Irritative Deposits from Stainless Steel Electrodes in the Preoptic Rat Brain Causing Release of Pituitary Gonadotropin.* (27009)

J. W. Everett and H. M. Radford[ø]

Department of Anatomy, Duke University School of Medicine, Durham, N. C.

Recently developed methods have made possible artificial induction of ovulation in female rats in which spontaneous ovulation has been blocked by certain drugs, notably pentobarbital. In rats exposed to controlled illumination 14 hours daily, the ovulation-inducing hormone is normally released from the adenohypophysis sometime between 2:00 and 4:00 P.M. on the afternoon of proestrus(1). The neurogenous stimulus which brings this about can predictably be blocked by suitably timed injection of pentobarbital(2). If, during the course of anesthesia, such an animal is subjected to electrical stimulation of the brain through electrodes resting in the tuber cinereum immediately over the median eminence, ovulation follows during the ensuing night(3). The procedure has also been effective in constant-estrous rats(4). Studies carried out in this laboratory(5) have shown that for inducing ovulation the preoptic region is at least as effective a stimulation site as the tuber cinereum. In fact, with currents of the characteristics that we have employed as standards, results with preoptic electrodes have been considerably more consistent than with electrodes close to the median eminence. In the preoptic region there is a greater latitude with respect to electrode position, suggesting that at this level the pathway in question is diffuse and that it becomes more restricted as it moves caudally into the tuber cinereum. Such an interpretation is in agreement with earlier observations by Hillarp(6).

The ovulation-inducing effect of preoptic stimulation is not blocked by atropine in dosage that will regularly block spontaneous ovulation(7), indicating that, like pentobarbital, atropine blocks the spontaneous event without involving the hypothalamico-hypophyseal system. When first encountered, the observation presented a paradox, for in earlier investigations(1,8) it had been concluded that the atropine-sensitive phase of the process leading to release of ovulating hormone lasts for about half an hour, concurrently with the discharge of hormone from the hypophysis. Complete discharge seemed dependent on continuous action of the atropine-sensitive mechanism throughout this time. Yet the electrical stimulation data disclosed that, under pentobarbital or atropine blockade, ovulation can be produced by a train of pulses lasting only 60 seconds, a clear suggestion of a triggering action. The observations recorded below, however, supply a reasonable explanation of the discrepancy by showing that, although the stimulus is introduced by electric current, the current produces an irritative focus which continues to act long after the electrode is withdrawn.

Methods. All animals used were adult females of our inbred stock. All had a vaginal smear history of at least two 4-day cycles in sequence and were again in vaginal proestrus on the day of experiment. Pentobarbital was injected intraperitoneally a few minutes before 2:00 P.M., the dose being 35 mg/kg body weight, an amount known to be uniformly effective in blocking spontaneous release of ovulating hormone. During the anesthesia, each animal was placed in the stereotaxic instrument with the aid of brief supplementary anesthesia with ether.

Electrodes were of several types. The standard one was a concentric, bipolar, stainless steel unit constructed of a 0.4 mm external diameter tube containing a Teflon-insulated 0.15 mm core. The unit was insulated externally with Formvar† or Epoxylite‡, both ele-

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ø Overseas Trainee, Division of Animal Physiology, Commonwealth Scientific and Industrial Research Organization, Australia. Present address: Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W., Australia.

† Formvar 15/95E, Shawinigan Resins Corp., Springfield, Mass.

‡ Epoxylite 6001-M Electrode Insulator, Epoxylite Corp., El Monte, Calif.

ments being bared at the tips. The core extended about 0.5 mm beyond the end of the tube. Characteristically, the core was the anode. For special purposes indicated later, concentric units of similar dimensions were constructed of platinum tubing and wire. For other special purposes parallel bipolar units were constructed of either reagent-grade iron wire# (analysis, 99.8% Fe; diam., 0.22 mm) or platinum wire (0.25 mm). For stiffness, one wire (the anode) was sheathed in glass and the other was insulated and cemented to the outside of the glass by dipping the assembly in Formvar repeatedly. The 2 wires were bare at the tips, with the cathode 1 mm shorter than the anode. In practice, this type of electrode was placed with the anode in the preoptic region and the cathode turned anteriorly.

