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Acquisition of Amygdala-Kindled Seizures in Female Rats: Relationship Between the Effect of Estradiol and Intra-Amygdaloid Electrode Location

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BUTERBAUGH, G. G. *Acquisition of amygdala-kindled seizures in female rats: Relationship between the effect of estradiol and intra-amygdaloid electrode location.* PHARMACOL BIOCHEM BEHAV **28**(2) 291–297, 1987.—Ovariectomized, adult female rats, with or without estradiol replacement, were kindled by daily amygdala stimulation. Kindling acquisition varied with the intra-amygdala site of stimulation. During stimulation of the medial (AME) or central (ACE) nucleus, the only effect of estradiol replacement (E), compared to non-replaced rats (nE), was to significantly decrease the number of trials with afterdischarge (AD) during early kindling (stage 0). In rats receiving stimulation of the cortical nucleus (ACO) or the baso-lateral group of nuclei (ABL), a similar effect of estradiol was extended through stage 1. In addition, nE rats with ACO or ABL electrodes required significantly more trials with AD and accumulated more than twice the sec of AD during the late stages of kindling, compared to E rats and regressed to lower stage responses between the first stage 4 and last stage 5 responses; regressed responses never occurred in E rats. Estradiol also significantly decreased the prekindling AD threshold of the AME and ACE. These results indicate that estradiol accelerates early stage kindling, likely by proconvulsive properties to increase excitability within immediate amygdala projections. During late kindling stages, estradiol may participate in reinforcing or sustaining the convulsive readiness of kindling circuits established during bilateral recruitment. The site of action for this latter effect of estradiol may reside within circuits accessed by stimulation of the ACO or ABL, and not the AME or ACE.

Kindled seizures Amygdaloid nuclei Estradiol Female rats Catamenial epilepsy Epilepsy Seizures Amygdala

AN increase in the frequency or severity of seizures that is consistently correlated with a phase of the menstrual cycle is termed catamenial epilepsy [23,34]. The changing levels of progesterone and estrogen during the menstrual cycle may contribute to the phenomenon [1]. An elevated estrogen/progesterone ratio during ovulatory cycles is linked with an increased incidence of generalized tonic-clonic seizures. Elevated estrogen levels during anovulatory cycles are correlated with increased frequency of partial and generalized seizures. The intravenous injection of conjugated estrogen has been shown to activate EEG epileptogenic activity [26]. Animal studies support these clinical observations by demonstrating epileptogenic properties of estradiol in a variety of species and models [27, 32, 33, 45, 46]. Widespread proconvulsive effects are supported by the findings that the minimal electroshock threshold in rats is lowest during estrus when circulating estrogen levels are high [48], and is decreased by the repeated administration of estradiol [49].

Kindling has been widely used as an animal model of epilepsy and involves low intensity stimulation of a specific brain region (e.g., amygdala) at regular intervals [13]. Initially, the stimulation produces an electrical afterdischarge with no behavioral correlates. As the process of kindling continues with successive stimulations, animals acquire progressively more severe electrical and motor seizures until a stable clonic convulsive response results. The fully kindled seizure is retained over long periods of stimulation-free time.

This laboratory has previously reported that the amygdala kindling model, using ovariectomized female rats, is sensitive to estradiol in that estradiol accelerates the overall rate

