

used. Blood samples were collected every two weeks and analyzed for levels of estradiol, progesterone and corticosterone. A significant decrease in lordosis scores across tests was observed despite comparable serum estradiol levels in initial and final samples. After nine weeks, the animals were assigned to either an adrenalectomy or a sham-adrenalectomy condition. Adrenalectomized animals had moderately higher scores than the sham group on subsequent tests, however, neither group differed significantly from the pre-adrenalectomy condition. Corticosterone and progesterone levels decreased over time. These results do not support the view that the decrease in lordosis was mediated by the adrenal.

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Table of Contents

	Page
Abstract	iii
Acknowledgements	v
List of Tables	vii
List of Figures	ix
Introduction	i
Experiment 1 - Method	26
- Results	32
- Discussion	61
Experiment 2 - Method	65
- Results	71
- Discussion	87
General Discussion	91
References	96
Appendix A	124
Appendix B	129
Appendix C	134
Appendix D	145
Appendix E	147
Errata	148

List of Tables

Table		Page
1	Treatment of animals in Experiment 1.	27
2	Summary table for the ANOVA of lordosis scores on ten weekly receptivity tests.	33
3	Summary table for the ANOVA of lordosis scores on ten weekly receptivity tests using data from WEEKLY estrogen treated animals and 0.5 ug EB DAILY animals.	38
4	Summary table for the ANOVA of lordosis scores on ten weekly receptivity tests using data from WEEKLY estrogen treated animals and DAILY 2 ug EB animals.	40
5	Summary table for the ANOVA of lordosis scores on ten weekly receptivity tests using data from DAILY 0.5 vs 2 ug estrogen treated animals.	41
6	Summary table for the ANOVA of darting/hopping on ten weekly receptivity tests.	43
7	Summary table for the ANOVA holding scores on ten weekly receptivity tests.	45
8	Summary table for the ANOVA of body weights prior to each of ten weekly receptivity tests.	46

Table

Page

- 9 Chart of significant main effects (estradiol treatment: EB; progesterone treatment: P; interruption: I;) and interactions for physiological variables. 53
- 10 Group means for statistically significant main effects and interactions presented in table 9. 55
- 11 Summary table for ANOVA of lordosis scores on test 1 through test 9 inclusive. 76
- 12 Summary table of ANOVA of darting scores on test 1 through test 9 inclusive. 77
- 13 Summary table for the ANOVA of lordosis scores on tests 10, 11 and 12. 78
- 14 Summary table for the ANOVA of darting scores on tests 10, 11 and 12. 79
- 15 Summary table for the ANOVA of lordosis scores before and after adrenalectomy. 80

List of Figures

Figures

Page

- 1 Mean lordosis scores in experiment 1 on weekly receptivity tests of ovariectomized female rats given only estradiol benzoate. 34
- 2 Mean lordosis scores in experiment 1 on weekly receptivity tests of ovariectomized female rats given progesterone (0.5 mg per week) and estradiol benzoate. 35
- 3 Bar graph depicting the differential facilitatory effects of 0.5 mg progesterone in the three treatment conditions of experiment 1. 37
- 4 Number of animals displaying darting/hopping behavior (n = 8) on each receptivity tests in experiment 1. 41
- 5 Number of animals displaying holding behavior (n = 8) on each receptivity test in experiment 1. 44
- 6 Mean body weights in experiment 1 on weekly receptivity tests of ovariectomized female rats given only estradiol benzoate. 48

- 7 Mean lordosis scores for receptivity tests 1 and 10 conducted prior to interruption of steroid treatment in experiment 1, and test 11 conducted after resumption of administration of exogenous steroid. 49
- 8 Number of animals displaying darting/hopping behavior on receptivity tests 1, 10 and 11. 50
- 9 Number of animals displaying holding behavior on receptivity tests 1, 10 and 11. 51
- 10 Mean lordosis scores on each receptivity test for animals in experiment 2. 72
- 11 Percent of animals displaying darting/hopping behavior on receptivity tests in experiment 2. 73
- 12 Percent of animals displaying holding behavior on receptivity tests in experiment 2. 74
- 13 Mean serum progesterone levels obtained from blood samples taken every two weeks during experiment 2 in ovariectomized and ovariectomized-adrenalectomized groups both before adrenalectomy and after (samples 1-4 and after (samples 5 and 6). 82

Figures

Page

- 14 Mean serum corticosterone levels obtained from blood samples taken every two weeks during experiment 2 in ovariectomized and ovariectomized-adrenalectomized group both before adrenalectomy (samples 1-4) and after (samples 5 and 6).

