BIOLOGY OF REPRODUCTION 22, 927–934 (1980)

Effects of Corticomedial Amygdala Lesions or Olfactory Bulbectomy on LH Responses to Ovarian Steroids in the Female Rat[1]

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ABSTRACT

Adult female rats received bilateral corticomedial amygdala lesions, olfactory bulbectomy, or sham surgery. Following ovariectomy, luteinizing hormone (LH) responses to estradiol benzoate (EB) and progesterone (P) treatment were assessed. With 4 weeks separating each injection, each rat received at 1200 h each of 3 doses of EB: 0.7, 3.5 and 7.0 μg/100 g BW. Oil or 2 mg of P was administered 72 h after EB treatment. Jugular blood samples (0.5 ml) were taken 5 times for each dose of EB: 43 h prior to, and 5, 29, 53 and 77 h after EB administration. Five hours after the 0.7 μg/100 g BW dose of EB, and at both 5 and 29 h after the 3.5 μg/100 g BW dose of EB, plasma LH levels were depressed in all groups, but subsequently recovered to near pre-EB levels. Plasma LH levels were also depressed 5 and 29 h after the 7 μg EB/100 g BW dose in all groups, but by 53 h LH levels were significantly elevated beyond pre-EB levels when analyzed without regard to surgical treatment. However, the rats with corticomedial amygdala lesions did not respond to this high dose of EB; plasma LH titers 53 h post-EB were only equivalent to pre-EB levels. Regardless of surgical treatment, all rats given P 72 h after EB treatment responded with elevated LH levels relative to LH levels in oil-treated controls. As the dose of EB increased from 0.7 to 7 μg/100 g BW, the magnitude of the P-induced rise in LH increased. Thus, although olfactory bulbectomy was without effect, corticomedial amygdala lesions attenuated LH elevations induced by EB but not those induced by EB plus P treatment. The corticomedial amygdala may play a role specifically in the positive feedback action of EB.

INTRODUCTION

The female rat displays a cyclic pattern of release by luteinizing hormone (LH) which is modulated by the feedback of ovarian steroids. While the medial basal hypothalamus (MBH) appears to be a neural site crucial to negative feedback of steroids on LH secretion, the anterior hypothalamic/preoptic area (AH/POA) is also apparently involved in the mediation of facilitatory feedback effects (Sawyer, 1975; Everett, 1977; Blake, 1978; Goodman, 1978). In addition, extrahypothalamic structures may exert a modulatory influence on the LH response to ovarian steroids, perhaps acting through the AH/POA to coordinate LH release with environmental stimuli, such as day length or copulation.

Two estrogen-sensitive regions (Pfaff and Keiner, 1973; Morrel et al., 1975) which have been implicated in the control of LH release in the female rat are the olfactory system and the corticomedial amygdala. Exposure to male odors can shorten the estrous cycle (Aron, 1974) and can induce ovulation in anovulatory rats exposed to constant light (Johns et al., 1978). Acute olfactory bulbectomy can prevent copulation-induced ovulation in the pentobarbital-blocked rat (Curry, 1974), and bulbectomy is known to increase sensitivity to estradiol benzoate (EB) in the ovariectomized rat as assayed behaviorally by the propensity to exhibit lordosis (Edwards and Warner, 1972; McGinnis et al., 1978; Tyler and Gorski, 1980). However, possible bulbectomy-induced alterations in steroid sensitivity as measured by LH release have apparently not been evaluated. The corticomedial amygdala appears, on the basis of the results of steroid

Accepted February 6, 1980.
Received July 3, 1979.
[1]Supported by NIH Grant HD-01182 and the Ford Foundation.
[2]Submitted in partial fulfillment of the requirements for the Ph.D. degree. Support was received from a UCLA Chancellor's Dissertation Fellowship and the Mental Health Training Program of the Brain Research Institute. Current address: Department of Biological Sciences, Northwestern University, Evanston, IL 60202.

implant (Terasawa and Kawakami, 1974; Docke et al., 1975; Kalra and McCann, 1975), electrophysiological (Velasco and Taleisnik, 1969; Kawakami et al., 1970, 1973; Kawakami and Terasawa, 1974; Carrillo et al., 1977) and lesion (Velasco and Taleisnik, 1971; Velasco, 1972) studies to be implicated in the facilitatory feedback of steroids on LH secretion. Furthermore, olfactory structures project to the amygdala and might influence LH regulation through this structure.

