Effects of Electrical Stimulation of the Amygdala on Gonadotropin Release and Ovulation in the Rat[1] (37036)

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It is well known that the hypothalamus is essential for the ovulatory surge of pituitary gonadotropin(s) in the rat. Extrahypothalamic areas in the limbic system have been implicated as modulators of the LH release. The role of the amygdala in this process has recently been reviewed by Sawyer (1) and Zolovick (2).

The amygdala appears to play a dual role in the control of the LH release. Stimulation of the amygdala in rats made persistent estrous by constant illumination often results in ovulation (3, 4). In contrast, amygdaloid lesions have resulted in early vaginal opening in the immature rat (5) and an elevation in circulating levels of LH in ovariectomized rats (6) and adult deermice (7). We now report an inhibitory effect of amygdaloid stimulation on LH release in acute experiments involving both cycling and ovariectomized rats.

Methods and Materials. Female Sprague–Dawley rats (Simonsen) weighing 200–300 g were maintained under standard conditions (8) with lights on from 0500–1900. Only rats showing 2 consecutive 4-day cycles were used. Stainless steel concentric bipolar electrodes were implanted unilaterally into the amygdala either between 1300 and 1500 on proestrus (acute) or bilaterally two or more weeks and at least 2 consecutive 4-day cycles prior to stimulation (chronic). In some rats of both groups the animals were anesthetized with ether 15 min before and throughout the stimulation period. In acute studies, control rats were placed under ether, shams received implants without stimulation, and others were injected with ovulation-blocking doses of pentobarbital (32 mg/kg; ip) at 1330–1345 or reserpine (1.0 mg/kg; sc) at 1215. It was necessary to maintain the reserpinized rats under light ether anesthesia for implantation. Electrical stimulation was applied unilaterally at 1445 in all chronic and between 1400 and 1600 in most acute rats (150 Hz, 100 μA, 0.5 msec, 30 sec on/off for 30 min) with bipolar concentric electrodes. A few acute animals were stimulated electrochemically with unipolar electrodes. Our implantation and stimulation techniques have been described (9). All electrodes were inserted diagonally from the side at an angle 15° off vertical to avoid damage to the stria terminalis. The occurrence of ovulation was tested by examining the oviducts microscopically for ova at noon the following day.

Three acute preparations were stimulated electrically at 1100–1130 on proestrus, and blood (1.5 ml/period) was continuously withdrawn from an intra-atrial cannula between 1000–1200, 1200–1400, 1400–1600, and 1600–1800 as described previously (10) for subsequent radioimmunoassay (RIA) of LH, FSH and prolactin. Three control rats were treated similarly without the electrical stimulation.

An additional group was ovariectomized and a bipolar elecrode implanted unilaterally

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TABLE I. Effects of Electrical Stimulation of the Amygdala (AME/ACO) on Ovulation in the Proestrous Rat.

Group	Drug	Electric stimulus	No. of rats	Number ovulating[a]
Acute	Ether	—	8	8
	Ether	+	10	0
	Pentobarbital[b]	+	9	0
	Reserpine[c]	+	5	0
Chronic	Ether[d]	+	8	8
	—	+	5	5

[a] All rats that ovulated had shed 7 or more ova.

[b] Includes one 20 μA, 60 sec and one 1 mA, 10 sec DC electrochemical (electrical stimulation of 30–90 min in the others).

[c] Includes one 20 μA, 60 sec and one 1 mA, 10 sec DC electrochemical stimulation.

[d] The contralateral amygdala was stimulated after 2 consecutive 4-day cycles had intervened following the stimulation without ether.

into the amygdala one week later. A chronic intra-atrial cannula (11) was introduced 2 weeks after electrode implantation. One week later, without anesthesia, blood (0.5 ml) was collected for RIA of LH immediately before and 30, 60, 90, and 120 min after the beginning of the 30-min stimulation period. RIA's were measured using the NIAMD kits (8, 12). All brains were perfused and prepared histologically (9) to verify electrode locations.

Results. Acute. All control and sham-stimulated rats placed under ether for 45–60 min between 1400–1600 ovulated (Table I). Three rats stimulated under ether, in which the electrodes were found outside the corticomedial amygdala in the caudate nucleus or central or basolateral amydala, ovulated in full (7 or more ova). In the remaining rats the electrodes were located, with histological verification, in the medial (AME) or cortical (ACO) amygdaloid nuclei. Stimulation of the AME/ACO under ether blocked ovulation, and the same stimulus in rats under an ovulation-blocking dose of pentobarbital or reserpine did not cause ovulation, *i.e.*, failed to reverse the blocking effects of the drugs.

Stimulation of the amygdala at 1100 on proestrus did not significantly alter the time for the proestrous "surges" of LH, FSH or prolactin or the quantity of the hormones released when compared with nonstimulated controls or values obtained from other studies in our laboratory using decapitated and cannulated rats (8, 10, 12).