83

- 15 Mean lordosis scores obtained by animals of experiment 2 that were selected on the basis of having reasonably stable estradiol levels.

85

- 16 Mean darting/hopping scores obtained by animals of experiment 2 that were selected on the basis of having reasonably stable estradiol levels.

86

Normal female rats are known to have four or five day cyclic fluctuation in plasma steroid hormones. At a predictable time during each of these cycles, the female rat will permit and often solicit mounting and intromission by a male rat. This relatively short period of receptivity, lasting several hours, called behavioral estrus, precedes and overlaps ovulation and thus may result in successful fertilization. Research into the mechanisms controlling ovulation and female mating behavior indicate that the phenomena are dependent on both the absolute levels of and the pattern of variation of the sex steroids: estrogens (including estradiol (E_2), estrone and estriol) and progesterone (P). In particular, there is a consensus that the absence of estrogens is associated with little or no female mating behavior and that the probability of such behavior increases with plasma levels of estrogens in a dose-dependent manner. Inhibitory effects of estrogens on female mating behavior have not been reported. In this study, the effects of chronic estrogen exposure on several behavioral and physiological variables will be evaluated and experiments suggesting that long-term estrogen exposure is associated with progressively lower levels of female mating behavior will be reported.

In order to interpret these studies some understanding of female mating behavior and of known mechanisms of estrogen action is required. A brief review will also be given of the central role of estrogens throughout ontogeny in the development and maintenance of female mating behavior. Finally, a review will be made of those few studies whose results suggest that the effects of acute and chronic estrogen exposure are distinguishable.

Hormonal control of lordosis: The mating behavior of the estrous female rat is composed of a complex series of acts. Quick running or darting movements, often accompanied by ear wiggling, end in an abrupt halt allowing the male to mount from the rear. As the male mounts, grasping the flanks with the forepaws and thrusting his pelvic region against the female, the female assumes a posture known as the lordosis response. The rear legs are extended and a marked dorsiflexion of the vertebral column elevates the head, rump and tailbase. The posture may be maintained or held for several seconds after the male has dismounted. This sequence of events, indicative of sexual receptivity in the female rat is known to be dependent on the steroid hormones, estrogen and progesterone, synthesized by both the ovaries and the adrenal glands. Experimental studies of the relationships between these steroids and female sexual behavior require precise statements as to the presence or absence of both sets of steroid secretion glands, the schedule and quantities of exogenous steroid administered and the conditions under which the behavior was observed.

Ovariectomy, and not adrenalectomy, abolishes the lordosis response. Sequential treatment with E_2 and P restores receptivity in the ovariectomized (OVX) female rat (Boling & Blandau 1939, Edwards, Whalen & Nadler 1968, Nequin & Schwartz 1971, Sodersten & Hansen 1977). Although repeated treatment of ovariectomized or ovariectomized-adrenalectomized (ADX) rats with E_2 alone can induce receptive behavior (Davidson, Smith, Rodgers & Block 1968, Davidson, Rodgers, Smith & Block 1968) treatment with E_2 followed by P is more

effective. In the intact female rat, the display of sexual behavior seen during estrus is dependent upon the synergism between a "priming" action of E_2 and a subsequent facilitatory action of P (Powers 1970). The necessity of prior estrogen priming for P to show this facilitatory effect in ovariectomized female rats has been demonstrated (Kow & Pfaff 1975).

Lordosis can be elicited in estrous female rats by manual stimulation of the pelvic region, by vaginal probing, as well as by the mounting of the male rat. Since a male rat often will not mount an unreceptive or ovariectomized female, certain studies make use of manual stimulation or vaginal probing to test for the lordosis response. These procedural alternatives for measuring the lordosis response may have particular significance for the interpretation of studies aimed at establishing the minimum amounts of exogenous hormone that must be administered to ovariectomized rats in order to restore the lordosis response. They may vary in reliability or have different hormonal requirements. Another factor to be taken into account when considering the results of such studies is the form of the exogenous estrogen used. Powers (1975) has shown that the long-acting esterified form of E_2 , called estradiol benzoate (EB), is more potent than the short-acting free-alcohol form (E_2) in stimulating lordosis behavior in response to P treatment. Furthermore, it has been found that plasma concentrations of E_2 are maintained at a higher level after administration of EB than after E_2 (Tapper, Greig & Brown-Grant 1974).