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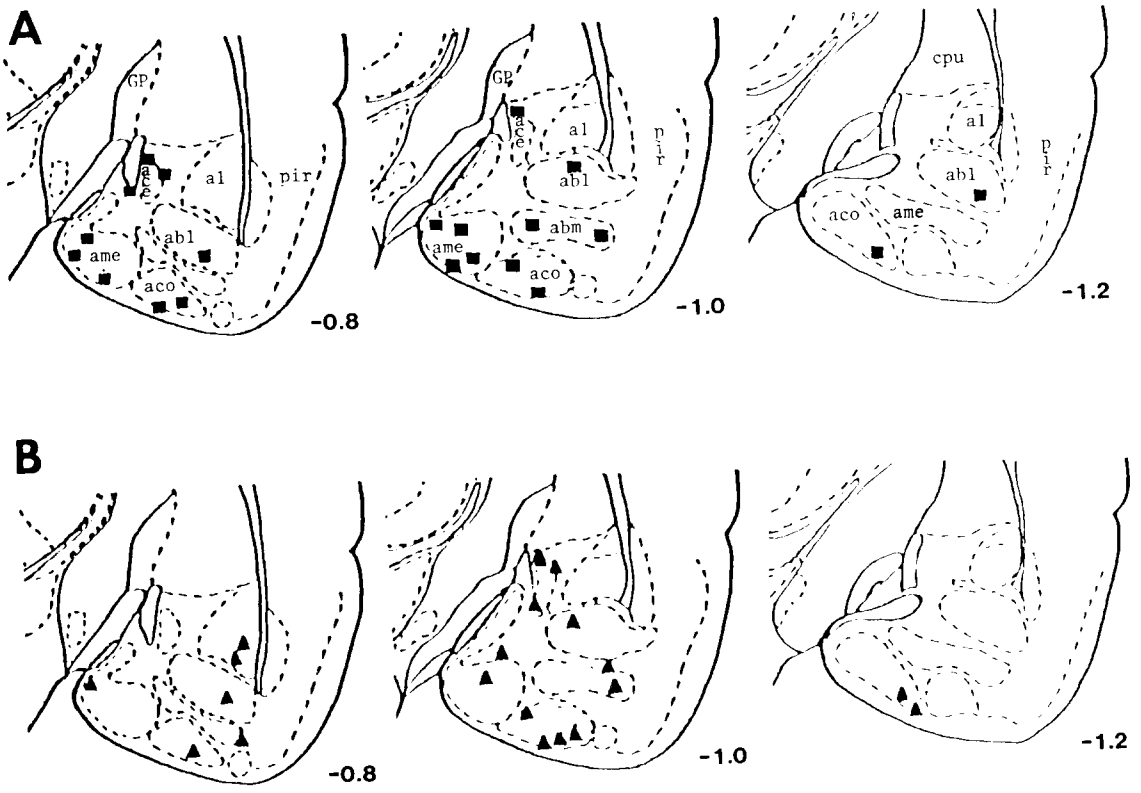

FIG. 1. Distribution of electrode tips within the amygdaloid complex in rats with and without estradiol replacement. Sections are adaptations from the Pellegrino and Cushman [36], −0.8, −1.0 and −1.2 mm from bregma. Abbreviations: Amygdaloid nuclei: (ABL, basolateral; ABM, basomedial; ACE, central; ACO, cortical; AL, lateral; AME, medial); CPU, caudate-putamen; GP, globis pallidus; PIR, pyriform cortex.

TABLE 1

SUMMARY OF THE ACQUISITION OF AMYGDALA-KINDLED SEIZURES IN OVARIECTOMIZED FEMALE RATS WITH AND WITHOUT ESTRADIOL REPLACEMENT

	Estradiol (N=20)	No Estradiol (N=21)
Duration of First AD (sec)	14.8 ± 2.2	11.0 ± 1.4
Duration of First Stage 5 Seizure (sec)	78.1 ± 4.8	86.8 ± 6.2
Total Trials to Kindle	11.6 ± 0.5	15.3 ± 0.5*
Total AD Sec to Kindle (sec)	593 ± 39	768 ± 80*

Data are expressed as mean ± s.e.m.
*Indicates significant from estradiol replaced rats, $p<0.05$.

of kindling [15]. Based upon this finding, a more detailed study of the influence of physiological levels of estradiol on the acquisition of amygdala-kindled seizures in female rats was initiated. The results indicate the influence of estradiol on the acquisition of amygdala kindled seizures is dependent upon the location of the electrode within the amygdaloid complex. In fact, this is the first report that a treatment effect (i.e., estradiol) on the acquisiton of amygdala kindled seizures can depend upon the site of amygdaloid stimulation.

METHOD

Animals

Adult, female, Sprague-Dawley rats, 250–275 grams, were housed in individual cages with unrestricted access to water and Purina Rat Chow. The rats remained in temperature and humidity controlled local animal care facilities equipped with a 13-hours-on (7:00 a.m. to 8:00 p.m.), 11-hours-off light cycle. The rats were transported to the laboratory one hour before experimentation and were returned to their home cages within one hour after use. Animals were weighed prior to surgery and at three to four day intervals during the kindling procedure.