Electric current was derived from a Grass S-4 stimulator and SIU-4 isolation unit. Although a wide variety of electrical parameters has been used, the following are standard: monophasic, rectangular 1 msec pulses, 100/sec, 100 μA, continuously for 60 sec or in trains of 30 sec at 30 sec intervals for 5 min. The current was routinely monitored by a cathode ray oscilloscope across a resistor in series with the preparation. The oscilloscope was calibrated at time of experiment with a precision microammeter (Simpson Model 269 VOM). Additional resistance of approximately 400 KΩ was introduced in the leads to the preparation to minimize the effect of electrode capacitance on the wave form and thus to produce an essentially rectangular wave on the monitor screen. The current in microamperes could then quickly be read from the height of the pattern. The relatively high *open-circuit* voltage of such a system is immaterial to the results described, as demonstrated by the fact that 0.05 msec pulses produce neither ovulation nor the characteristic histologic reaction described below. Conversely, both effects are readily obtained in circuits that lack the high peripheral resistance and hence utilize correspondingly low open-circuit voltage.

For experiments requiring introduction of minute volumes of fluid into the brain, the injection was accomplished by means of a tuberculin syringe driven by a machine screw. A length of polyethylene tubing (Clay-Adams PE-50) led from the syringe to a glass cannula 0.4-0.5 mm in diameter and 6-8 cm long, attached to the electrode carrier of the stereotaxic apparatus. The system was filled with mineral oil lightly colored with Oil Red O, after which the aqueous solution intended for injection was drawn far into the cannula. Delivery of the desired volume of fluid was measured directly by excursion of the end of the oil column between calibration marks.

The results of all experiments were determined the next morning by direct observation of presence or absence of eggs in the Fallopian tubes(2). In seemingly negative cases the ovaries were preserved for histologic examination. The brain was fixed by perfusion with 10% formalin in physiological saline and subsequent immersion in the mixture for at least 24 hr. Blocks containing tracks of electrodes or cannulae were serially sectioned at 25μ and stained in Luxol Fast Blue—Cresyl Fast Violet(9). In special cases, for the Prussian Blue test, 1% potassium ferrocyanide was added to the perfusion medium and the brain sections were counterstained with Darrow Red(10).

Tissue Reaction to Stimulating Current. Histologic study of the brains from the many animals subjected to electric currents of widely varied parameters disclosed that in all cases of ovulation there was a zone of electrolytic damage about the electrode tips and that in negative cases the area affected tended to be significantly smaller if recognizable at all. Characteristically, in the damaged areas (Figs. 1 and 2) outright coagulation was restricted to a small spot immediately at the end of the electrode track. Outside of this, there was a more extensive zone of mild inflammation in which few nerve cells were to be found; some nerve fibers could still be recognized but their myelin staining was greatly diminished; neutrophilic leukocytes were scattered in small numbers throughout. In 43 representative brains, tracings of the affected areas were made by projection and their diameters were measured perpendicular to the electrode track. The marked difference between positive and negative cases can easily be seen in Table 1. Only 3 of the 16 negative specimens had lesion diameters significantly larger than the elec-

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trode track itself. Size of the lesion was, in general, correlated with amount of electricity delivered, expressed in coulombs.

TABLE I. Relation Between Amount of Electrolytic Damage of Preoptic Tissue and Induced Release of Pituitary Ovulating Hormone.

Diam. of lesion (mm)	Experimental results		
	Ovulation	Partial* effect	Negative
<.3‡	0	3	13
.3-.39	4	2	1
.4-.49	0	0	2
.5-.59	10	2	0
.6-.69	5	0	0
.7-.79	1	0	0

* Fewer than 7 ova in Fallopian tubes.
‡ Minimal, not significantly larger than electrode track.

Effect of Direct Current. Because of the monophasic character of the pulses, it seemed possible that we were dealing with an effect of metals secondary to erosion of the anode. In a small series of experiments, in fact, the Prussian Blue test clearly demonstrated the presence of iron at the end of the electrode track.

This fact, coupled with the relation of result to size of lesion and quantity of electricity. called for a test of direct current. This was immediately successful. Among 14 rats, currents ranging from 150 μA to as little as 10 μA for as short a time as 20 sec gave uniformly positive results. Even 15 μA for 10 sec gave full ovulation in 5 of 6 rats and partially ovulated the other (6 tubal ova, 4 unruptured follicles). By contrast, 7.5 μA for 10 seconds failed to ovulate any of the 5 rats thus treated.