In the present experiment, several components of ovarian steroid feedback effects on LH release following olfactory bulbectomy or corticomedial amygdala lesions were examined. Effects of chronic bulbectomy or amygdala lesions on ovarian cyclicity as determined by vaginal smears were studied first, and then the animals were ovariectomized. Chronically gonadectomized females were used to allow comparison of these data with those of previous reports on the effects of limbic lesions. A classical protocol of steroid treatment (Taleisnik et al., 1969) was employed for the same reason. Portions of this study were presented in abstract form (Vitale and Gorski, 1978).

MATERIALS AND METHODS

Female Sprague-Dawley rats were housed singly under reversed lighting conditions (light on 0500–1900 h) with food and water available ad libitum. Vaginal smears were taken daily for 2 weeks. Rats were 4 day cycles then received one of three surgical treatments. Surgery was conducted under methohexital sodium (Brevital) anesthesia before the onset of the afternoon critical period for LH release. Stage of the estrous cycle at the time of surgery was randomly distributed within each treatment group. Bilateral olfactory bulbectomy (BULBX) was performed on 23 rats, using a cut and suction technique. A knife was inserted at the junction of the bulbs with the frontal cortex. The tissue anterior to the cut was then aspirated and the resultant cavity was filled with Oxycel for hemostasis. Twenty-two rats received bilateral electrolytic lesions of the corticomedial amygdala (AMYG). A platinum electrode with the blunt tip uninsulated was used to deliver 2.5 mA of anodal current for 10 sec. Coordinates, based on de Groot (1959), were: 0 mm anterior to bregma, 3.5 mm lateral, and 8.1 mm below the dura. Sham surgery (SHAM) consisted of drilling through the skull to the dura over the olfactory bulbs (n = 9) or amygdala (n = 9).

Vaginal smears were taken daily for 3–5 weeks postsurgery until rats were ovariectomized. Three weeks after ovariectomy, tests for LH response to steroids were initiated. Each rat received s.c., in ascending order, each of 3 doses of EB at 1200 h: 0.7, 3.5 and 7.0 μg/100 g BW. Doses were given at 4 week intervals. The regimen for taking plasma samples for LH assay was the same under each dose of EB. Blood (0.5 ml) was drawn via jugular puncture under ether anesthesia at 1700–1745 h on 5 days (Samples 1 through 5). The first blood sample was taken 43 h prior to EB injection. Additional samples were taken 5, 29, 53 and 77 h after EB treatment. Each surgical group was then divided into an oil or a progesterone (P) subgroup. Rats in the oil subgroup received 0.05 ml sesame oil 72 h after EB was administered; those in the P subgroup received 2 mg P in 0.05 ml oil at that time. Blood sample 5 was taken 5 h after the oil or P injection.

Plasma samples were stored at −40°C until assayed. LH was assayed using the methods of Niswender et al. (1968) and the instructions supplied in the NIAMDD kit. The reference preparation was LH-RP-1, which has a biological potency of 0.03 × NIH-LH-S1. Antibodies were purchased from Antibodies, Inc., Davis, CA. Plasma samples were assayed in duplicate, and the values averaged. Because of the large number of samples involved, three consecutive assays were done, with grouping based on dose of EB. Within and across assays the order of treatment-groups and times of sampling were counterbalanced. The same preparations of anti-LH, anti-rabbit gamma globulin and iodinated LH were used for all 3 assays. Analysis of variance was used to analyze overall treatment effects, followed by Scheffe, Chi-square and F tests.

Animals were sacrificed under ether anesthesia and perfused with 10% formalin. Brains from rats in the AMYG group were frozen and 60 μm sections were stained with thionin for lesion verification. Brains from rats in the BULBX group were examined under a dissecting scope fitted with a grid reticule. The aspiration boundaries of each brain, as seen from ventral, dorsal and lateral perspectives, were then drawn on analysis sheets which had outlines of the intact brain as seen from each perspective superimposed on a grid background.