Animal Preparation

Rats were anesthetized with ketamine HCl, 100 mg/kg IP, and ovariectomized via bilateral flank incisions. A stainless steel, bipolar electrode, 0.25 mm diameter (No.303/2; Plastic Products, Roanoke, VA), 0.5 mm vertical tip separation (in-

TABLE 2

INFLUENCE OF ELECTRODE PLACEMENT ON THE RATE OF AMYGDALA-KINDLING IN OVARIECTOMIZED RATS FEMALE WITH AND WITHOUT ESTRADIOL REPLACEMENT

	Trials With AD to Kindle		Sec of AD to Kindle	
	(Hormonal Treatment)			
Amygdala Region	Estradiol	No Estradiol	Estradiol	No Estradiol
A. Central	12.3±1.4	13.3±1.9	638±37	594±78
Medial	13.3±1.9	13.0±1.0	591±66	487±17
B. Basolateral	12.2±0.9	17.1±0.8*	616±99	929±53*
Cortical	9.6±0.7	15.4±0.5*	531±71	819±78*

Data are expressed as mean ± s.e.m., N=5–7 per group. *Significant from estradiol-replaced rats, $p<0.05$.

sulated except for cut tip) was stereotaxically implanted into the right amygdaloid complex. The basic coordinates were 1 mm posterior and 4.8 mm lateral to bregma, and 7.8 mm ventral to dura (incisor bar 5 mm above the intra-aural line). In some cases, these were adjusted to increase the number of animals with medial or central electrode locations. The electrodes were inserted into a pedestal (No. 303; Plastic Products) and the assembly affixed to the skull with two additional anchoring screws and dental acrylic.

Estradiol Replacement Procedure

Capsules were constructed from 10 mm lengths of Silastic tubing (0.058 in i.d. × 0.077 in o.d.; Dow-Corning Corp., Midland, MI) sealed at each end with Dow-Corning Silastic Elastomer. Each capsule contained a 5 mm length of a packed mixture of 10% 17 β-estradiol in cholesterol (Sigma Chemical Co., St. Louis, MO) routinely reported to maintain serum estradiol levels of 30–40 pg/ml [29,35]. Identically constructed control capsules contained only cholesterol.

Immediately following electrode implantation, each rat received a single capsule containing estradiol/cholesterol or cholesterol (randomly determined) implanted subcutaneously in the dorsal neck region. Some rats received capsules containing 1% estradiol in cholesterol to maintain lower serum estradiol levels (9–10 pg/ml; [29]). Other rats received two 10% estradiol capsules to maintain higher serum estradiol levels of 45–60 pg/ml [15].

Amygdala Kindling Procedure

Rats were acclimated to the Plexiglas stimulation cages (30×30×64 cm) on days eight and nine following surgery. On day 10, daily kindling stimulations (1 sec of 60 Hz biphasic, square-wave pulses, 1 msec pulse duration; Grass Model S88 stimulator coupled with 2 Grass PSIU6 stimulus-isolation, constant-current units) were begun. The amygdala AD response was recorded with a Grass Model 7 polygraph. An electronic relay disconnected the polygraph during stimulations. Stimulations were always delivered between 9:00 and 11:30 a.m., at the same time each day for each rat.

During the first stimulation session, the afterdischarge (AD) threshold was determined with an ascending series of 10 μA incremental stimulations at four minute intervals. Thereafter, each animal received kindling stimulations at twice AD threshold current in order to normalize stimulation intensity between animals. Convulsive responses during kindling were identified using the stage 0–5 paradigm of Racine [40] and animals were considered kindled after two consecutive stage 5 responses.

Post-Kindling Procedures

Twenty-four hours following completion of kindling, the AD threshold was determined in all rats using the above procedure. The following day, the estradiol capsule in 10 randomly selected rats was replaced with a control cholesterol capsule; the remaining estradiol-replaced rats received fresh estradiol capsules to control for the surgery (light ether anesthesia.). Twelve days following kindling, all animals were administered suprathreshold (4×threshold) stimulation to verify retention of the stage 5 response.

Electrode Tip Placement Verification

Rats were deeply anesthetized with pentobarbital and perfused (intracardiac) with 0.9% saline followed by phosphate-buffered (pH 7) 10% formalin saturated with potassium ferrocyanide. Anodal current was passed through each lead of the bipolar amygdala electrode and the brains removed and frozen-sectioned. The center of the resulting blue spot was used to identify the electrode tip within the amygdala.

