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Ovulation in Persistent-Estrous Rats after Electrical Stimulation of the Brain.*† (23480)

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The fact of neural regulation of ovulation in rabbits and certain other animals classed as "reflex ovulators" has been well known for many years. Direct evidence has been obtained by the induction of ovulation through electrical stimulation of the hypothalamus(1, 2). More recently, ovulation has been reported in rabbits and cats after stimulation of the amygdaloid complex(3,4). Indirect evidence indicates that *spontaneous* ovulation as exemplified by the rat is likewise regulated by the hypothalamus(5). However, until very recently there was no direct evidence for this from stimulation of the brain. Critchlow(6) is the first to demonstrate it, using proestrous rats in which the "spontaneous" mechanism was blocked out with pentobarbital. To be effective, his electrodes had to be near the median eminence. To produce oyulation in rats by stimulation elsewhere in the nervous system, other means are required to block the spontaneous intrinsic stimulus. One such method is to subject the rat to continuous illumination for a few weeks(7,8,9). In time such an animal usually enters a state of persistent estrus. She can ovulate in response to coitus(9) and in this respect is like the rabbit, cat, etc. For comparison with them she would theoretically serve as a more suitable preparation than the pentobarbital-blocked rat. We wish to record the fact that such preparations can be made to ovulate by electrical stimulation of the brain through electrodes implanted at a distance from the hypothalamus.

Materials and methods. Adult female albino rats over 6 months old were exposed to continuous illumination until a persistent vaginal estrus had become established. Of this group, 12 rats formed the eventual series of acceptable experiments. A bipolar electrode was placed in the brain of each animal by use of a Horsley-Clarke stereotaxic instrument and Krieg's coordinates. The electrodes were fashioned from 0.32 mm Nichrome wire, stretched, and insulated with baked enamel. The spacing of the two wires at the tip was <0.05 mm. In 11 rats the electrode was aimed for the amygdaloid complex; in the remaining rat it was inserted into the septal region. It was fixed to the skull by a bridge made of an 18 mm wound clip clamped to the temporal crests and filled-in beneath with zinc dental cement. The operation was performed under ether anesthesia to allow rapid recovery. Soon afterward the rat was placed in a shoulder harness to which wires from the electrode were anchored before leading off to the stimulator. She had free range of movement within the radius of the wires. Stimulation was carried out after recovery from the anesthetic. The source of stimulus was an electronic impulse generator with independently controlled frequency and intensity sec-

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TABLE I. Summary of Brain Stimulations and Results.

Electrode		Result		
Location	Proof	Interval	Evidence	Score
Amygdala	Sec*	24 hr	11 tubal ova	+
"	Gr obs†	"	12 *Idem*	+
"	"	"	9 "	+
"	Sec	"	9 "	+
"	"	"	8 "	+
"	Gr obs	11 day	1 mm corp lut	?
"	Sec	"	*Idem*	?
"	Gr obs	24 hr	No tubal ova	—
"	"	"	*Idem*	—
"	"	"	"	—
"	Sec	5 day	No corp lut	—
Septum pellucidum	"	48 hr	Many new corp lut	+

* Stained sections of formalin-fixed brain.
† Gross observation of formalin-fixed brain (see text).

tions. The wave form which it delivers is a rapidly rising spike from a condenser discharge, with exponential decay during ca. 3 msec. The frequency and intensity varied from individual to individual. The practice was to administer as much as the animal would take, short of convulsions, during periods of 30-60 minutes. The usual frequency was 25-30 c/sec. The voltage ranged as high as 10-15 v, reaching 20 v in one instance. The instrument was not always dependable, however, and more reliance was placed on the overt response of the animal than on the setting of the voltage dial. The results were assessed at autopsy, which was carried out on the first day after stimulation in 8 rats, on the second day in 1 rat, on the fifth day (proestrus) in 1 rat and on the eleventh day of anestrus in 2 rats. In the 8 rats the fallopian tubes were searched for ova and the ovaries were examined for freshly ruptured follicles(5). In the others, if corpora lutea were present, the ovaries were serially sectioned for histological examination. The intact brain of each animal was preserved entire in 10% formalin. Six were imbedded in celloidin, sectioned at 26 μ and stained by the Luxol fast blue-Cresyl fast violet technic of Klüver and Barrera(10). Otherwise, only gross observations were made. Usually a spot of discoloration could be seen on the ventral surface, marking the location of the electrode tip. If doubt existed, progressive thin slices were made with a razor blade until the electrode track was found.