RESULTS

Histology

The amygdala lesions were found to be limited to the basal aspects of the cortical and medial amygdaloid nuclei. The point of maximum lesion size fell between +0.2 mm AP and −1.4 mm AP (de Groot, 1959) for all but one lesion. Data from 1 rat were excluded from analysis because of failure to observe lesioned tissue. Two typical lesions are presented in Fig. 1. Examination of the electrode tracts revealed very faint scars, since the animals had been lesioned 5 months prior to sacrifice. The electrode tracts typically coursed just lateral to the stria terminalis dorsally and did not appear to transect it. Due to the coordinates used, however, the electrode could have damaged the stria terminalis at its origin. A photograph of a brain from a rat in the BULBX group and a summary of the aspiration boundaries of this

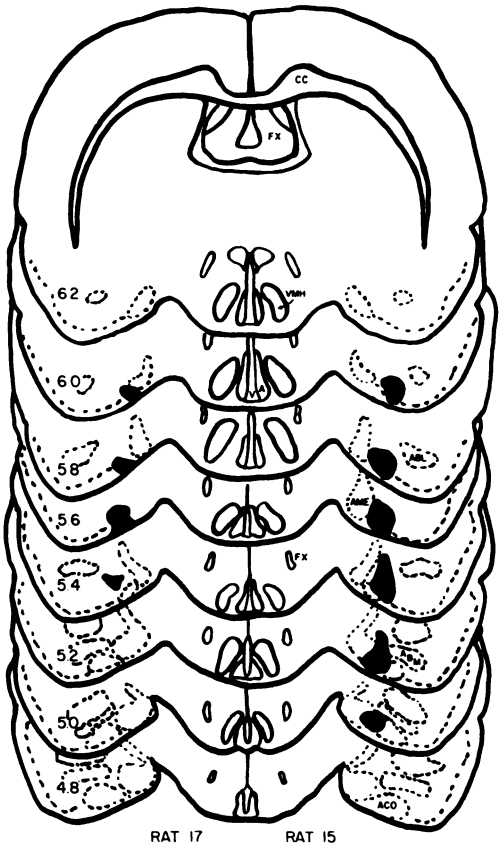

FIG. 1. Examples of representative corticomedial amygdala lesions. The lesion of Rat 17 (left) is typical of the smaller lesions, while that of Rat 15 (right) is typical of the larger lesions in this study. Abbreviations: A, arcuate; AMB, amygdala mediobasal nucleus, ABL, amygdala basolateral nucleus; ACO, amygdala cortical nucleus; AME, amygdala medial nucleus; CC, corpus callosum; Fx, fornix; V, third ventricle; VMH, ventromedial hypothalamus. Sections are modified from the Pellegrino and Cushman atlas (1967).

group are presented in Fig. 2. In general, there was no or minimal damage to the frontal cortex, and a thin layer of bulb tissue frequently remained on the ventral surface. The peduncle was left intact in most cases. One brain showed very variable boundaries, with substantial bulb tissue remaining unilaterally. Data from this rat were excluded from analysis.

Vaginal cycles of 8 rats were disrupted postsurgery; periods of diestrous smears lasting from 3 to 19 days were observed in rats in the AMYG and sham-operated groups. Chi-square tests showed no significant difference in the frequency of irregular cycles over lesion groups. There were no significant group differences in body weight at any point in the experiment.

LH Response to EB

0.7 μg EB/100 g BW. Figure 3a depicts plasma LH levels before and after administration of the 0.7 μg EB/100 g BW dose for Samples 1 through 4. An analysis of variance (Surgical Group by Time) was performed. There was a significant effect of Surgery ($F = 3.799$, $P = 0.023$) and Time ($F = 43.153$, $P<0.001$), but no interaction ($F = 1.7$, $P = 0.119$). However, an F test on LH titers at Sample 1 revealed a significant effect of surgical group on the pre-EB LH levels ($P<0.01$). Because this significant pre-EB group difference precluded the direct comparison of groups at each time, separate analyses of variance were done on each surgical group over time. Then, Scheffe's tests were applied to each set of data. All three surgical groups showed a significant effect of time at $P<0.001$. However, Scheffe's test revealed slight differences among the groups in the response to EB over time. The SHAM group was slightly more sensitive to the negative feedback effects of a low dose of EB than were either of the other groups. Samples 3 and 4 were both significantly lower than Sample 1 only for the SHAM group ($P<0.05$). All three surgical groups showed significant ($P<0.05$) negative feedback at 5 h post-EB (Sample 2).