Data Analysis

Significant differences in AD number were tested by the Mann-Whitney U-test. All other tests of significance were done with Student's *t*-test, unpaired, two-tailed.

RESULTS

The acquisition of amygdala-kindled seizures in all ovariectomized female rats used in this study with (one 10% estradiol capsule each) or without estradiol replacement is summarized in Table 1. Rats without estradiol replacement required significantly more trials with AD to kindle (132%), and accumulated significantly more sec of AD during kindling (130%), compared to rats with estradiol replacement. The duration of the first kindling AD or the first stage 5 AD were not significantly different in rats with and without estradiol.

Regional Electrode Distribution and Kindling Rates

When the location of the electrode within the amygdala was considered, altered kindling rates were found to correlate with specific anatomical regions of the amygdala (Fig. 1). Three of these regions were the central (ACE), medial (AME) and cortical (ACO) nuclei. A fourth region comprised the ABL complex of nuclei including the basolateral (ABL), basomedial (ABM), and lateral (AL) nuclei.

ACE and AME electrode placements. Similar overall rates of kindling (total number of trials with AD and accumulated AD sec) were found in rats with and without estradiol replacement (Table 2A). Although the number of stage 0 responses was significantly greater in rats without estradiol (Fig. 2A), this difference was not sufficient to change the overall rate of kindling. Rats with estradiol had longer AD durations during stage 0 compared to rats without estradiol. Although this difference did not reach statistical significance, the combination of fewer, but longer ADs led to no

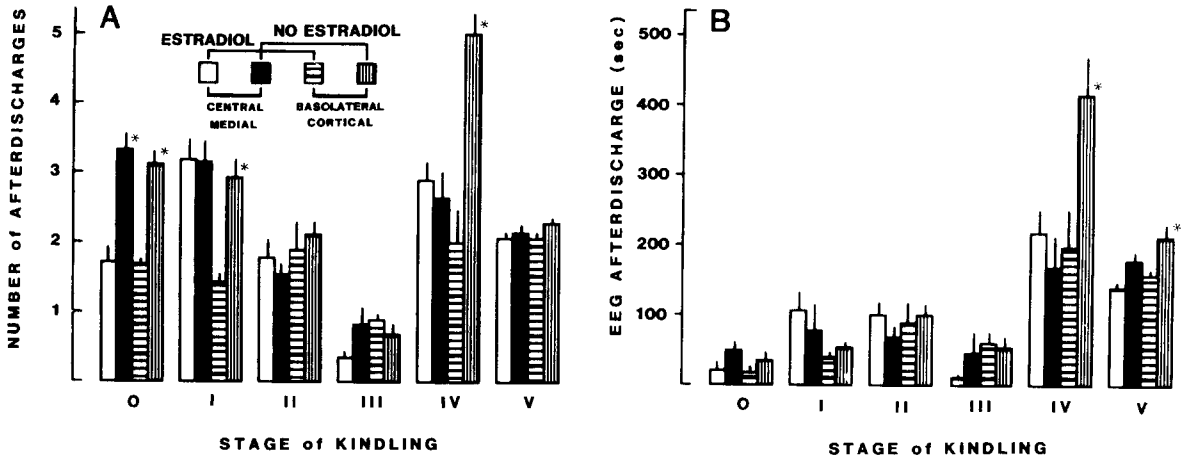

FIG. 2. Per-stage analysis of amygdala kindling as influenced by estradiol replacement and electrode location. (A) Number of trials with afterdischarge (AD) accumulated during each stage of kindling. (B) Sec of AD accumulated during each stage of kindling. Data from rats with electrodes within the central or medial nucleus, or within the basolateral nuclei or cortical nucleus, are combined because of no significant within-group differences. The bars represent the mean±s.e.m. ADs or AD sec during each stage of kindling, N=8–14 per group. *Indicates significant from estradiol-replaced rats with the same electrode distribution, $p \leq 0.05$. Stage 0=no motor response or motor arrest; Stage 1=licking, facial clonus, chewing and/or eye blinking; Stage 2=stage 1 response + head nodding; Stage 3=unilateral forelimb clonus; Stage 4=rearing with bilateral forelimb clonus; Stage 5=stage 4 response + repeated falling and ear adduction [40].