Results with Platinum Electrodes. It has been reported that in contrast to stainless steel electrodes, electrodes of platinum-iridium are not subject to erosion when imbedded in brain tissue(11). In an attempt to avoid the effects noted above, concentric electrodes were prepared from platinum (see Methods). Eleven rats were stimulated with this type of electrode placed in the preoptic region. The characteristics of the stimulating current in each case were such that ovulation would have been induced in essentially every rat had stainless steel electrodes been used. Only 1 rat ovulated completely, 1 rat had a single ovum, and 9 rats were negative. (Table 2).

TABLE II. Attempted Induction of Ovulation with Platinum Electrodes.

Stimulus: rectangular, monophasic, 1 msec pulses, 100/sec		Results		
Current (μA)	Time	Ovulation	Partial effect	Negative
150	5 min*	0	1	1
100	5 " *	1	0	5
100	60 sec	0	0	3

* 30 sec "on," 30 sec "off" each min.

Microinjection of $FeCl_3$. By means of the microsyringe, with the end of the glass cannula resting in the preoptic region, small amounts of 5% aqueous $FeCl_3$ were injected into the brain substance (Table 3). In control animals, like amounts of 5% NaCl were injected, with pH adjusted in most cases to 1.3 to correspond with the pH of the $FeCl_3$. Ovulation was induced in all rats that received 1 μl $FeCl_3$ but it occurred in only one of the 8 rats that received acidified NaCl. Histologic study disclosed a pronounced inflammatory reaction to $FeCl_3$ in areas measuring 3-4 mm in diameter in the case of 1 μl injections and 1-1.5 mm in the rats that received 0.1 μl. There was comparatively much less reaction to NaCl, but it was nevertheless significant, especially when NaCl was acidified. Diameters of the affected zones were between 0.5 and 1 mm.

TABLE III. Microinjections into Preoptic Tissue.

Treatment	Results	
	Ovulation	Negative
1. μl 5% $FeCl_3$	8	0
.1 " " "	2	2
1. μl 5% NaCl, pH 5.3	0	4
1. " " " pH 1.3	1	7

Electrodes of "Reagent" Iron vs. Platinum. The electrodes employed were the parallel bipolar units made of reagent iron or platinum wires. Eight proestrous, pentobarbital-blocked rats were subjected to 100 μA D.C. for 30 sec through the iron wire, and 7 rats were subjected to 500 μA D.C. for 30 sec through platinum. Seven of the former rats ovulated and all of the latter were negative. The lesioned areas were about equal in size; the larger amount of current was used in the platinum experiments to insure this. But while the iron electrodes produced the familiar zone of inflam-

mation with minimal coagulation (Fig. 3), the chief effect of the platinum electrodes was to coagulate (Fig. 4) and there was hardly any tissue reaction outside the coagulum.

Discussion. Hillarp and associates(12) described prolonged behavioral after-effects of bilateral electrolytic lesions placed basally in the lateral preoptic region of the rat brain. Beginning 20 to 30 minutes after recovery from ether anesthesia, rats of either sex developed a strong drive to male-type copulatory behavior, which lasted several hours. The electrode material was nichrome, another iron-containing alloy. Electrodes of nichrome may be effectively substituted for stainless steel in preoptic excitation of the ovulation mechanism (Riley and Everett, unpublished). It is reasonable to assume that both the behavioral effect observed by Hillarp and the ovulation phenomenon described here result from *irritative* action of local deposits of metallic compounds, perhaps not only of iron but of other metals as well. We conclude that in both circumstances the irritation continues long after withdrawal of the electrode, furnishing a reasonable solution to the paradox earlier mentioned in relation to the atropine blocking experiments.

The data suggest that preoptic neurons which control release of ovulating hormone, while easily subject to this grossly unphysiological type of excitation, are difficult to excite by more strictly electrical stimuli. Other possible interpretations should be considered, however. It may be that electrical stimulation (i.e., through platinum electrodes) should continue throughout the time needed by the pituitary gland to discharge the amount of hormone necessary for ovulation. Hypophysectomy experiments, at varying intervals after electrolytic stimulation of the brain, have disclosed that such hormone release requires between 30 and 40 minutes under ether anesthesia and between 45 and 60 minutes under pentobarbital(13). The optimal pulse form and frequency may differ from those tested. Perhaps the virtue of the electrolytic stimulus is that its irritative after-effect extends radially through a considerable volume of brain tissue, stimulating fibers throughout the damaged zone or at its periphery; pulses administered through platinum electrodes, as presently designed, may actuate relatively few fibers.

