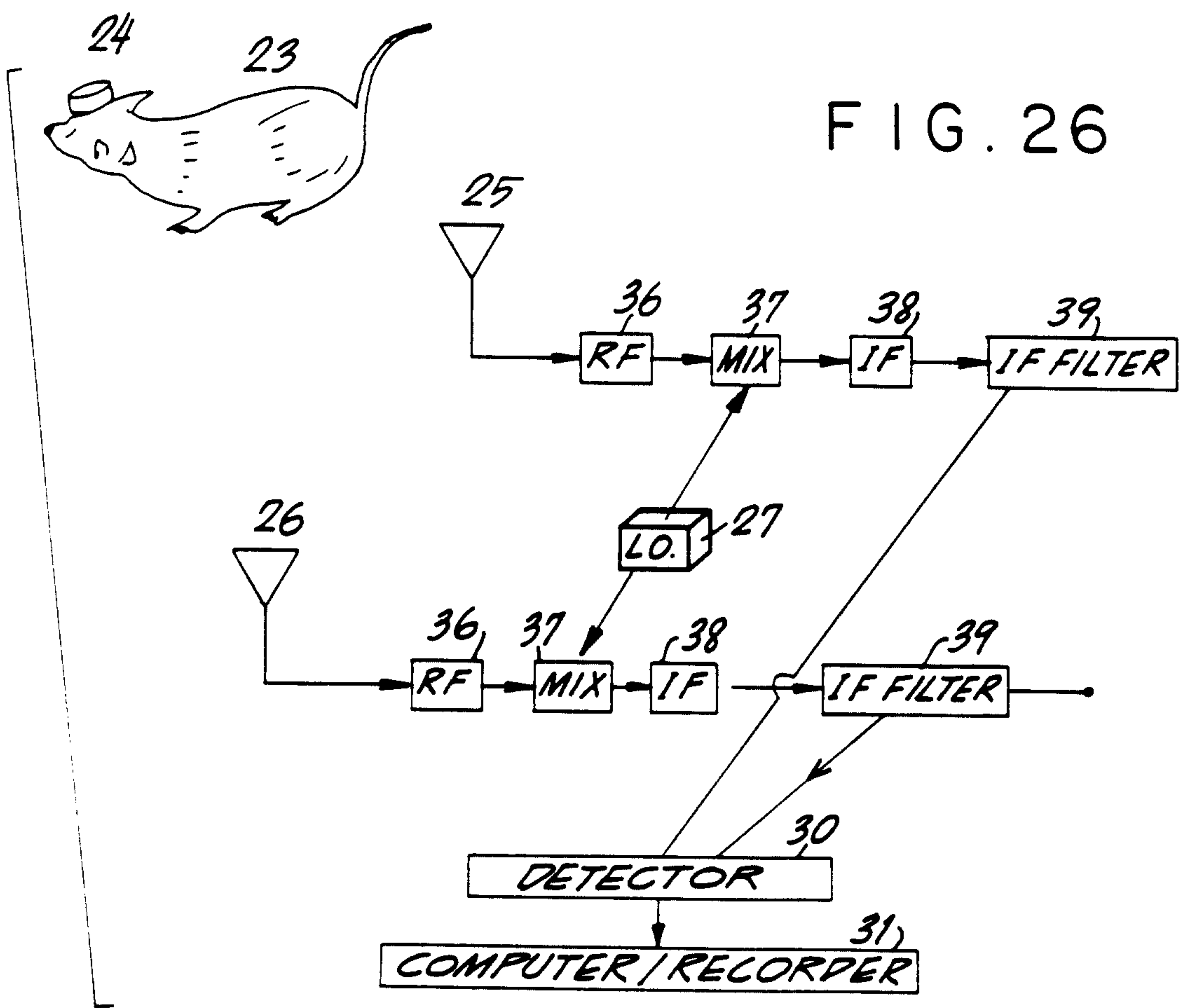


FIG. 26

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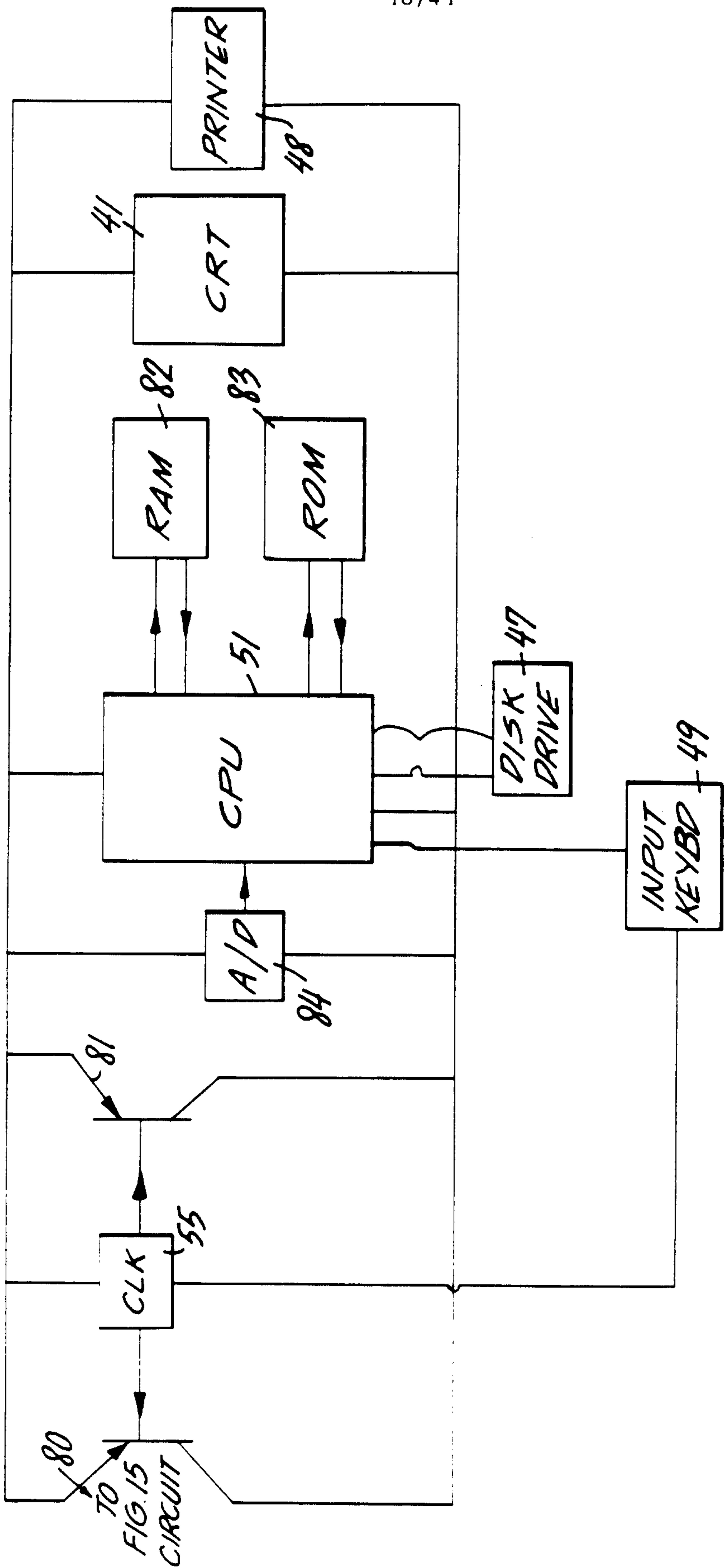


FIG. 27

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METHOD FOR CHANNEL O

1. CHROMATOCHART V1.07
2. RUN IDENTIFICATION/FILES OPTIONS
3. TITLE \$ AUTO
4. DATE \$ 9/25/85
5. TIME \$ AM
6. OPERATORS
7. CONDITIONS\$ V
8. DISK FILENAME.S,D \$ AUTO, S6, D2
9. SAVE DAT.FILE (Y:N) \$ Y
10. SAVE BAS.FILE (Y:N) \$ Y
11. SAVE PEK.FILE (Y:N) \$ Y
12. GRADIENT FILENAME.S,D\$
13. STANDARD FILENAME, S,D\$ _____
- 14.
15. DATA ACQUISITION OPTIONS
16. ADALAB SLOT (0:7) : 2
17. RAM SLOT (0:7) : 0
18. RAM ADDRESS, K (1:255): 1
19. RUN LENGTH, K (1:64): 1
20. FIRST RUN # (1:127): 13
21. # REPEAT RUNS (0:127): 0
22. AVERAGE # POINTS (1:8) # 4
23. SUM # POINTS (1:8) : 2
24. CHART DELAY (1:127) : 10
25. CHART WIDTH, 100'S (1:4) : 4
26. CHART Y SCALE (1:255) : 20
27. CHART OFFSET (-9999:9999) : -10
28. CHART HARDCOPY (Y:N) \$ Y
- 29.
30. INTEGRATION/REPORT OPTIONS
31. SMOOTHING WIDTH (3:15) : 9
32. INIT. SLOPE THRESH. (1:32767) : 10000
33. MINIMUM BASELINE WIDTH (1:99) : 70
34. INITIAL PEAK WIDTH (1:99) : 10
35. WIDTH % CHANGE (1:100) : 70
36. SHOULDER % WIDTH (1:100) : 100
37. MIN. PEAK AREA (1:32767) : 100
38. REVIEW BASELINE (Y:N) \$ N
39. TIME UNITS IN REPORT (SEC:MIN) \$ SEC
40. REPORT PRINTER SLOT #(0:7) : 1
41. CHART HARDCOPY (Y:N) \$ Y
42. CHART WIDTH, 100'S (1:4) : 2
43. CHART X SCALE (1:20) : 1
44. CHART Y SCALE (-255:255) : 20
45. CHART OFFSET (-9999:9999) : -10

FIG. 28(a)

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EVENT CONTROL SCHEDULE

EVT	SECONDS	TYPE	BIT	ON/OFF	GOTO
-----	---------	------	-----	--------	------

1	0	STATUS	1		
2	1	SWITCH	2	OFF	
3	121	SWITCH	1	OFF	
4	136	STATUS	5		
5	185	STATUS	1		
6	242	SWITCH	1	ON	
7	243	SWITCH	2	ON	
8	244	SWITCH	3	OFF	
9	245	SWITCH	3	ON	
10	400	STATUS	1		
11	401	STATUS	0		

FIG. 28(a)

SH. 2 OF 2

METHOD FOR CHANNEL 0

1. C H R O M A T O C H A R T V1.07
2. RUN IDENTIFICATION/FILES OPTIONS
3. TITLE\$ VIVO
4. DATE\$ 4/8/86
5. TIME\$ AM
6. OPERATOR \$
7. CONDITION\$ V
8. DISK FILENAME,S,D\$ VIVO,S6,D2
9. SAVE DAT.FILE (Y:N) \$ Y
10. SAVE BAS.FILE (Y:N) \$ Y
11. SAVE PEK. FILE (Y:N) \$ Y
12. GRADIENT FILENAME,S,D \$
13. STANDARD FILENAME,S,D \$ _____
- 14.
15. DATA ACQUISITION OPTIONS
16. ADALAB SLOT (0:7) : 2
17. RAM SLOT (0:7) : 0
18. RAM ADDRESS, K(1:255) : 1
19. RUN LENGTH, K (1:64) : 1
20. FIRST RUN # (1:127) : 1
21. # REPEAT RUNS (0:127) : 0
22. AVERAGE # POINTS (1:8) # 4
23. SUM # POINTS (1:8) : 2
24. CHART DELAY (1:127) : 10
25. CHART WIDTH, 100'S (1:4) : 4
26. CHART Y SCALE (1:255) : 20
27. CHART OFFSET (-9999:9999) : -10
28. CHART HARDCOPY (Y:N)\$ Y
- 29.