TABLE 3

THE INFLUENCE OF ESTRADIOL REPLACEMENT DURING EARLY AND LATE KINDLING STAGES ACCORDING TO ELECTRODE PLACEMENT

	Trials With AD (Hormonal Treatment)		Sec of AD (Hormonal Treatment)	
	Estradiol	No Estradiol	Estradiol	No Estradiol
A. Central Medial Electrodes				
Stages 0–2	6.8±0.6	7.9±0.5	225±33	195±23
Stages 3–4	6.0±0.4	5.4±0.3	376±40	365±47
B. Basolateral Cortical Electrodes				
Stages 0–2	5.1±0.5	8.1±0.6*	177±22	185±19
Stages 3–4	5.4±0.3	8.2±0.4*†	405±41	703±55*†

The data indicate the number of trials with AD and total sec of AD accumulated during the early stages (0, 1 and 2) and late stages (3, 4 and 5) of kindling. Data are expressed as mean ± s.e.m., N=7–14 per group.

*Indicates significantly different from estradiol replaced rats, $p<0.05$.

†Indicates significantly different from corresponding rats with central or medial electrode placements, $p<0.05$.

TABLE 4

AD THRESHOLDS IN OVARIECTOMIZED RATS WITH AND WITHOUT ESTRADIOL REPLACEMENT ACCORDING TO THE REGIONAL DISTRIBUTION OF ELECTRODE SITES

	Pre-Kindling AD Threshold (Hormonal Treatment) μAmps		Post-Kindling AD Threshold (Hormonal Treatment) μAmps	
Amygdala Region	Estradiol	No Estradiol	Estradiol	No Estradiol
Central and Medial n.	39.5± 2.8	66.7±11.2*	40.5±2.8	66.7±10.2*
Basolateral and Cortical n.	59.0±10.5	58.2± 5.5	62.5±8.7	52.9± 3.9

Data are expressed as mean±s.e.m., N=7–14 per group.
*Significant from estradiol-replaced rats, $p<0.05$.

significant difference in the total AD sec accumulated during stage 0 (Fig. 2B) or combined stages 0, 1 and 2 (Table 3A).

ABL and ACO electrode placements. Rats with ABL electrodes and without estradiol accumulated significantly more trials with AD (140%) and sec of AD (151%) to complete kindling compared to rats with estradiol (Table 2B). Rats with ACO electrodes showed similar increases of 160% and 154%, respectively.

During stages 0 and 1, ABL/ACO rats without estradiol accumulated significantly more trials with AD (Fig. 2A). However, the tendency toward longer stage 0 and 1 AD durations in estradiol replaced rats led to no significant differences in total stage 0 or stage 1 sec of AD (Fig. 2B) or in combined stages 0, 1 and 2 (Table 3B).

The later stages of kindling were particularly sensitive to the absence of estradiol in ABL/ACO rats. Significantly more stage 4 responses were accumulated in rats without estradiol compared to rats with estradiol (Fig. 2). The increased number of stage 4 responses, combined with the tendency for longer stage 4 ADs in rats without estradiol (83±13 sec) compared to rats with estradiol (68±12 sec), resulted in significantly more sec of AD accumulated in rats

without estradiol compared to those with estradiol during stage 4 (Fig. 2B) and combined stages 4 and 5 (Table 3B).

In addition to the greater number of stage 4 responses, eight of the 14 rats kindled in the absence of estradiol (3—ACO and 5—ABL electrodes) regressed to lower stage responses (stage 1, 2 or 3) during stimulation sessions between the first stage 4 and first stage 5 responses. The regressions ranged from a single lower response to as many as three responses which alternated with stage 4 responses. Short AD durations (20–40 sec) accompanied these regressed responses. Three of these rats, and three additional rats without estradiol (2—ABL and 1—ACO electrodes), also showed one or two stage 4 responses (with typical long AD durations) following the first stage 5 response. In contrast, all rats with estradiol replacement progressed smoothly through kindling stages 4 and 5 to the completion of kindling, with no regressed reponses.