Results. The results of all acceptable experiments are assembled in Table I. In 11 rats the tips of the electrodes rested in the amygdaloid complex. Five of them had tubal ova on the day after stimulation. Two others possibly ovulated, but the information is indirect on account of the delayed autopsy time. Four failed for no evident reason. In one rat the electrode was aimed at the septum pellucidum; sections of brain later demonstrated that the tip lay in the lateral ventricle impinging on the dorsal lateral aspect of the septum and also in contact with the caudate nucleus. Two days after stimulation her ovaries had new corpora lutea about 36 hours old, as judged from their histological appearance.

Proximity of amygdala and lateral hypothalamus makes it possible that in several cases the positive result may have come about through spread of the stimulating current into the hypothalamus. However, the one rat that was stimulated in the septal region and one of the amygdala group (#2) can hardly be explained on that basis. In the former the distance to the hypothalamus would be too great. In the latter there were few motor signs, in spite of the fact that the electrode tips were just lateral and ventral to the internal capsule. Spread would thus appear to be ruled out.

Seven faulty experiments, in which the electrode was not firmly attached to the skull or the electrical connections were poor or the electrode was not deep enough, may be considered as controls in the sense that they show that mere insertion of the electrode does not serve as an adequate stimulus. None of them ovulated.

The results confirm by a somewhat different method the observation by Critchlow(6) that ovulation can be induced in rats by electrical stimulation of the brain—this in a species that normally ovulates spontaneously. Although the present experiments are only preliminary, they show clearly that the persistent-estrous rat in continuous illumination is a suitable experimental animal for explora-

tion of neural elements of the LH-release apparatus higher than the median eminence. The chief limitation would appear to be the fact that in some strains of rats (*e.g.* our "normal" strain derived from Osborne-Mendel stock) the persistent-estrous state often cannot quickly be induced until the animal is 6 months old.

Summary. Persistent estrus was induced in female rats by continuous illumination. Through "permanent" bipolar electrodes, stimulation was administered after recovery from anesthesia to either the amygdala (11 rats) or the septum pellucidum (1 rat). The latter and 5 of the former ovulated within 24 hours.

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"Transfer" of Complement from One Antigen-Antibody Complex to Another.*† (23481)

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It has been shown that some of the I^{131} labelled complement incorporated with antigen-antibody-I^{131} complement precipitates is released when these precipitates are resuspended in fresh sera containing active complement(1). This behavior was attributed to an equilibration between the I^{131} complement in the precipitates and the free complement present in the fresh sera. It was of interest to determine whether the complement components still maintain their original activities after the reaction with an immune complex. There is some evidence in the literature indicating that complement components once fixed may maintain some of the original ability to participate in the hemolysis of sensitized erythrocytes(2,3).

The purpose of the present investigation was to determine whether or not components of complement can "transfer" from one immune complex to an unrelated one and if so, what are the conditions which govern this "transfer." As an index of complement activity, use was made of the precipitation of soluble antigen-antibody complexes by complement(4,5). Antigen-antibody complexes (soluble or insoluble) containing complement were mixed with soluble I^{131} bovine serum albumin (I*BSA)-rabbit anti BSA complexes which were prepared in the region of excess antigen. The subsequent precipitation of the soluble I*BSA-anti BSA complexes was employed as a measure of the "transfer" of complement to these complexes. Although complement appears to be responsible for the precipitation of soluble immune complexes by normal sera, there is a possibility that other substances present in the sera may play a role. It has previously been inferred that

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