FIG. 28(b)

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- 30. INTEGRATION/ REPORT OPTIONS
- 31. SMOOTHING WIDTH (3:15) : 5
- 32. INIT. SLOPE THRESH. (1:32767) : 500
- 33. MINIMUM BASELINE WIDTH (1:99) : 1
- 34. INITIAL PEAK WIDTH (1:99) : 80
- 35. WIDTH % CHANGE (1:100) : 70
- 36. SHOULDER % WIDTH (1:100) : 80
- 37. MIN. PEAK AREA (1:32767) : 100
- 38. REVIEW BASELINE (Y:N)\$ N
- 39. TIME UNITS IN REPORT (SEC:MIN) \$ SEC
- 40. REPORT PRINTER SLOT #(0:7) : 1
- 41. CHART HARDCOPY (Y:N)\$ Y
- 42. CHART WIDTH, 100'S (1:4) : 2
- 43. CHART X SCALE (1:20) : 1
- 44. CHART Y SCALE (-255:255) : 20
- 45. CHART OFFSET (-9999:9999) : -10

EVENT CONTROL SCHEDULE

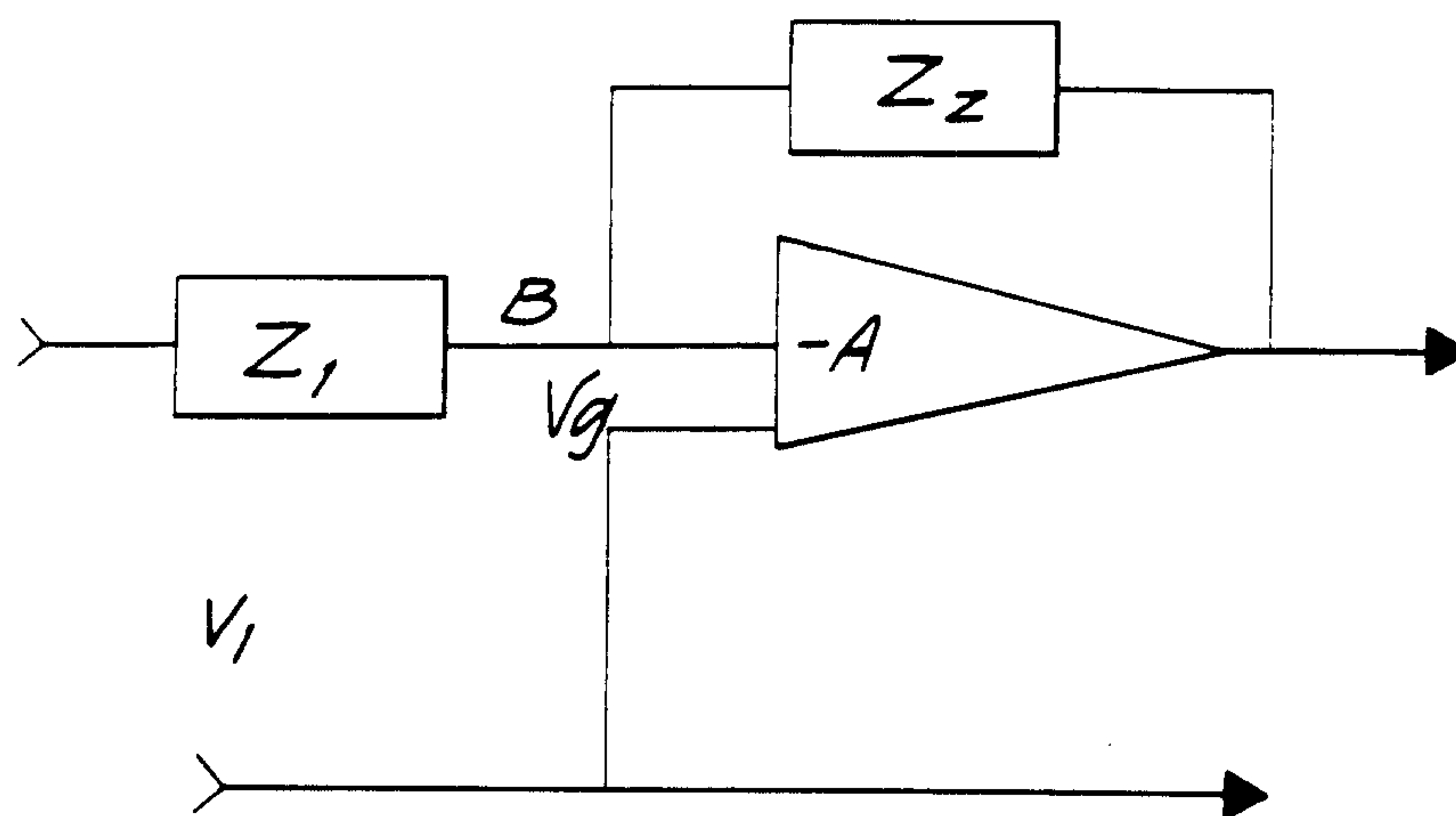
EVT	SECONDS	TYPE	BIT	ON/OFF	GOTO
1	0	STATUS	1		
2	1	SWITCH	2	OFF	
3	121	SWITCH	1	OFF	
4	136	STATUS	5		
5	185	STATUS	1		
6	242	SWITCH	1	ON	
7	243	SWITCH	2	ON	
8	244	SWITCH	3	OFF	
9	245	SWITCH	3	ON	
10	400	STATUS	1		
11	401	STATUS	0		

FIG. 28(b)

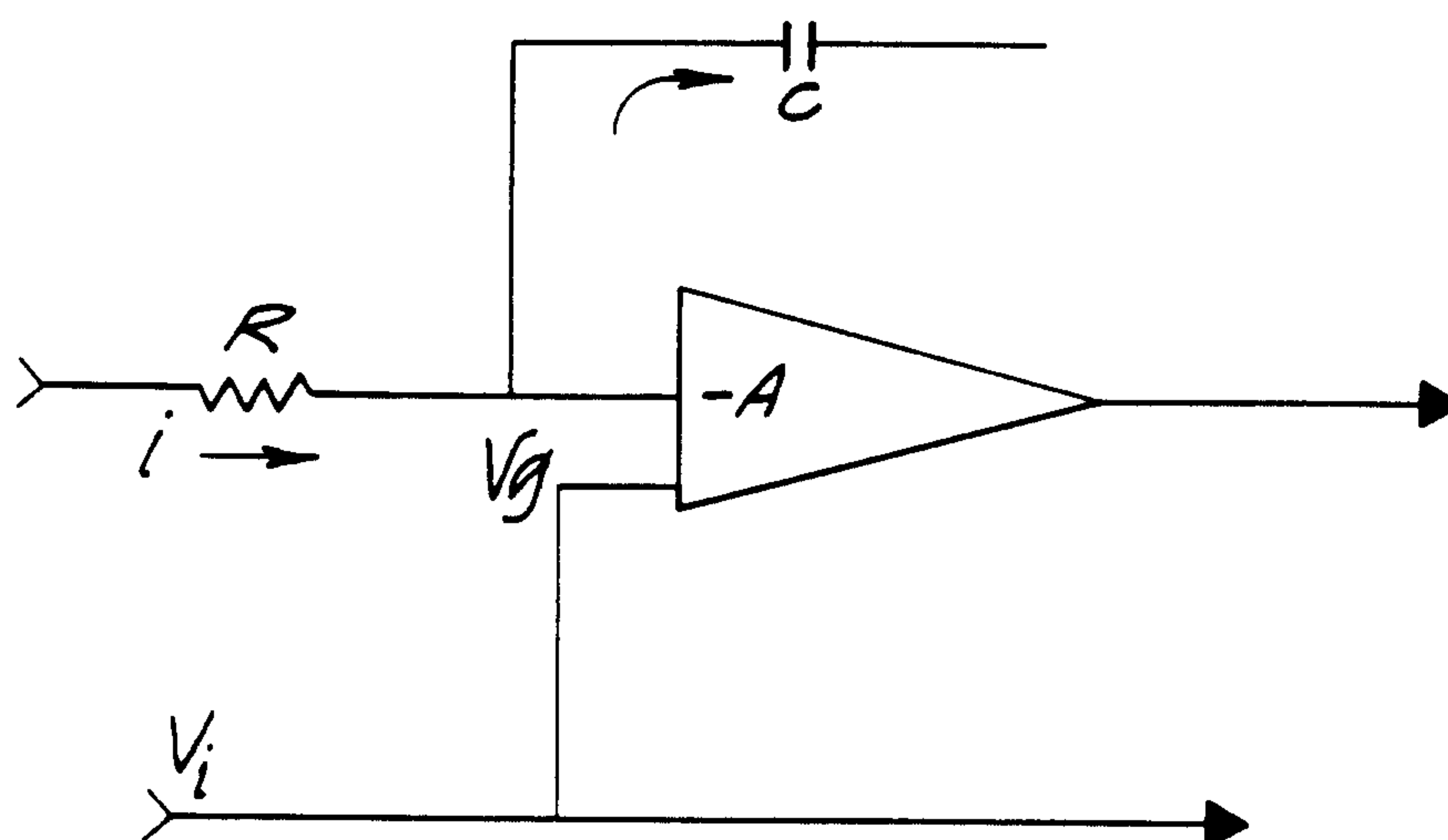
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THE BASIC OPERATIONAL AMPLIFIER CIRCUIT

$$V_o = -\frac{Z_2}{Z_1} V_i$$

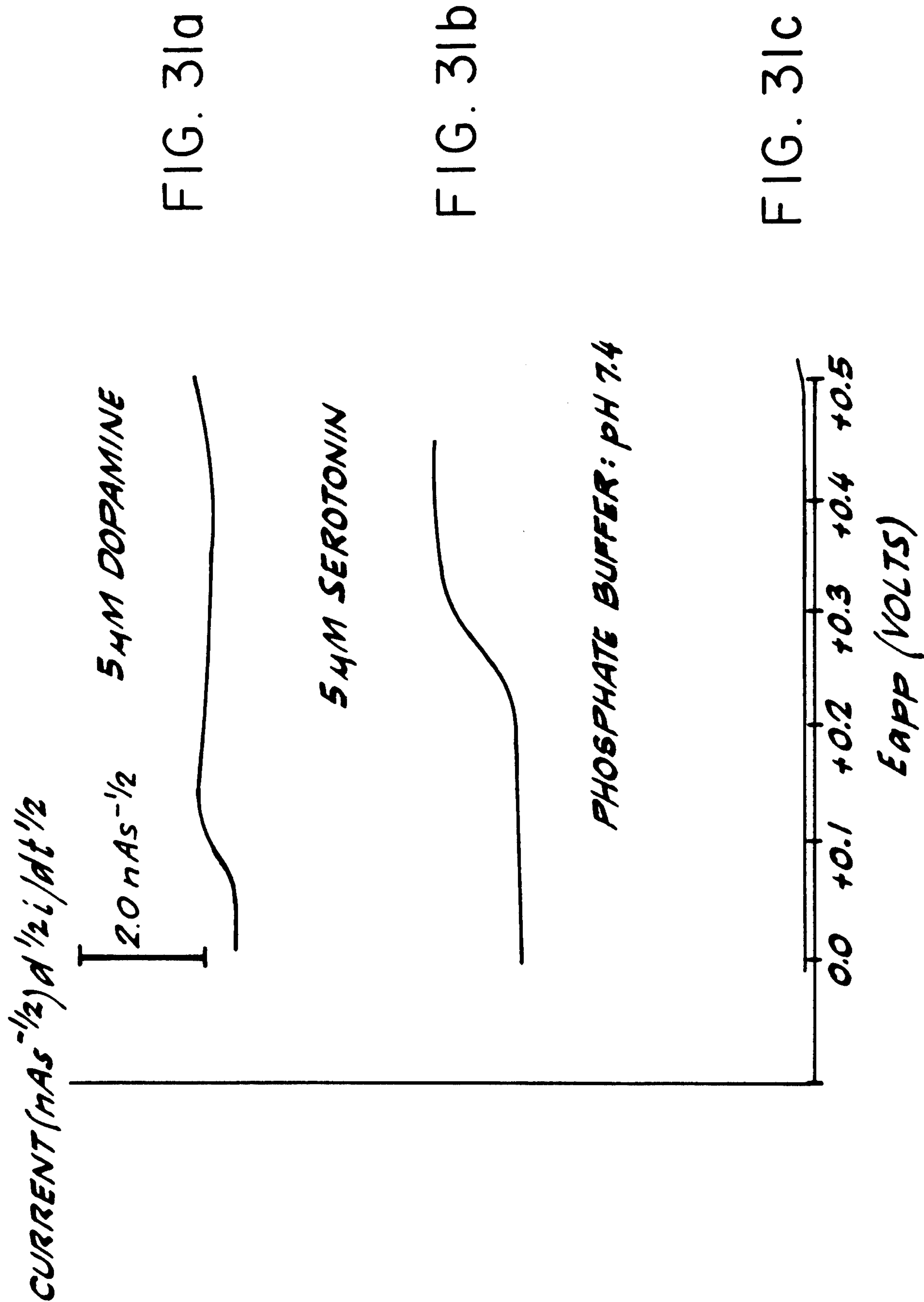
GENERALIZED OPERATIONAL AMPLIFIER CIRCUIT.