3.5 μg EB/100 g BW. LH levels prior to and following the 3.5 μg EB/100 g BW dose are presented in Fig. 3b. Analysis of variance (Surgical Group by Time) revealed a significant main effect of time ($F = 53.128$, $P<0.001$), but neither a surgical group effect nor an interaction. There were no significant surgical group differences at Sample 1, the pre-EB sample (F test, $P = 0.69$). Regardless of surgical group, LH levels in Samples 2 and 3, while not differing from each other, were both significantly lower than levels in Sample 1 ($P<0.01$) or Sample 4 ($P<0.01$; Scheffe's test). Levels in Samples 1 and 4 did not differ from each other. Thus, 3.5 μg EB/100 g BW was effective across surgical groups in suppressing LH 5 and 29 h after EB administration.

7.0 μg EB/100 g BW. LH levels prior to and following the 7.0 μg EB/100 g BW dose are presented in Fig. 3c. Analyses of variance (Surgical Group by Time) revealed a significant main effect of Time ($F = 41.1961$, $P<0.001$) and Surgical Group ($F = 3.704$, $P = 0.026$); the

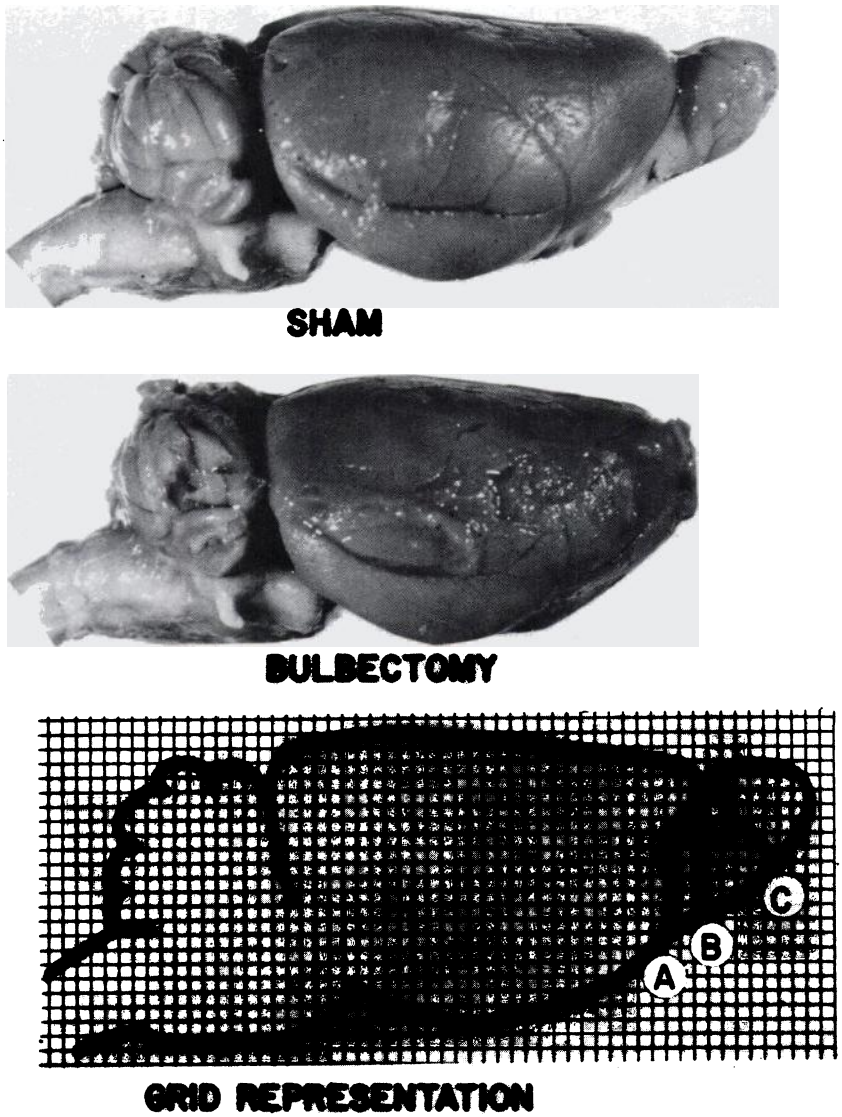

FIG. 2. Lateral view of the brains of a sham-operated and bulbectomized rat. The lower figure illustrates the grid system used to record the extent of bulbectomy and summarizes tissue removal for this study: A, the most posterior aspiration boundary; no tissue caudal to this was aspirated in any animal. B, representation of the aspiration boundary shown above, which also represented the mean extent of bulbectomy, i.e., tissue anterior to this line was removed in the typical rat. C, the most anterior aspiration boundary, indicating the minimal damage inflicted in any animal.