Chronic. All rats ovulated whether or not stimulation at 1445 was performed under ether (Table I).

Plasma LH levels were transiently depressed after electrical stimulation in ovariectomized rats (Fig. 1). LH started to decline by 60 min after the onset of stimulation and reached its lowest values at 90 min ($p < 0.05$), and levels returned to prestimulation values within 120 min.

Discussion. Neither ether nor the added stress of implantation early on the afternoon of proestrus inhibited ovulation. However, electrical stimulation of the AME/ACO under

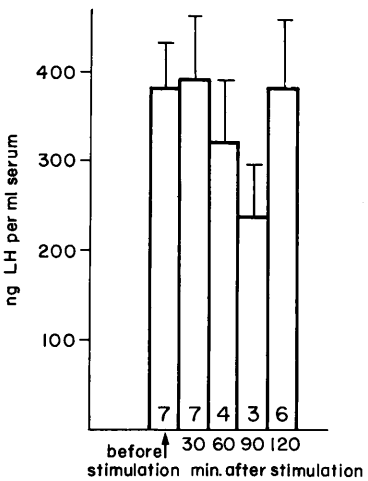

FIG. 1. Serum LH levels of ovariectomized rats after electrical stimulation of the amygdala. Mean ± SEM. The numbers within the bars represent the number of animals in each group.

these "acute" conditions resulted in failure of ovulation. In the chronic preparation under ether, electrical stimulation failed to block ovulation. Nevertheless, an inhibitory effect of amygdaloid stimulation was observed in the unanesthetized chronic animal after ovariectomy, *i.e.*, a significant depression in serum LH levels 90 min after stimulation. Inhibitory influences of the amygdala on gonadotropic secretion have been inferred heretofore from experiments involving lesions (5–7) and localized implants of estrogen (6).

Stimulation on the morning of proestrus did not advance, block or diminish the proestrous surges of any of the three gonadotropins suggesting that the ovulatory blockade under ether in acute studies effected only a temporary inhibition of the neural triggering mechanism(s). This view is supported by the brevity of suppression in plasma LH levels after amygdaloid stimulation in chronic ovariectomized rats. It is possible that this depression would have been more marked and prolonged if electrochemical stimulation had been employed to activate a greater population of neurons.

There is no doubt that the amygdala can also exert facilitatory influences on gonadotrophic secretion (1, 2). Bunn and Everett (3) and Velasco and Taleisnik (4) were able to induce ovulation by amygdaloid stimulation in acute rat preparations but their animals were mostly in persistent estrous and their stimuli were generally electrochemical. Our rats, on the other hand, were proestrous cycling animals and our stimuli weak electrical pulses. Proestrous rats may be more susceptible to the stress of ether and sterotaxic surgery than are persistent estrous animals.

The persistent estrous state commonly induced by continuous illumination also occurs after anterior deafferentation of the medial basal hypothalamus. Some of the fibers entering the hypothalamus anteriorly are necessary for spontaneous ovulation and others appear to exert an inhibitory influence on LH release in both the rat (8) and the hamster (13). It is possible that constant illumination may inactivate such inhibitory fibers which play an important role in the depressive influence of the amygdala on LH release. This would allow free reign for activation of stimulatory impulses from the amygdala to induce LH release.

In his review Sawyer (1) proposed that the medial amygdala contained both facilitatory and inhibitory neurons relative to pituitary activation. He also referred to somewhat analogous experiments in female rabbits (unpublished work of Saul and Sawyer) in which the acute animal preparation under ether, which would ovulate in response to hypothalamic stimulation, completely failed to ovulate following electrical stimulation of the amygdala at parameters which were practically 100% successful in ovulating the chronic unstressed rabbits. The present experiments in the rat show that such stimuli, applied acutely, not only fail to stimulate but that they actually inhibit cyclic ovulation. Surgical stress and ether must somehow inhibit facilitatory neurons or activate inhibitory neurons (1) to the extent that the cyclic stimulus in the rat or electrical stimulation in the rabbit amygdala fails to release an ovulatory surge of LH.

Summary. Electrical stimulation of the amygdala in acute experiments under ether on the afternoon of proestrus in cycling rats blocked ovulation whereas similar stimulation on the morning of proestrous did not alter the afternoon surges of the gonadotropic hormones. "Acute" stimulation on the afternoon of proestrous also failed to overcome the ovulatory blockade exerted by pentobarbital or reserpine. Furthermore, serum LH levels were temporarily reduced by stimulation of chronic electrodes in the amygdala of ovariectomized rats, but such "chronic" stimulation did not inhibit the proestrous rat from releasing an ovulatory surge of LH. The results indicate that the amygdala can exert an inhibitory influence on LH release in acute experiments in cycling rats and under chronic conditions in ovariectomized rats.

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