$$V_o = -\frac{1}{RC} \int V_i dt$$

AN OPERATIONAL AMPLIFIER INTEGRATOR

FIG. 29

(PRIOR ART)



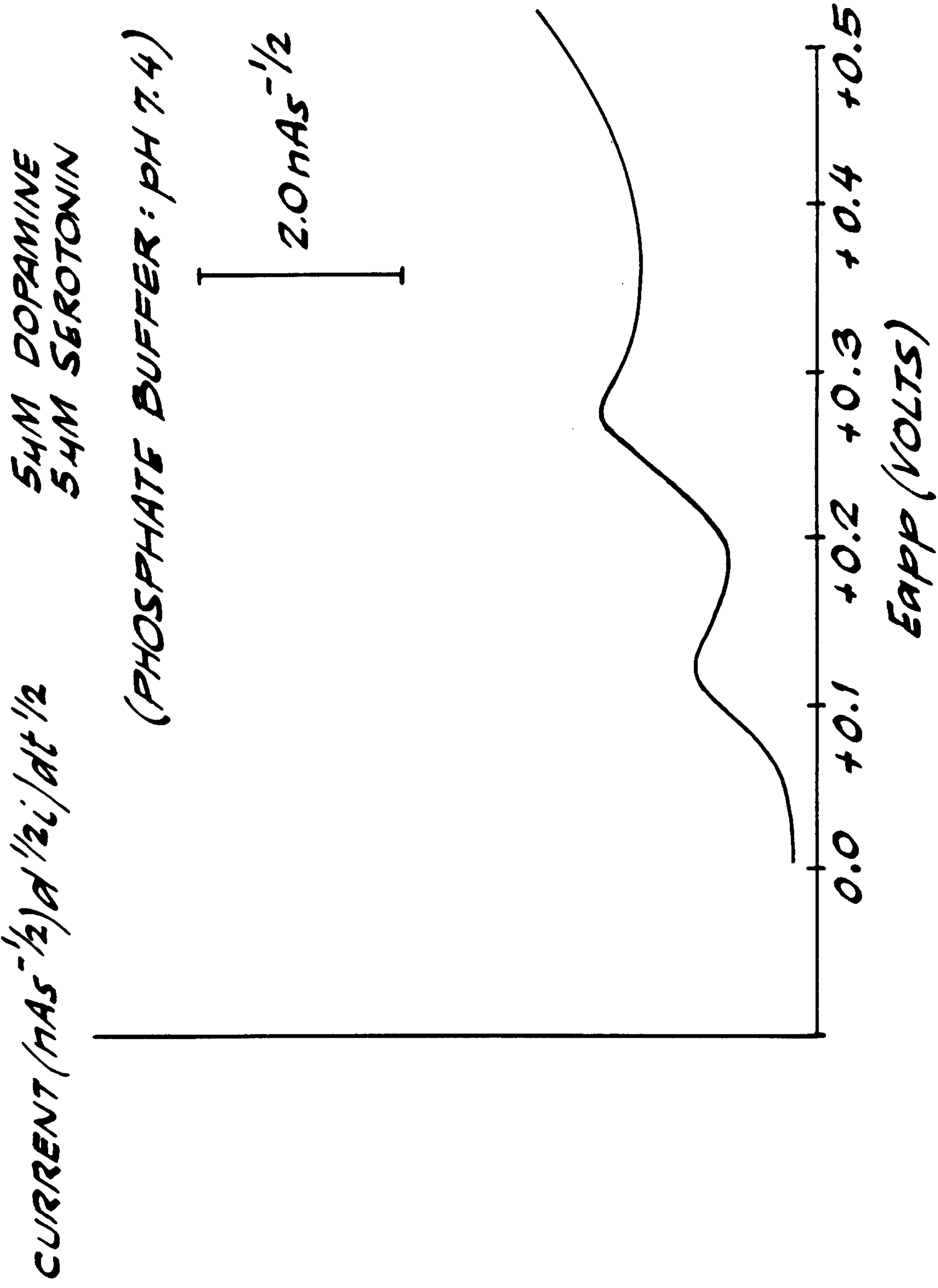
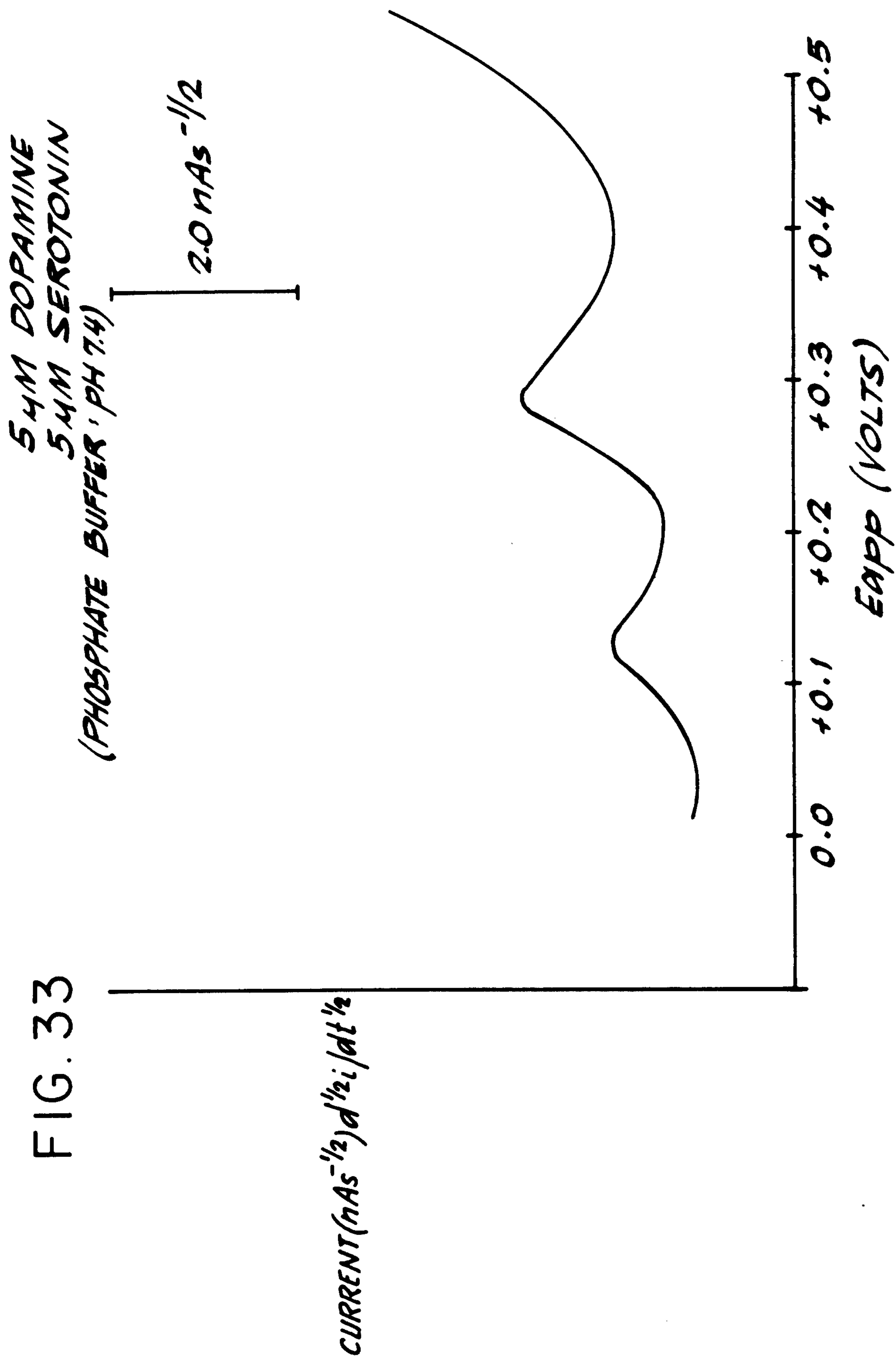
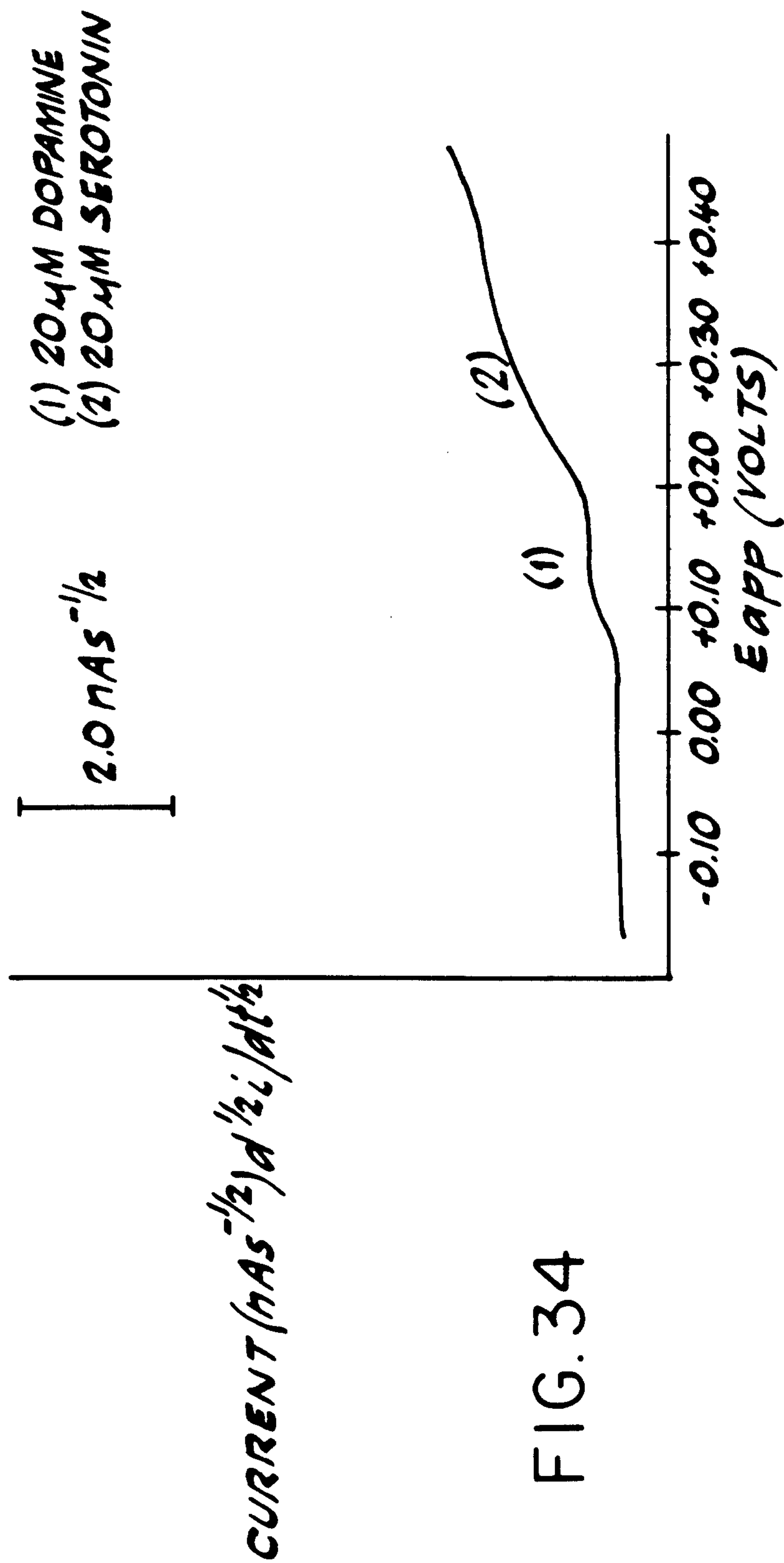