interaction was not significant ($F = 1.889$, $P = 0.084$). There were no significant surgical group differences at Sample 1 ($F = 0.754$, $P = 0.52$). Scheffe's test applied to the time effect without reference to surgical group revealed significant ($P<0.05$) differences between all pairs of samples except Samples 2 and 3 (5 and 29 h post-EB). Thus, 7 μg EB/100 g BW produced a significant depression of LH titers 5 and 29 h later regardless of surgical group, but by 53 h the overall LH titers were significantly elevated above pre-EB levels. Dunnett's test applied to the main effect of surgical group indicated that amygdala-lesioned rats consistently had lower LH levels compared with those of the SHAM group controls. This differential response was most pronounced 53 h post-EB (Sample 4) at which time there was a marked increase in LH titers in the other two groups which presumably contributed to the overall significant

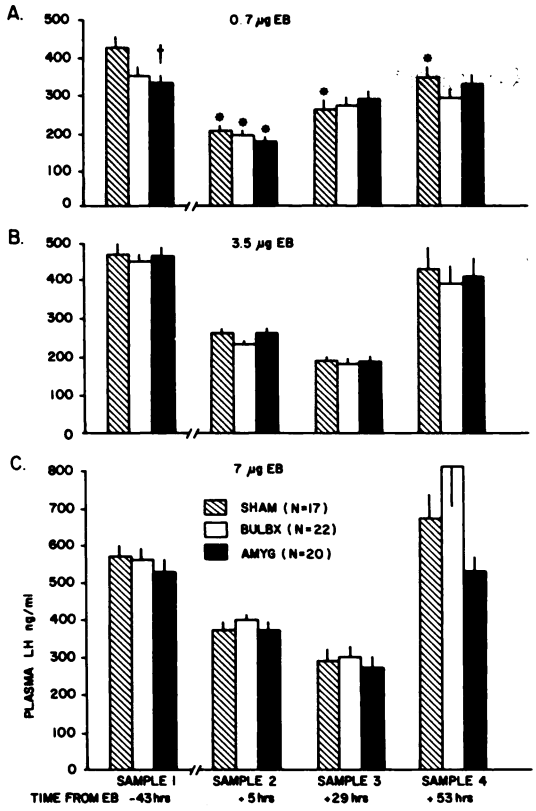

FIG. 3. Mean plasma LH levels (+ SEM) in afternoon (1700–1745 h) blood samples obtained from ovariectomized sham-operated (SHAM), bulbectomized (BULBX) and amygdala-lesioned (AMYG) rats following a single injection of: A, 0.7 μg; B, 3.5 μg; and C, 7.0 μg estradiol benzoate (EB) per 100 g BW. See text for results of statistical analyses of B and C. †Significantly different ($P<0.05$, F test) from SHAM group values at that time. *Significantly different ($P<0.05$) from pre-EB LH levels.

difference between LH values in Samples 1 and 4.

Examination of the results of the Surgical Group by Time ANOVA revealed a large mean squares (within group), which resulted in a large critical difference under the Scheffe test. A considerable contribution to this variance can be traced to LH levels at Sample 4 (53 h post-EB). Since the raw data indicated that a number of rats showed very high LH levels at Sample 4, the LH value of each rat at Sample 4 was expressed in ratio to its Sample 1 value. An arbitrary cutoff point of 1.5 was assigned to identify rats showing substantial elevation of LH at Sample 4, as shown in Fig. 4. Chi square tests were then applied to test whether the proportion of rats showing elevated LH at Sample 4 varied by surgical group; both the SHAM and BULBX groups had a significantly greater proportion of rats showing elevated LH levels compared with those of the AMYG group ($\chi^2 = 7.425$, $P<0.01$ and $\chi^2 = 9.56$, $P<0.01$, respectively). The SHAM and BULBX groups did not differ from each other ($\chi^2 = 0.471$, $P = 0.5$).

This same criterion was applied to the data from Samples 1 and 4 following treatment with 0.7 μg and 3.5 μg EB/100 g BW, but very few animals met this criterion and there were no significant differences among these groups.