Regional Electrode Distribution and Threshold Changes

One other correlation with the electrode site was a change in the threshold for evoking AD. In rats with ACE or AME electrodes, estradiol replacement was associated with a significant 40% lower AD threshold compared to rats without estradiol (Table 3). The AD threshold was not significantly different in rats with electrodes within the ABL or the ACO, with and without estradiol. Post-kindling AD thresholds were not significantly different from the pre-kindling AD thresholds in all groups. Moreover, AD evoked at threshold stimulation always resulted in generalized seizures in kindled rats.

Retention of Kindled Seizures

After 12 stimulation-free days following the completion of kindling, all rats which had been kindled during estradiol replacement, and in which estradiol replacement was continued, responded with the fully kindled stage 5 response. Eight of the 10 rats kindled during estradiol replacement, and in which estradiol was removed following kindling, responded with a stage 5 seizure. The other two rats in this category (ACE and ABL electrodes) showed a stage 2 response; the following day a stage 5 response was obtained in these two rats. All rats kindled in the absence of estradiol, and continued without estradiol, responded with stage 5 responses.

Capsules Containing 1% or 10% Estradiol

Rats implanted with a 1% estradiol capsule and with the electrode tip within the ACE/AME or ABL/ACO nuclei (N=3–4 each), required 12.8±1.6 and 15.5±1.9 trials with AD to kindle, respectively. Total AD accumulation was 625±76 and 912±98 seconds, respectively. These measures of the rate of acquisition of amygdala kindled seizures are not significantly different from those of rats receiving no estradiol replacement (see Table 2). Rats implanted with two 10% estradiol capsules and with electrode tips in the ACE/AME or ABL/ACO nuclei (N=3–5 each) required 13.2±1.2 and 11.2±1.5 trials with AD to kindle, respectively. Total AD accumulation was 479±54 and 543±32 seconds, respectively. These values are not significantly different from those of rats implanted with a single 10% estradiol capsule (see Table 2).

Body Weight Changes

All animals used in these experiments showed an increase in body weight over the duration of the experiments. The largest increments occurred in rats receiving no estradiol (54.2±2 g) and those implanted with a 1% estradiol capsule (62.3±3 g). Significantly less body weight was gained by rats receiving estradiol replacement from one capsule (15±4 g) or two (12.3±2 g) 10% estradiol capsules. Slowed weight gain in rats receiving estradiol replacement similar to that used in the present study has been previously reported (Biegon *et al.* [3]). There was no correlation between electrode position and body weight gain.

DISCUSSION

The results demonstrate that the continuous replacement of estradiol to ovariectomized female rats at levels within the physiological range facilitates amygdala kindling. More importantly, the effect of estradiol was found to be markedly dependent upon the site of amygdala stimulation. Although differences in kindling rates have been related to the intra-amygdaloid site of stimulation [12,24], this is the first report that a treatment effect on seizure acquisition is clearly dependent on the site of amygdala stimulation. Others have reported that repeated estradiol injections to intact young rats did not alter amygdala kindling [44]. However, 84% of their electrode placements were within the central or medial amygdaloid nucleus; the present study found little difference between rats with and without estradiol replacement during kindling by stimulation of these nuclei. Although other factors such as age and method of estradiol administration may have contributed to their different findings, the present results suggest that electrode position is the dominant factor.

The effect of estradiol to decrease the AD threshold of the AME in ovariectomized rats [46] was confirmed by the present study and extended to include the ACE region. These local threshold changes may have contributed to the limited influence of estradiol on the earliest stage of kindling during ACE/AME stimulation. The greatest impact of estradiol on kindling, however, was during stimulation of the ABL or ACO areas in which the AD threshold was not altered by estradiol. Therefore, it is likely that the site-dependent effects of estradiol on kindling reported here relate to differences in circuitry accessed by amygdala stimulation sites rather than as a result of local alterations in thresholds.

During the early stages of kindling, seizure activity is limited to the amygdala and its ipsilateral projections [10]. The well-documented excitatory or proconvulsive properties of estradiol [11, 19, 25, 47–49] may have facilitated seizure propagation within this spatially limited seizure network. A major amygdala efferent pathway, the stria terminalis (ST), has prominent origins within the ACE and AME of the amygdala [16] and carries inhibitory fibers to the ventromedial hypothalamus (vmH) [8,42]. Lesioning the ST increases the rate of amygdala kindling limited to the initial stage of kindling [9] similar to the effects found here during ACE/AME stimulation. On the other hand, the ABL regions project heavily into the ventral amygdalofugal pathway (VAF) which projects to the vmH [6,18]; the ACO also accesses the vmH by way of the medial portion of the ventral ST [10]. Lesioning the VAF retards amygdala kindling [41], suggesting it is a major efferent pathway for seizure propagation from the amygdala. Estradiol facilitates neuronal excitability within the vmH [20], including VAF target neurons [39]. Therefore, the ability of estradiol to facilitate early kindling, including the greater effect during ABL/ACO stimulation, may relate to a relatively limited ef-

fect of estradiol within the vmH, a region more or less accessed by all amygdala regions.