FIG. 32





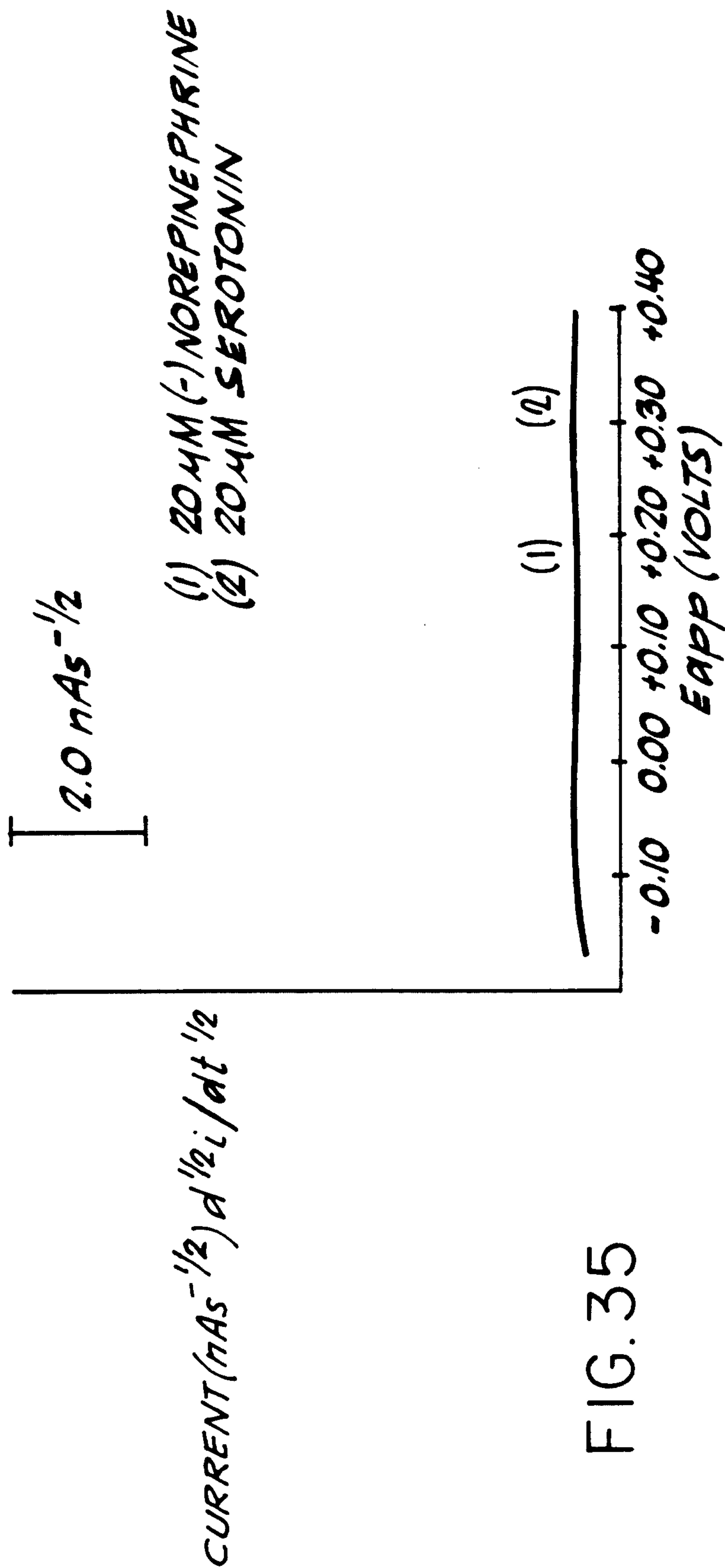


FIG. 35

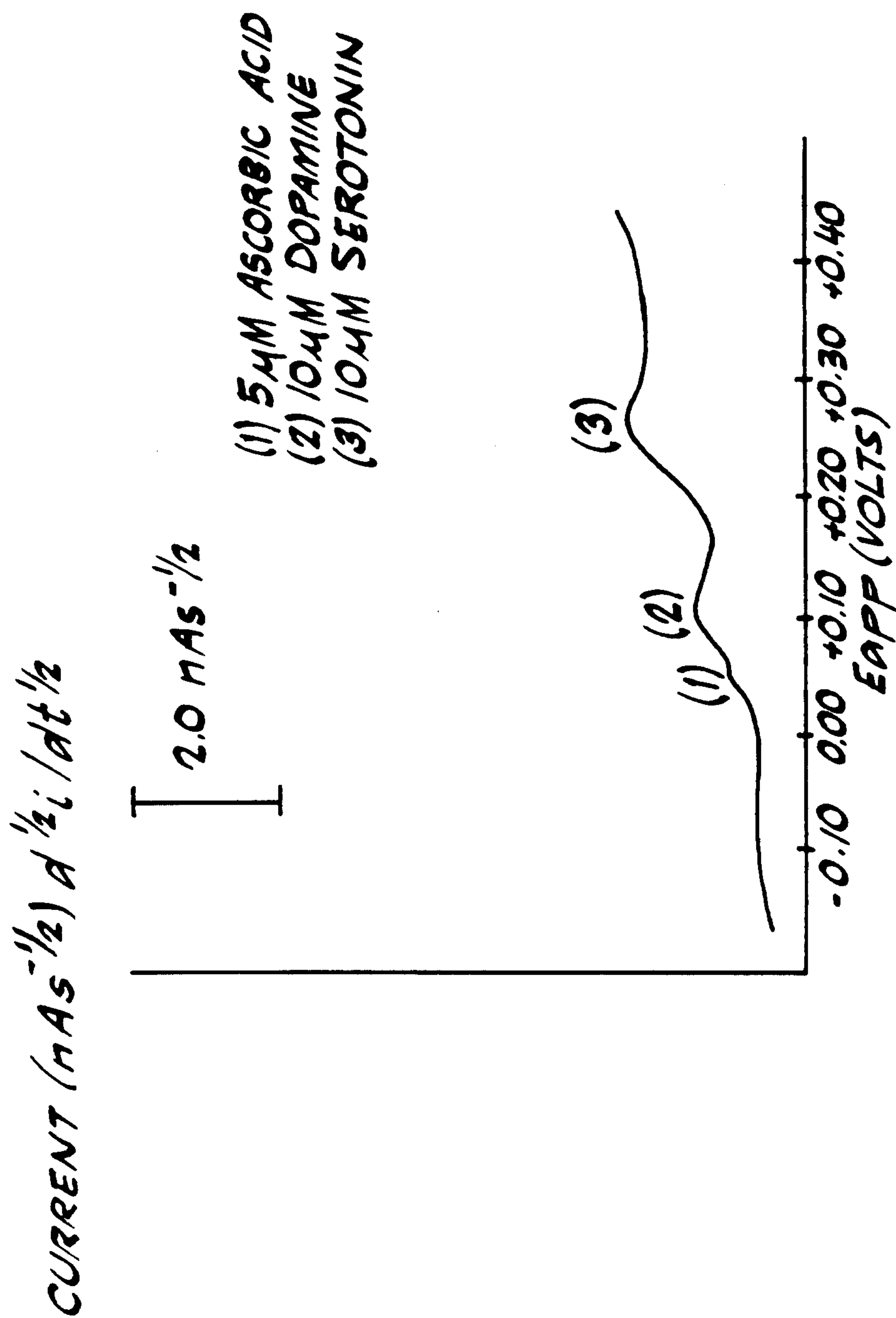


FIG. 36

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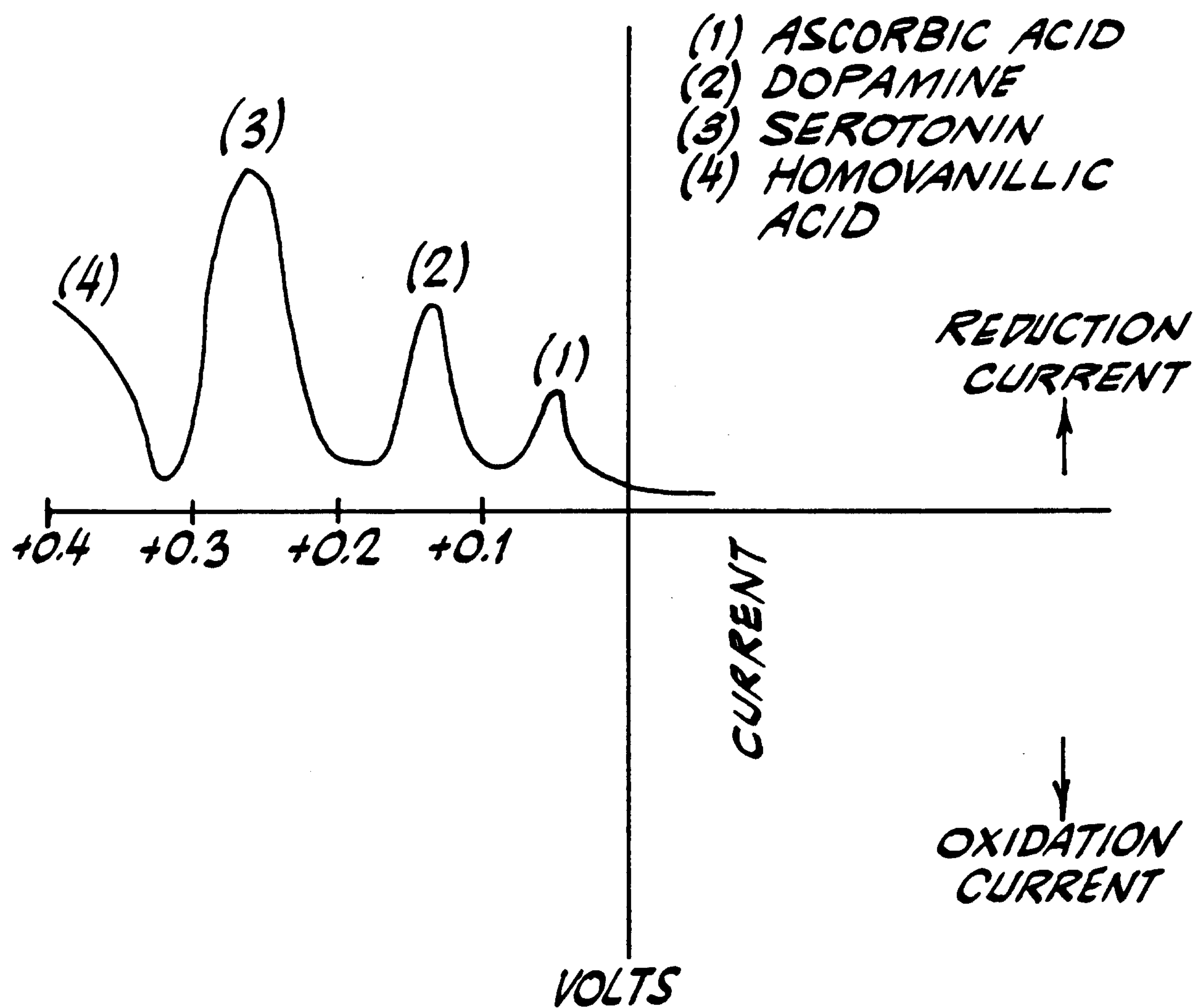