In causing the discharge of ovulating hormone under pentobarbital sedation, stimulation by electrolysis has been effective not only in the preoptic region, but somewhat more caudally in the anterior hypothalamus and, in preliminary experiments, more rostrally in the medial parolfactory area, in parts of the nucleus accumbens, and in septal nuclei dorsal and anterior to the anterior commissure. Anatomical and physiological characterization of the neurons involved in the phenomenon will be objectives of future study. It is appropriate to note here that consistent failure has resulted from our attempts to provoke ovulation by (unilateral) electrolytic stimulation of tuberal regions, using small currents that would be uniformly effective in more rostral locations, and that although the mere insertion of an electrode into the hypothalamus has been reported to release enough LH to be detected by the ovarian ascorbic acid depletion test(14), this is clearly not adequate to produce significant luteinization under the conditions of our experiments.

Finally, the present findings urge great caution in use of electrodes of stainless steel or nichrome for stimulation. In reporting either stimulation experiments or the short-term effects of electrolytic lesions, not only should the electrical parameters be specified, but the physical and chemical characteristics of the electrode itself should be described.

Summary. When ovulation was induced by means of stainless steel electrodes in the preoptic area, delivering pulses of varied characteristics, a prominent zone of mild inflammation appeared at the end of the electrode track. When ovulation failed, the zone was characteristically small or absent. Electrolysis with direct current, 10 μA or more for 20 sec or longer, proved uniformly effective in causing ovulation. Substitution of platinum electrodes for stainless steel usually gave negative results. Microinjection of $FeCl_3$ solution into the preoptic tissue induced ovulation, while injection of similar amounts of acidified NaCl did not. Finally, electrolysis stimulated ovulation when electrodes of reagent grade iron wire were used, but not when similar electrodes of platinum were employed. It is concluded that iron and perhaps other metals electrolytically deposited from electrodes of stainless