It is clear that proconvulsive properties of estradiol (i.e., to decrease the number of ADs, but not total seizure experience) do not completely explain the late kindling results in ABL/ACO rats. During the protracted stage 4 acquisition period in rats without estradiol, the tendency for longer AD durations and the almost two-fold increase in total EEG AD accumulation differs sharply from the results during early kindling. All of these retained the fully kindled seizure response after 12 stimulation-free days, indicating that the fully kindled state had been reached. Therefore, while estradiol was not required for late kindling to occur, its absence obviously decreased the efficiency of the process. Stage 1 or 2 responses in fully kindled rats show 2-deoxy-D-glucose uptake patterns resembling partially kindled rats [10]. Therefore, the regressed responses observed in many rats without estradiol were likely due to a return to the spatially localized seizures of partial kindling. These regressions and the observation that many ovariectomized rats without estradiol fail to "kindle" during repeated pentylenetetrazol injections [15], emphasize that the lack of estradiol was associated with unstable conditions for late kindling. Whether the optimized conditions for kindling maintained by estradiol relate to genomic functions involving changes in protein synthesis [28,39], or is due to some other property of estradiol which maintains convulsive readiness within the involved circuits awaits further study.

The marked difference in the effects of estradiol between ABL/ACO and ACE/AME rats during late kindling suggests that AD originating from the ABL and ACO utilizes additional circuitry that is not as readily accessed by AD from the ACE and AME. This additional circuitry may include sites of action which enable the effects of estradiol to optimize the final acquisition phase of kindling. The lateral entorhinal cortex receives projections from the amygdala, primarily from the ACO [14,22] and ABL regions [2,21]. In turn, the lateral entorhinal cortex projects to widespread areas including the hippocampus, the pyriform cortex and endopyriform nucleus and the prefrontal cortex, areas implicated as having a major role in limbic kindling or seizure production [7, 37, 38, 43]. These areas are potential sites mediating the influence of estradiol on late seizure acquisition.

It is of particular interest that ablative lesions of the orbital or prefrontal cortex lead to a profile of behavioral response regressions and slowed late kindling during amygdala stimulation suggesting that these cortical regions, with widespread projections, reinforce or sustain kindling circuits forming elsewhere in the brain during the later stages of kindling [5]. Estradiol has been shown to enhance neocortical excitability in several species [17, 27, 32, 33]. Therefore, estradiol may maintain the conditions necessary for anterior neocortical regions to provide the required level of facilitation to motor regions involved in the final stages of kindled seizure acquisition. The absence of estradiol would then interfere with the reinforcing influence of the cortex, perhaps by disrupting a convergence of seizure activity [4] from cortical areas (or other structures) into regions of the brain which may be crucial to kindled seizure acquisition, such as the brain stem and mesencephalic reticular formation [10,30]. Further study of the effect of estradiol on late kindling acquisition may provide clues in support of the hypothesis that the generation of limbic seizures requires positive feedback in a network involving many regions [31].

In conclusion, the results of this study suggest complex interactions of estradiol with the process of acquisition of amygdala kindled seizures in ovariectomized female rats. Further studies are needed to better understand these interactions of estradiol with seizure processes and to establish the relationship of these effects of estradiol in rats to catamenial epilepsy in humans. It should be pointed out, however, that not all women with a seizure disorder experience catamenial exacerbation of seizure frequency or severity. Based upon the present results, catamenial symptoms may depend upon the location of the seizure focus, the circuitry recruited during seizure development or the presence or location of secondary epileptic foci. The present results also add to the evidence that the rate of amygdala kindling varies with the intra-amygdaloid site of stimulation, supporting the need for close attention to electrode placement when interpreting amygdala-kindling data [24] especially when treatment effects are compared.

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