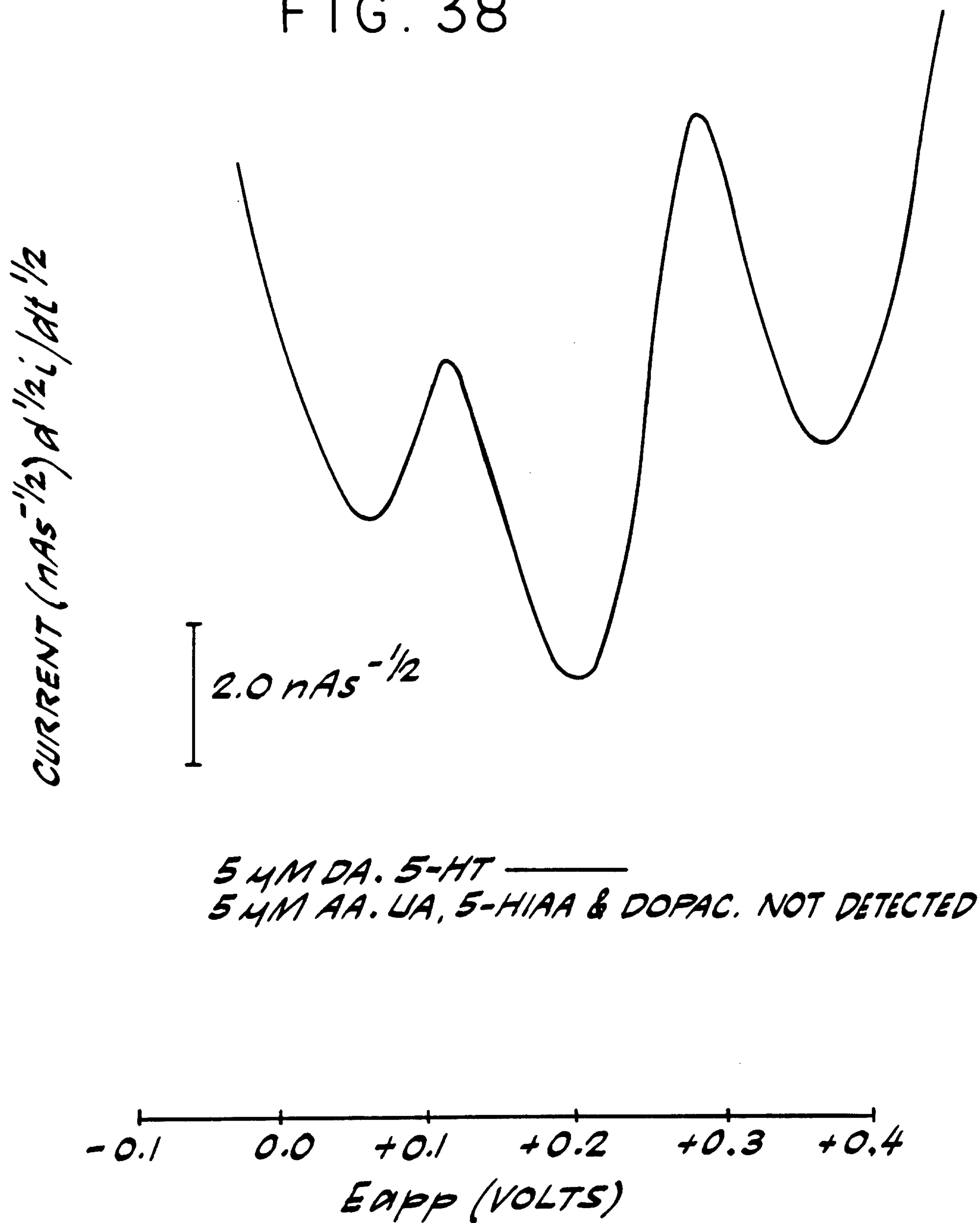


FIG. 37

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FIG. 38



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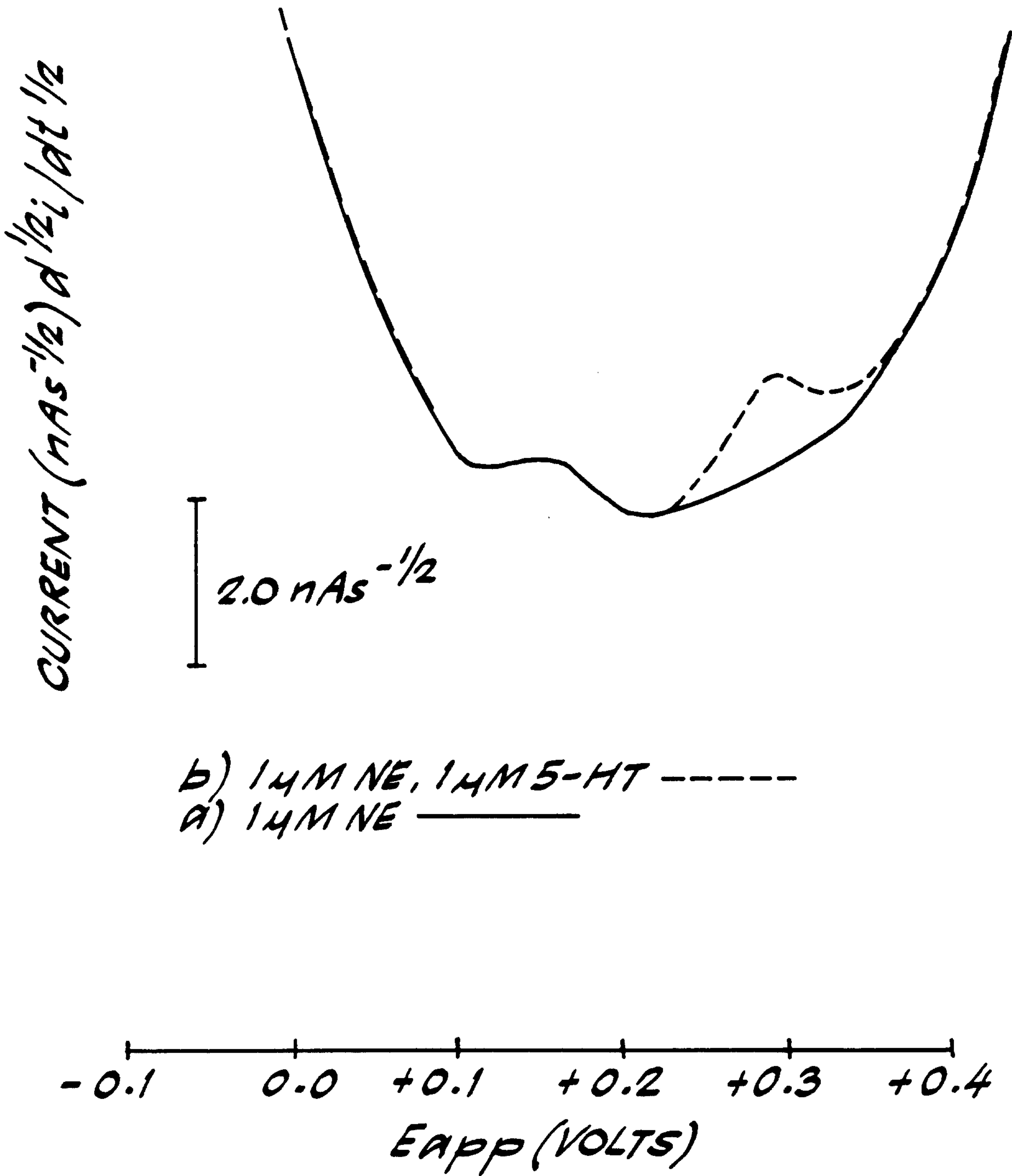