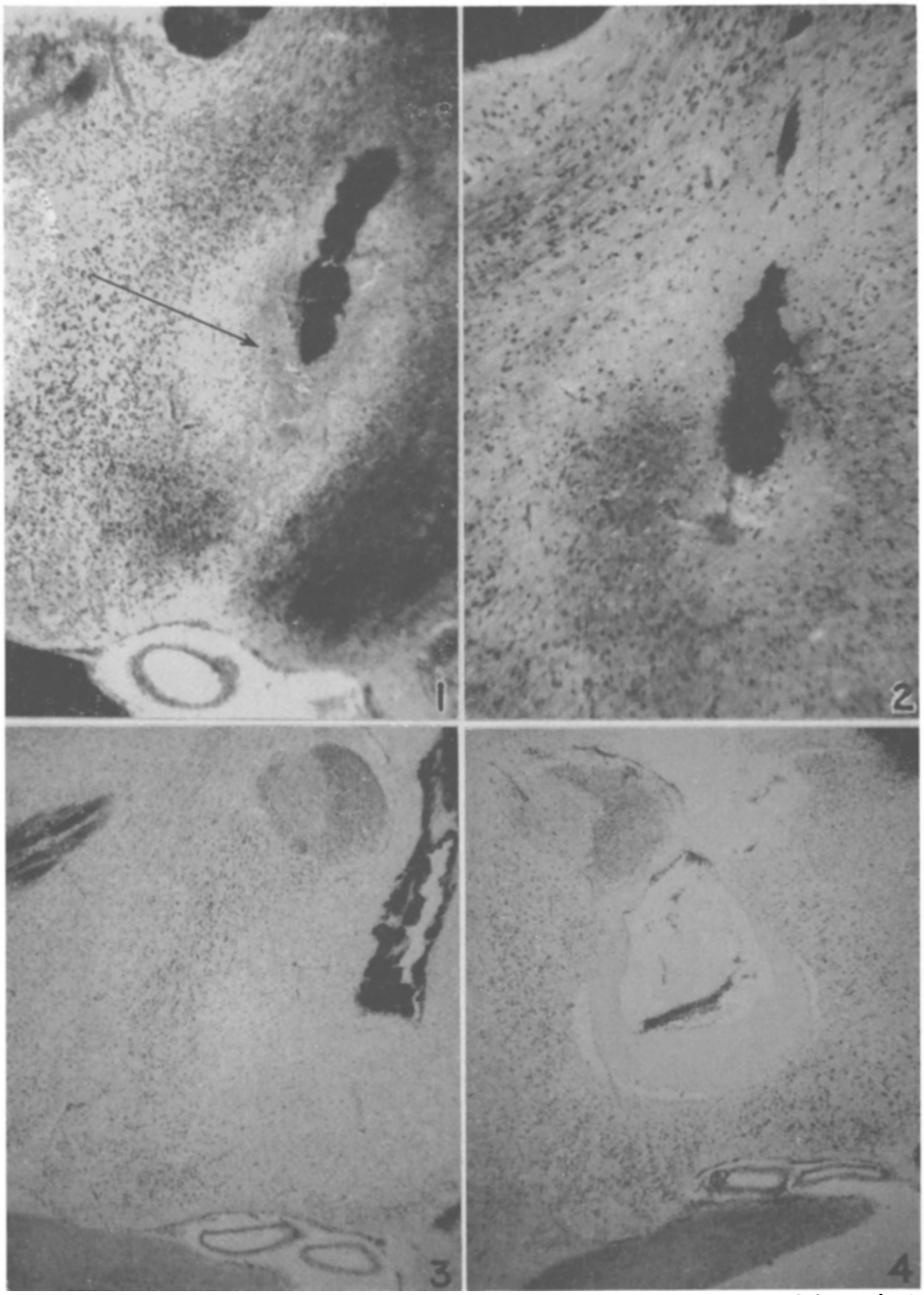

FIG. 1. Parasagittal section of preoptic area (right) and anterior hypothalamic area (left) in brain of an experimental rat, showing tissue reaction (arrow) about the end of the electrode track. Concentric stainless steel electrode. Ovulation induced by monophasic, rectangular pulses, 1 msec duration, 100/sec, 100 μA, 60 sec. Anterior commissure above, optic chiasma lower left, diagonal band to right and somewhat down. Luxol Fast Blue—Cresyl Fast Violet. 50 X.

FIG. 2. Higher magnification of a similar zone of electrolysis. 100 X.

FIG. 3. Electrolytic effect of anodic direct current (100 μA, 30 sec) delivered by an electrode of reagent iron. Ovulation induced. 35 X.

FIG. 4. Electrolytic effect of anodic direct current (500 μA, 30 sec) delivered by an electrode of platinum. No ovulation 35 X.

steel (or nichrome) produce irritative foci whose stimulative action on certain neurons continues long after the flow of electric current has ceased.

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Effect of Cold Acclimation on Vitamin A Metabolism.* (27010)

EDITH PORTER AND E. J. MASORO
Department of Physiology, Tufts University School of Medicine, Boston, Mass.

Rodahl and Moore(1) reported that the liver of the polar bear contains a very large amount of vit. A, and concluded that the toxic effects noted in man following his ingestion of polar bear liver are symptoms of hypervitaminosis A. They also mentioned that similar findings are sometimes seen with the liver of seals.

It would seem most likely that this high hepatic Vit. A content of polar bear and seal livers is related to the nature of the diet of these animals. However, it is also possible that low environmental temperature may play a direct role. To test this possibility, experiments designed to determine the effect of cold acclimation on hepatic vit. A content were carried out with rats.

Experimental. Adult male rats of the Wistar strain were fed *ad libitum* for 10 days a standard diet of the following composition: 25% casein, 10% lard, 51% dextrose, 3% salt mix (2), 5% cellulose, 6% brewers yeast, 0.04% cod liver oil concentrate, 0.01% alpha-tocopherol. After a period of dietary stabilization, the rats were divided into the following 4 groups: Group A was fed the standard diet *ad libitum* for 4 to 5 months while living at 25°C; Group B was fed the standard diet *ad libitum* for 4 to 7 months while living at 0−2°C; Group C was fed the standard diet minus the cod liver oil concentrate *ad libitum* for 4 months while living at 25°C; Group D was fed the standard diet minus the cod liver oil concentrate *ad libitum* for 4 months while living at 0−2°C. In the case of Groups A and B the diets contained 34 units of vit. A per gram of diet, thus providing an abundance of the vitamin. In the case of Groups C and D the rats were administered orally 40 units of vit. A as cod liver oil concentrate in carrier corn oil once a week, thus providing just over the minimum vit. A requirement for rats(3).

At the end of the experimental period the rats were killed by decapitation. The liver was excised, weighed, wrapped in aluminum foil and quickly frozen in liquid nitrogen. The livers were stored in the frozen state at −30°C until analysed for vit. A content.

The method of Ames *et al*(4) was used for vit. A analysis. These authors point out that when levels of vit. A are below 10 γ per gram of liver, turbidity problems can interfere with the assay. In many of the livers analysed in the present work, levels of less than 10 γ of vit.

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