LH Responses to Oil and P

There were no differences between oil and P groups on Samples 1 to 4 (i.e., prior to administration of oil or P) at any EB dose. Data from Sample 5 are presented in Fig. 5. The analysis of variance (Surgical Group by Oil vs P by Dose of EB) demonstrated a significant oil vs P effect ($P<0.001$), a significant dose of EB effect ($P<0.001$) and a significant interaction of dose by oil vs P ($P<0.001$). Regardless of surgical group, P elevated LH levels relative to those of oil-treated controls. The size of the priming dose of EB determined the magnitude of the response to P, and this was not dependent on surgical group.

DISCUSSION

In this study, three components of steroid feedback effects on afternoon LH levels were examined in olfactory bulbectomized or amygdala lesioned rats: EB-induced suppression of LH, EB-induced facilitation of LH, and EB plus P-induced facilitation of LH. Bulbectomy did not alter vaginal cyclicity, nor did it alter the LH response patterns following administration of EB or P in ovariectomized rats. However, in female rats bulbectomy typically results in increased behavioral sensitivity to EB as assayed by the propensity to exhibit lordosis (Edwards and Warner, 1972; McGinnis et al., 1978; Tyler and Gorski, 1980). Thus, the effect of bulbectomy on LH release in response to EB differs from its effect on behavioral sensitivity to EB. While sexual receptivity and the release of LH are normally temporally coordinated events, distinct extrahypothalamic mechanisms may contribute to the regulation of each.

In contrast, rats receiving small corticome-

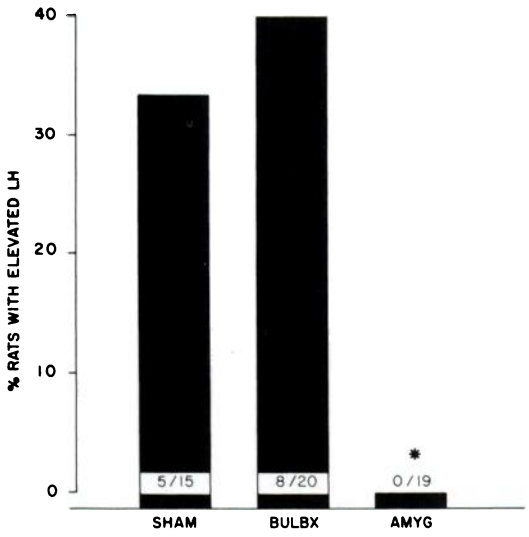

FIG. 4. The influence of sham surgery (SHAM), bulbectomy (BULBX) or amygdala lesions (AMYG) on the ability of an injection of 7.0 μg estradiol benzoate (EB)/100 g BW to elevate plasma LH levels in ovariectomized rats 53 h later (1700–1745 h). LH values at least 50% greater than those obtained prior to EB treatment were considered elevated.

dial amygdala lesions showed a clear deficit in the facilitatory feedback effects of 7 μg EB/100 g BW on LH. Since neither the negative feedback effects of EB nor the facilitatory feedback effects of EB plus P treatment were substantially altered, the lesion effect is apparently specific to the disruption of the ability of a single bolus of EB to facilitate LH.

Steroids act at both neural and pituitary levels to suppress or facilitate LH release (for review see Blake, 1978). In particular, the MBH has been identified as a site at which EB acts to inhibit LH release (Blake et al., 1974). It may also be a site through which P acts in the EB-primed rat to inhibit daily LH elevations (Blake, 1977). Since EB-induced inhibition of tonic LH release remains following surgical isolation of the MBH (Blake, 1978), other neural structures may not be involved in the inhibiton of LH by EB. However, extrahypothalamic structures may modulate the positive feedback effects of steroids on LH. In fact, several studies have implicated the corticomedial amygdala region in this regard. Lesions of the medial amygdala or transection of the stria terminalis on proestrus block ovulation, but cyclic ovulation eventually returns (Velasco and Taleisnik, 1971; Velasco, 1972). Stimulation of the corticomedial region may induce ovulation (Velasco and Taleisnik, 1969; Kawakami et al., 1970) and gonadotropin release (Kawakami et al., 1973; Kawakami and Terasawa, 1974) and synchronize the time of the proestrous LH surge (Carillo et al., 1977). Estrogen implants in the medial amygdala advance ovulation in cycling adults (Terasawa and Kawakami, 1974; Docke et al., 1975) and induce gonadotropin release (Kalra and McCann, 1975). Although these results demonstrate a role of the corticomedial amygdala region in the control of ovulation, in the present study animals did not suffer from acute surgical trauma, and LH levels were evaluated under several steroidal conditions. Thus, we have been able to define more precisely the role of the corticomedial amygdala in the control of gonadotropin release.