FIG. 39

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FIG. 40

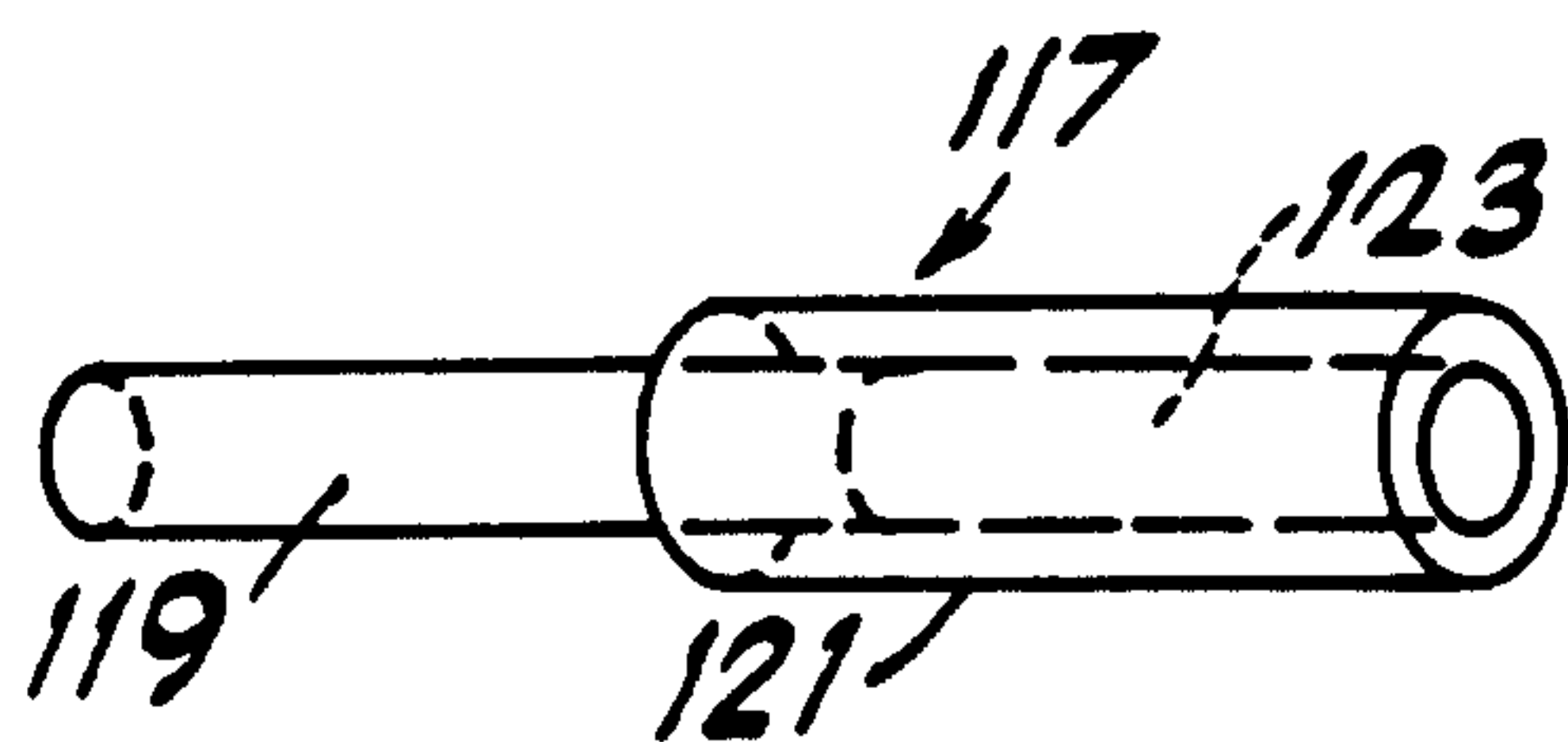
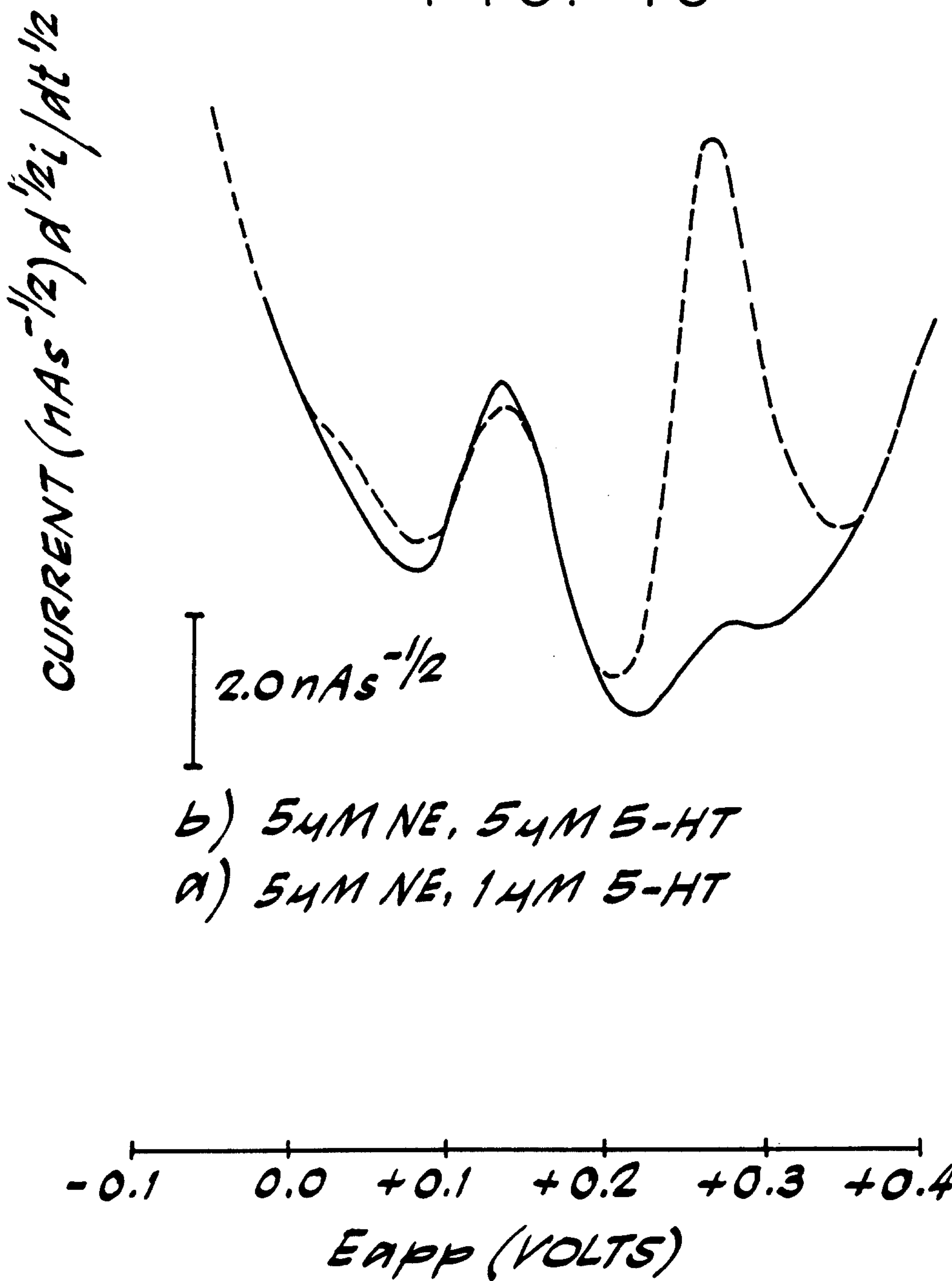


FIG. 44

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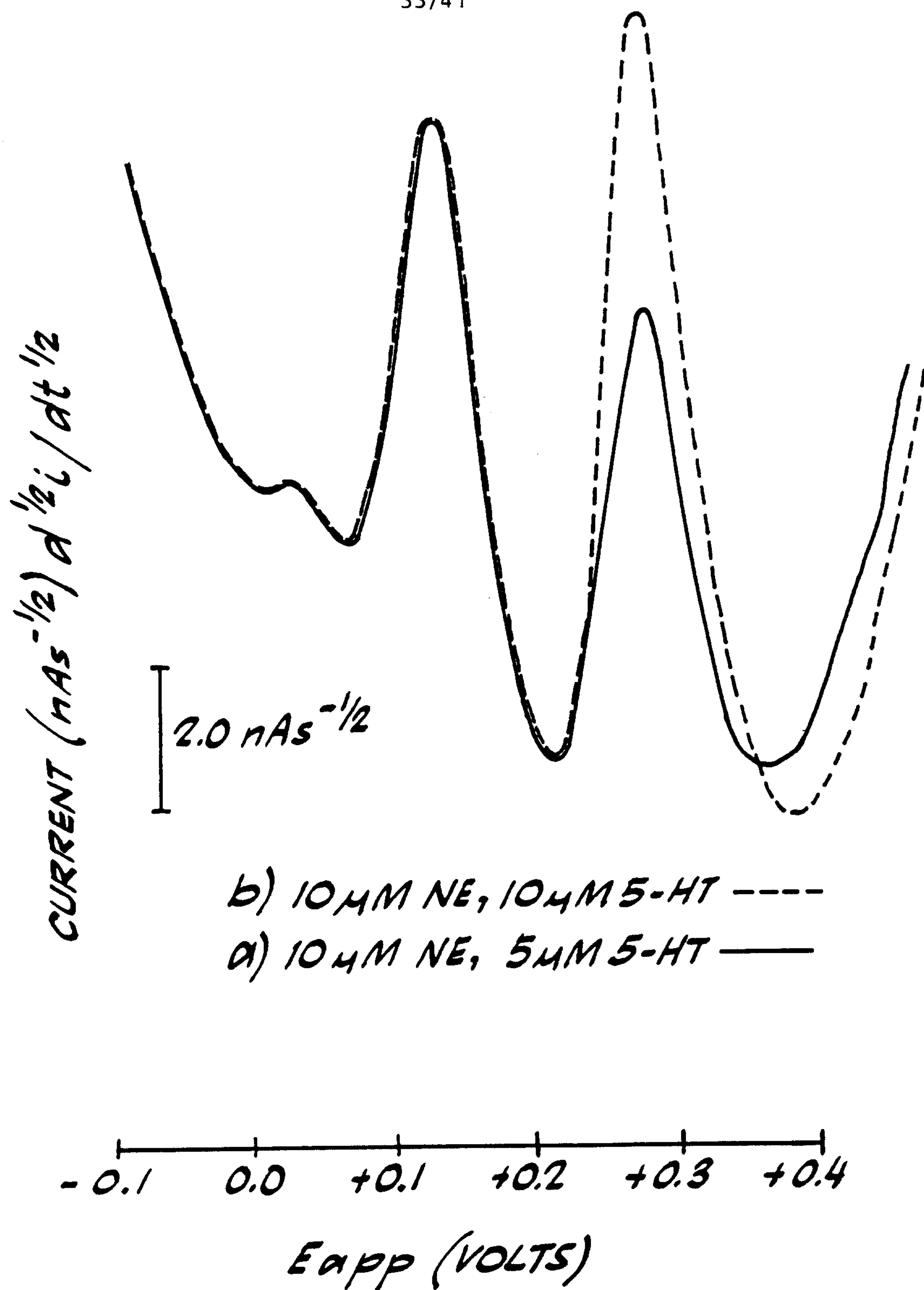
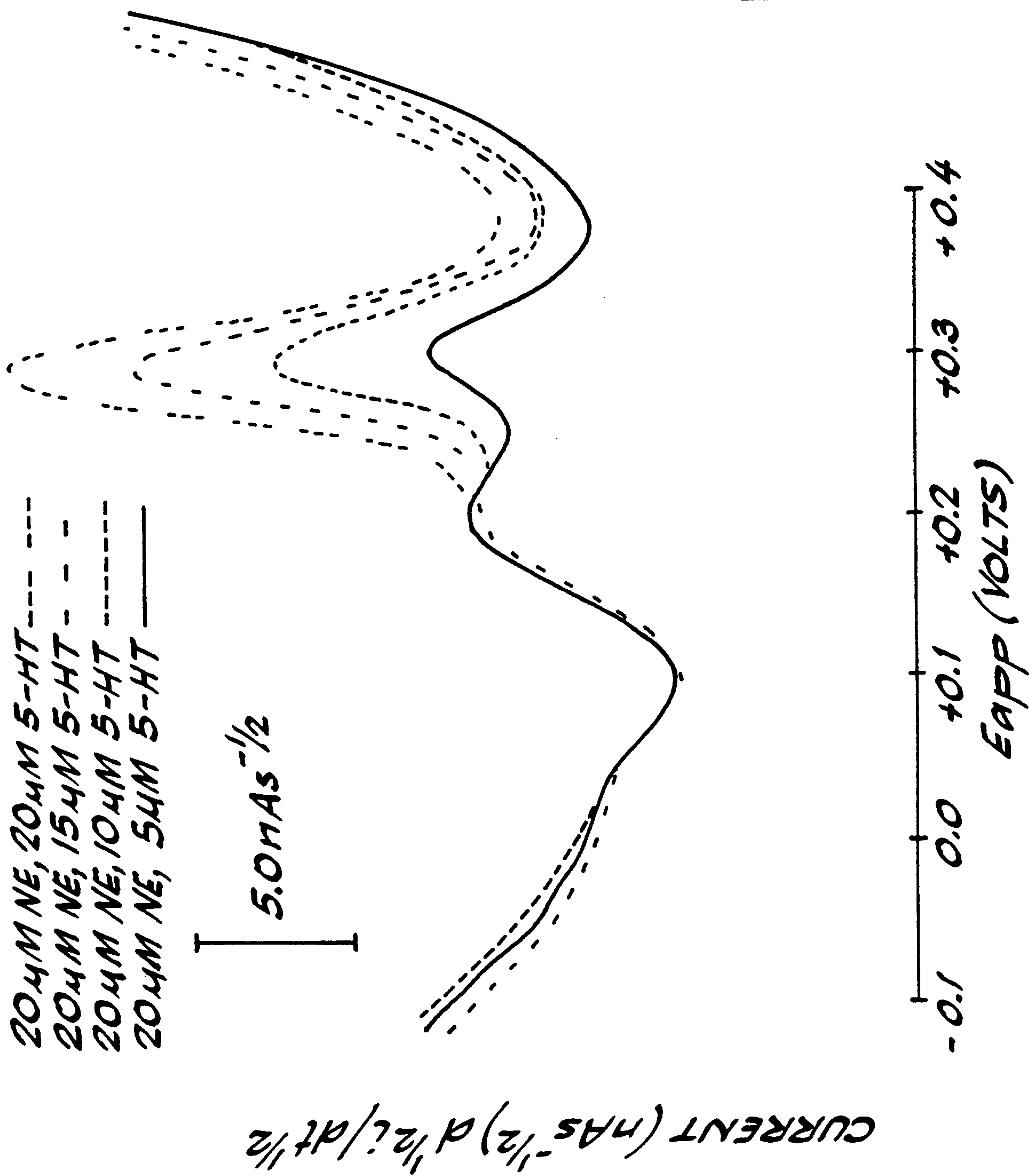


FIG. 41

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FIG. 42



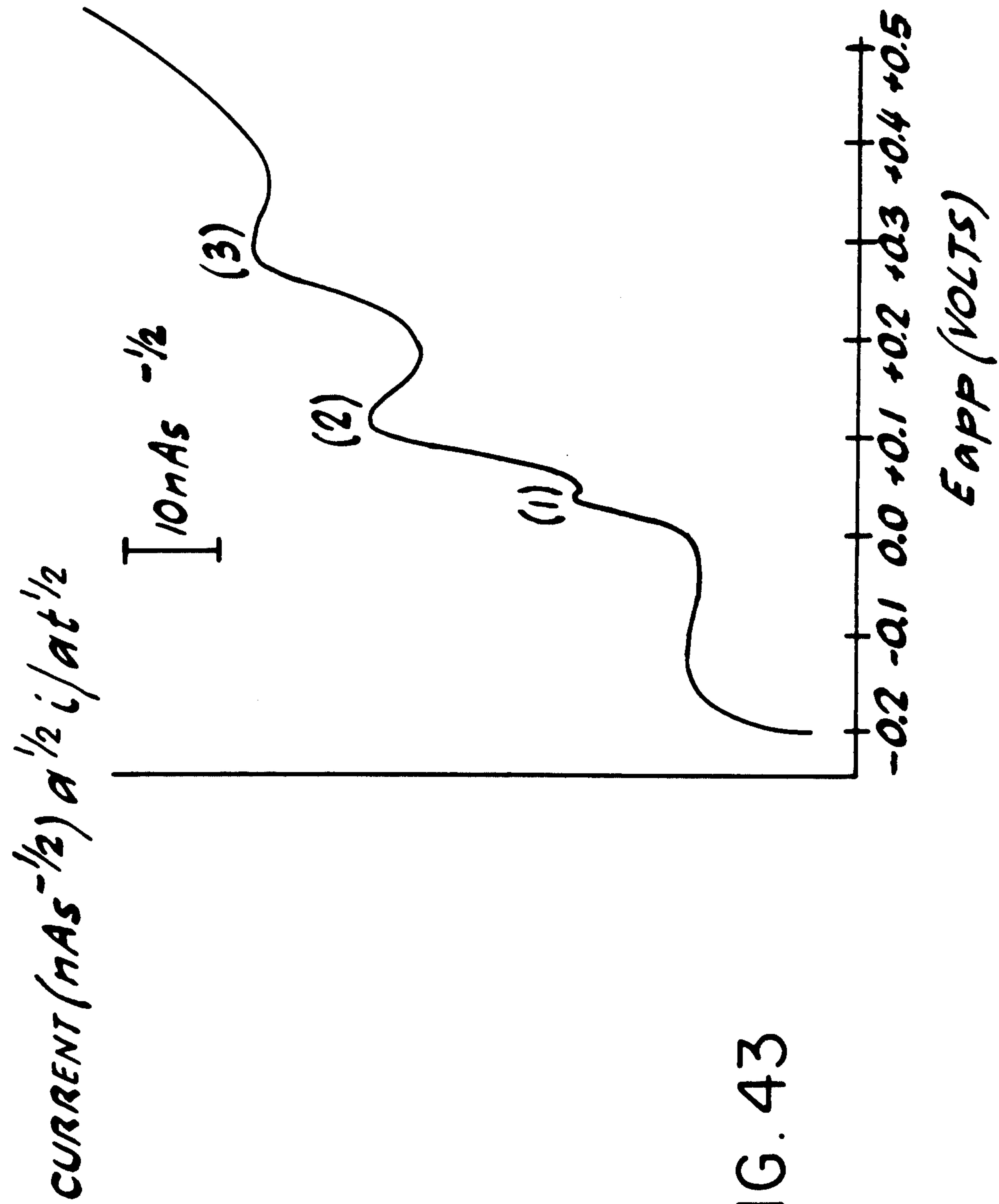
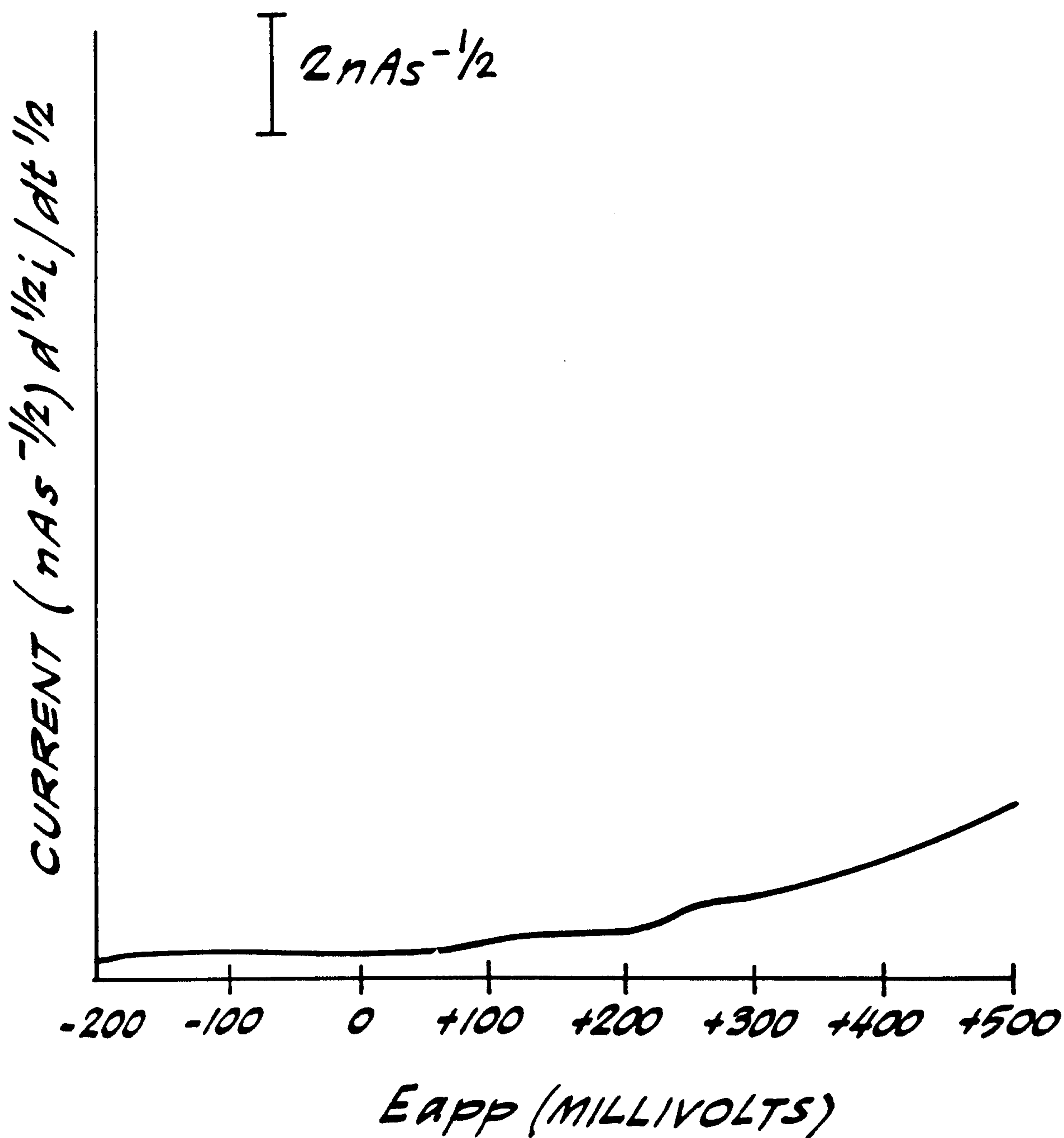


FIG. 43

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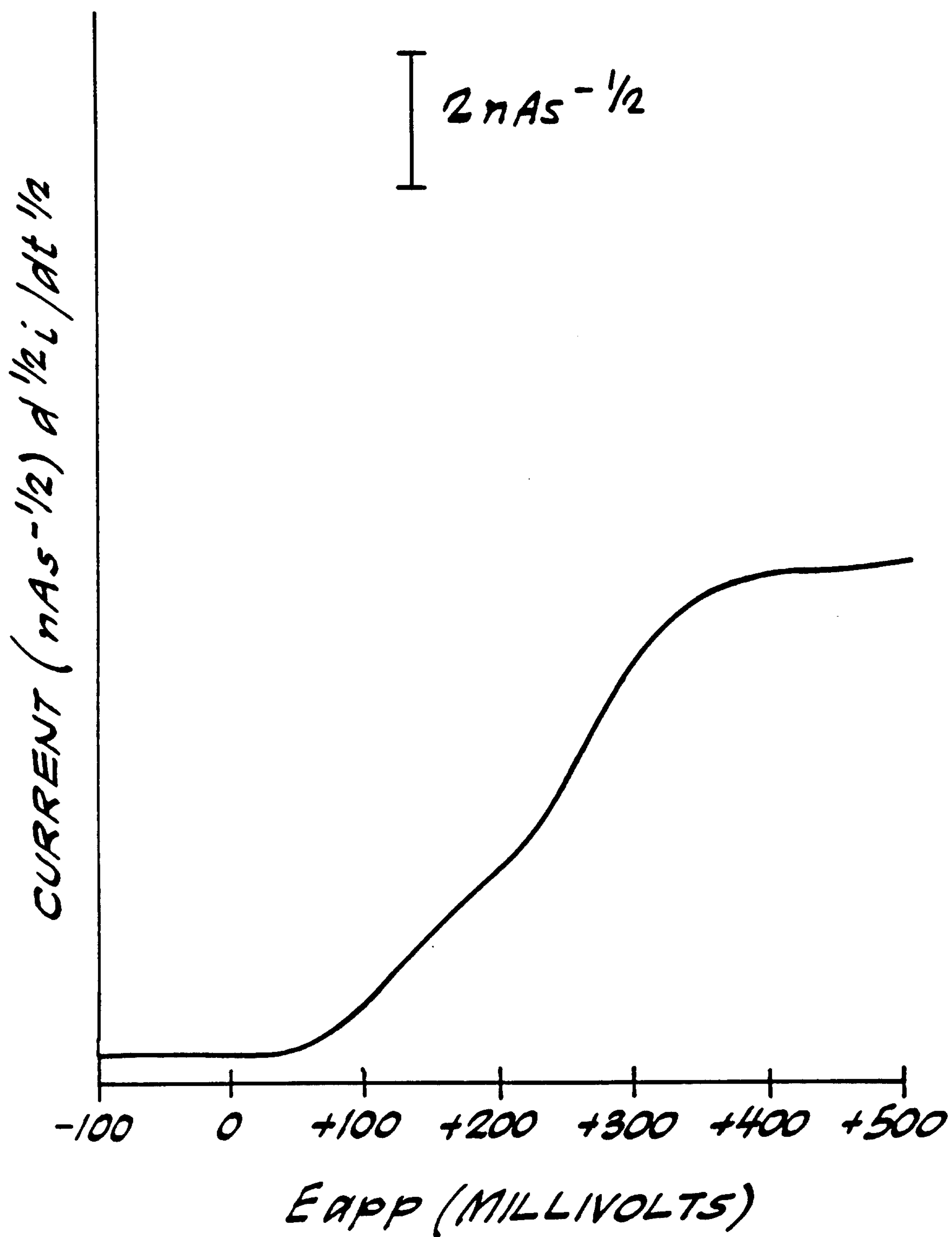
FIG. 45