It is especially noteworthy that the LH response of the AMYG group animals to P was equivalent to that of the other groups. Recently, Moguilewsky and Raynaud (1979) demonstrated that estrogen induces an increase in P receptors in the pituitary and hypothalamus but not in the amygdala. This information is concordant with the data on LH release presented here and may indicate that the amygdala, which can influence the facilitatory feedback effects of EB on LH, may not be necessary for the facilitatory feedback action of combined steroid treatment.

Even in the amygdala-lesioned rats, one must assume that after ovariectomy, exogenous EB continued to act at the level of the pituitary to sensitize it to LHRH (Blake, 1978). Such a change in pituitary sensitivity might obscure a subtle deficit in EB-induced LHRH release. In this experiment the criterion chosen to define an elevation in LH was a 50% increase over pre-EB LH levels measured at the same time of day. Clearly, LH levels were elevated 53 h post-EB relative to levels 5 or 29 h post-EB in all groups of animals, but it is impossible to distinguish between the facilitation of LH secretion and the termination of inhibitory feedback. However, at 53 h post-EB only the animals in the AMYG group failed to exceed their basal, pre-EB, LH values. Since the LH levels that the AMYG group animals did achieve were not significantly different from their own pre-EB LH values, this deficit does not seem to reflect enhanced sensitivity to the negative feedback effects of estrogen on LH. However, it is not clear whether the small corticomedial amygdala lesions prevented EB facilitation of

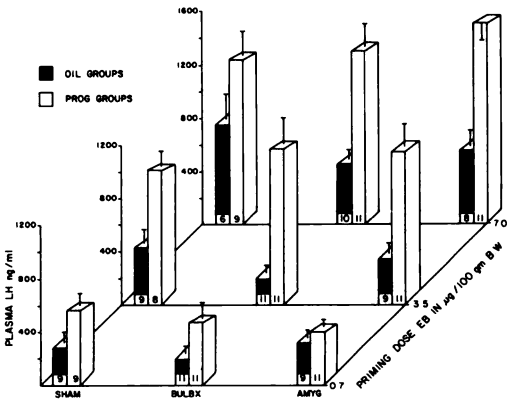

FIG. 5. The effect of progesterone (P) or oil (O) injections in sham-operated (SHAM), bulbectomized (BULBX) and amygdala-lesioned (AMYG) rats given 72 h after each of 3 doses of estradiol benzoate (EB; 0.7, 3.5 and 7.0 µg/100 g BW). Plasma was obtained 5 h after P or O injection. The numbers at the base of each bar indicate n animals in each group. These are the same animals in which the temporal response to only EB treatment is illustrated in Fig. 3. Regardless of surgical treatment, the mean LH level of P-treated rats was greater than that for O-treated rats ($P<0.001$, analysis of variance, Group by Dose EB by O vs P); as the dose of EB increased, the mean LH level following P treatment also increased ($P<0.001$). The SEM is presented at the top of each bar.

LH release altogether, or merely diminished sensitivity to EB. The latter seems more plausible and could be tested by using higher doses of estrogen. In any case, the present data do indicate that small amygdala lesions can produce a specific, well defined disruption in the modulation of LH release.

In this study, the electrode track in rats in the AMYG group was lateral to the dorsal stria terminalis, but may have damaged the stria at its origin. Although we cannot definitively attribute the observed effect on LH release to the lesions rather than to hypothetical damage to the stria terminalis, we have shown that a discrete intervention in the amygdala system disrupts a process which may participate in the control of ovulation.

The AH/POA and MBH exert powerful regulatory influences over reproductive behavior and gonadotropin release. However, limbic neural structures, the influence of which may not be essential for successful reproduction to occur, may still normally contribute to its regulation. Structures such as the amygdala or olfactory bulb may constitute part of a "fine tuning" mechanism to allow for the optimal coordination of hormonal and behavioral events with environmental stimuli.

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