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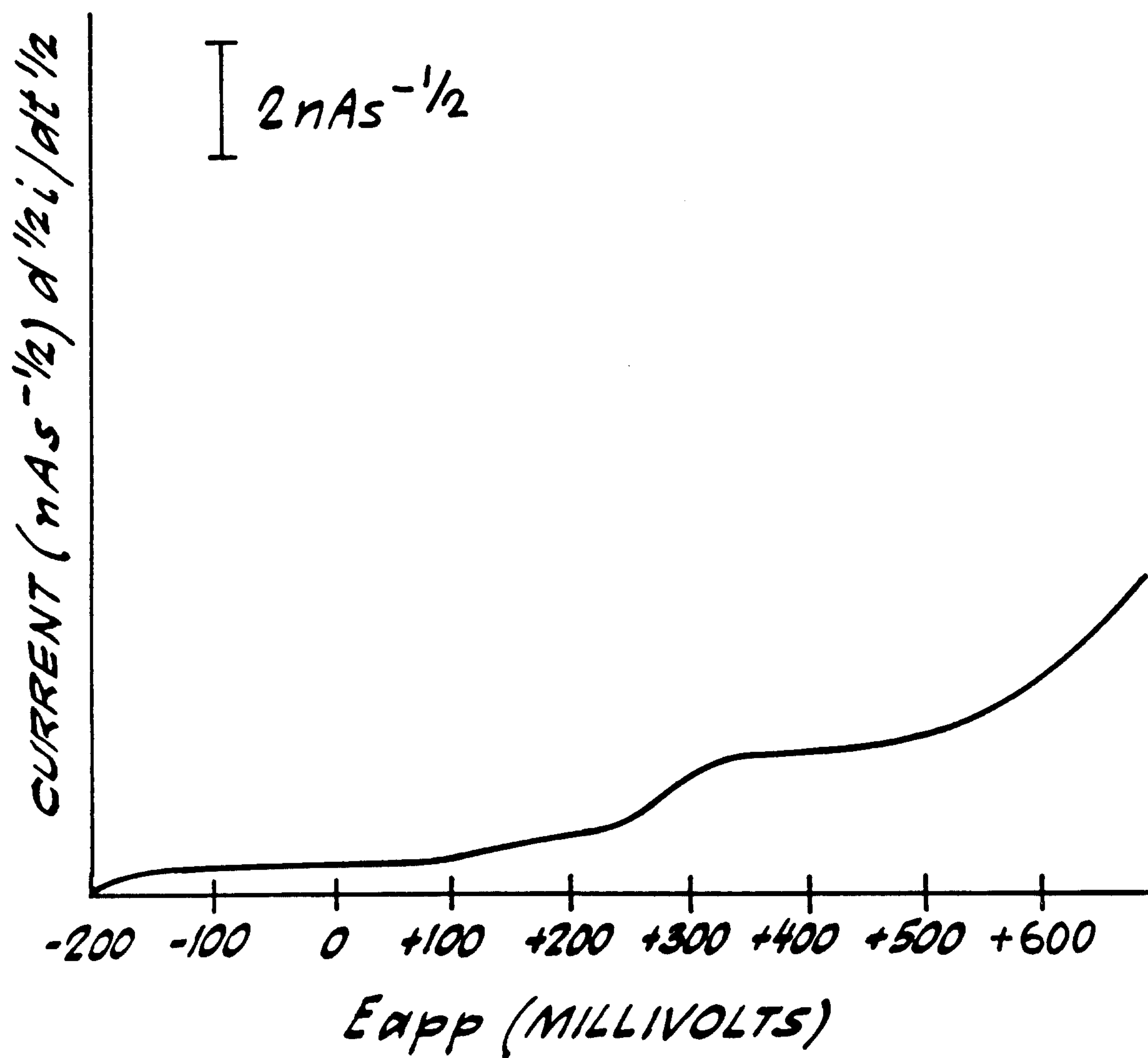
FIG. 46



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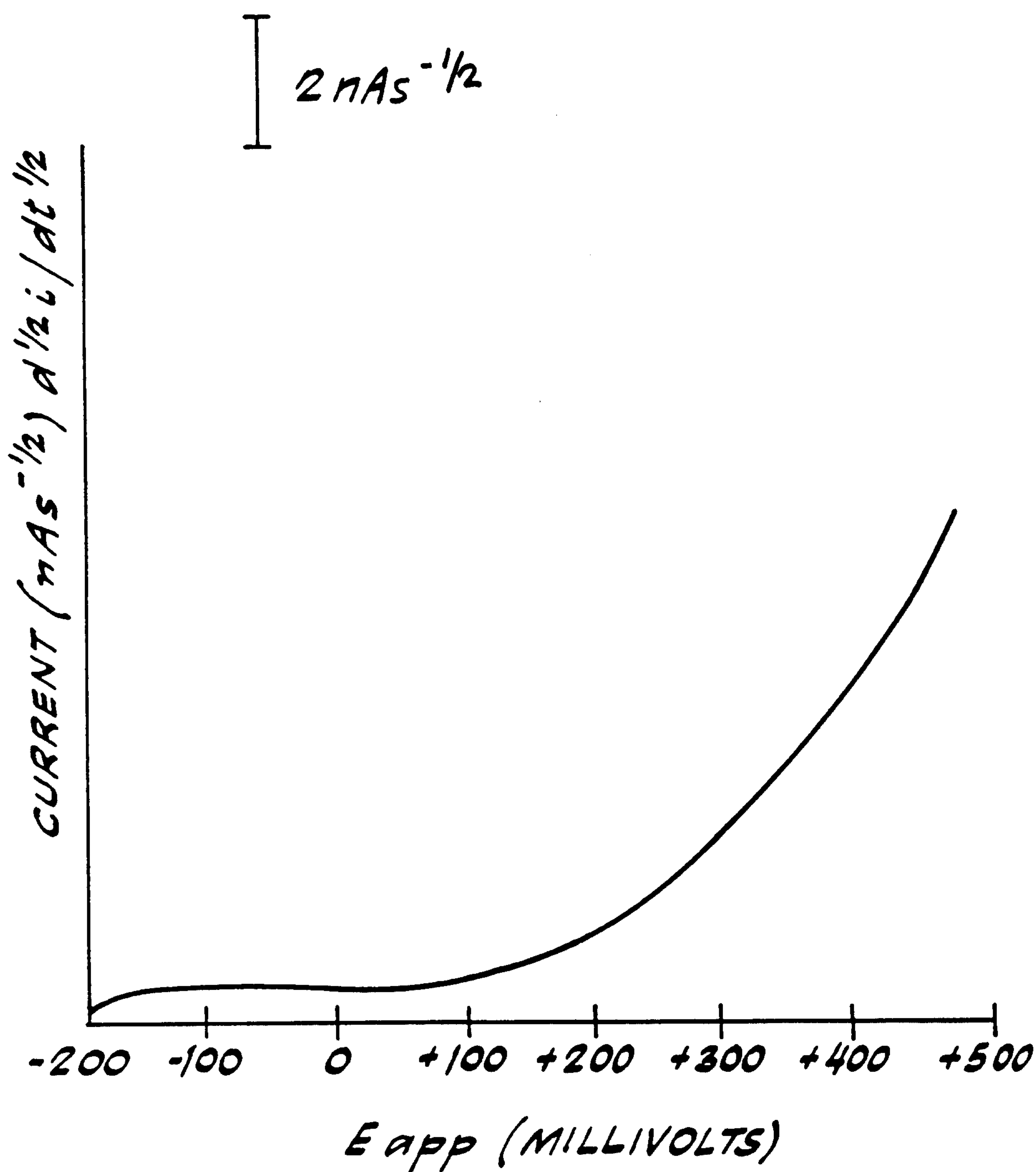
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FIG. 47



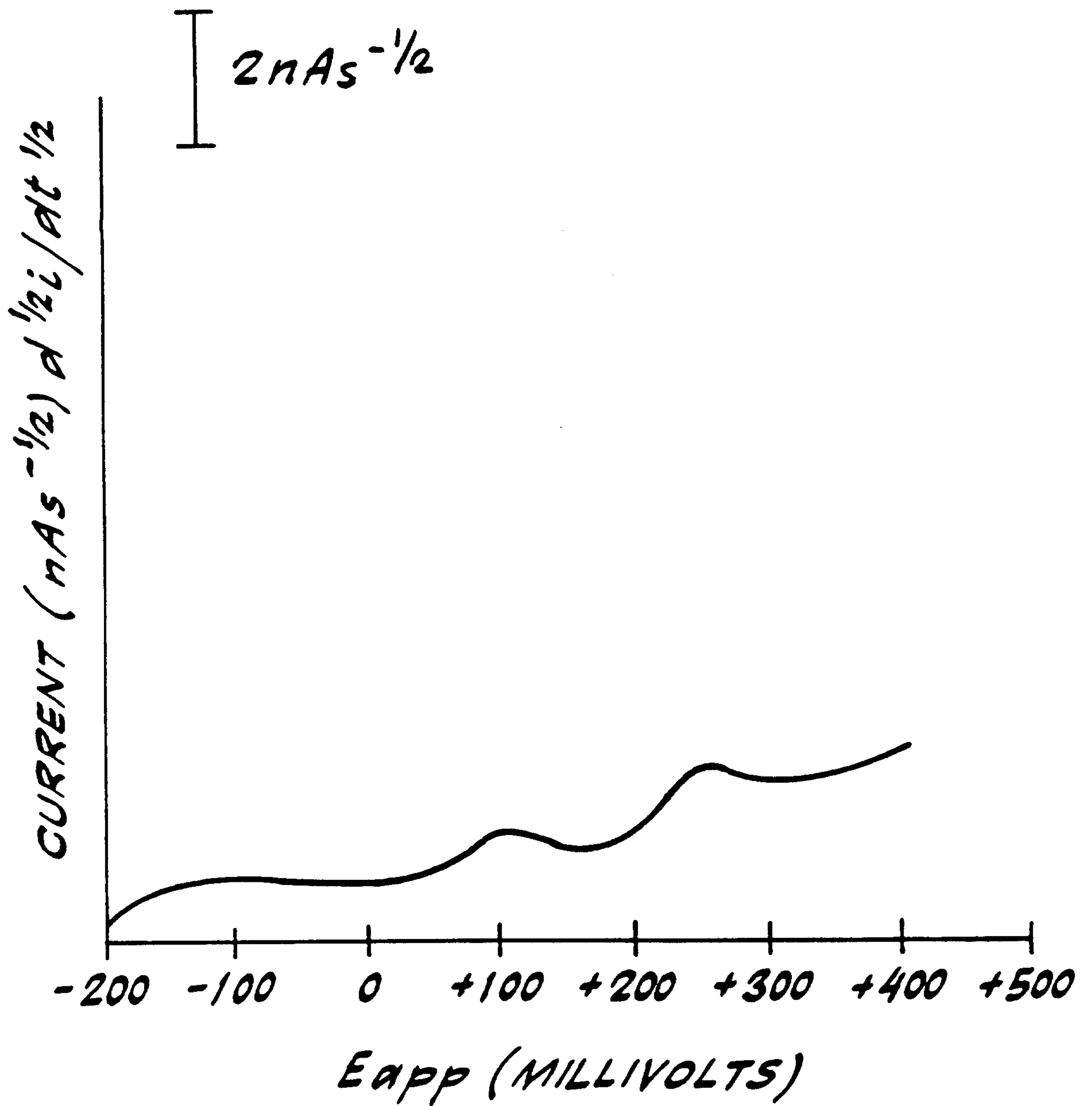
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FIG. 48



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FIG. 49



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FIG. 50