Using EB and testing for lordosis response with stud males, Davidson, Smith, Rodgers & Bloch (1968) showed that in ovariectomized rats complete recovery of lordosis occurred within twelve days of daily injections of 0.8 ug EB. This finding was soon extended to the ovariectomized-ADX rat (Davidson, Rodgers, Smith & Bloch 1968). Recently, it was reported by the same laboratory that using silastic implants of E_2 and stud males for lordosis tests, a single thirty minute exposure to the implant was sufficient to restore lordosis in ovariectomized rats injected with 0.5 mg of P three days after the onset of treatment with E_2 (Johnston & Davidson 1979). However, in other studies in which manual stimulation of ovariectomized and ovariectomized-ADX rats was used, it was found that the minimum exposure to E_2 was twenty-four hours even when larger implants were used (Ydstebo & Sodersten 1977, Hansen, Sodersten, Srebro 1978). Although such conflicting results may be partially explained by the procedural differences and by the diurnal rhythm in responsiveness to E_2 in ovariectomized, ovariectomized-ADX and intact rats (Hansen et al 1978), there is nevertheless no consensus in the literature as to the required duration of E_2 presence in plasma prior to the observation of lordosis. Some studies indicate that an extended E_2 presence in plasma is required before lordosis may be observed (Ydstebo & Sodersten 1977). Other studies suggest that E_2 has a "trigger-like" action which sets off a sequence of events which may then run their course in the absence of plasma E_2 (Morin, Powers & White 1976; Hough, Ho, Cooke & Quadagno 1974; Whalen & Gorzalka 1973). It appears, however, that the crucial factor is the intraneuronal concentration of E_2 in cells with the appropriate steroid receptor.

Plasma E_2 concentration may therefore be of only indirect significance in a description of the mechanisms underlying the lordosis response.

Several studies have demonstrated that under certain conditions ovariectomized rats will demonstrate lordosis in the absence of exogenous steroids. For example, in hormonally untreated ovariectomized rats, vaginal probing alone (Komisaruk & Diakow 1973), hypophysectomy alone (Crowley, Rodriguez-Sierra & Komisaruk 1976), basal hypothalamic implants of prostaglandins (Rodriguez-Sierra & Komisaruk 1978) and peripheral administration of luteinizing hormone releasing factor (Moss & McCann 1973, Pfaff 1973) result in significant increases in the lordosis response when testing is done with manual stimulation rather than with stud males. These studies, however, do not demonstrate that lordosis is possible in the complete absence of E_2 and P since it has been shown that adrenal venous plasma of ovariectomized rats has significant amounts of both steroids (Shaikh & Shaikh 1975). In summary, there are many possible conditions for observing lordosis in the ovariectomized rat. There appears to be no evidence in the literature that lordosis is possible in the complete absence of prior E_2 exposure.

Finally, it should be mentioned that in the female rat, ovariectomized as an adult, there are at least two distinct effects of E_2 on lordosis. When E_2 is given in small repeated doses, little or no lordosis is observed. However, a subsequent dose of P results in a dramatic increase in lordosis responding. In this situation E_2 is said to "prime" the animal for lordosis. When ovariectomized or ovariectomized-ADX rats are "primed" with low doses of estrogens and

said to "prime" the animal for lordosis. When ovariectomized or ovariectomized-ADX rats are "primed" with low doses of estrogens and then given a large dose of EB instead of P, a "facilitatory" action of E_2 is observed resembling that found after similarly timed injections of P (Kow & Pfaff 1975). It appears that EB implants in the medial reticular formation or medial preoptic area are functionally equivalent to the large subcutaneous EB injection; it was found in an earlier study that a significant increase in lordosis occurred when such implants followed three days of injections with low doses of EB (Ross, Claybough, Clemens & Gorski 1971). Taken together these studies suggest that different mechanisms may underly the priming and the facilitating action of estrogens. It should be noted that the results of studies using only single large doses of EB or of other long lasting forms of estrogens may be due to both a priming and a facilitating action of the estrogen. What is clear from all these studies is that E_2 does not, in any sense, directly elicit the behavior. Whatever the mechanism of action of E_2 , it can be said to have a permissive action, setting the stage for the elicitation of the behavior by other stimulus events.

Mechanisms of Estrogen Actions: Estradiol is known to exert at least some of its effects on lordosis by a mechanism that involves binding to a steroid-specific cytosol receptor. Subsequent translocation of the steroid-receptor complex to the cell nucleus, and binding to chromatin, leads to an alteration in both nucleic acid and protein synthesis (Baulieu, Alberga, Jung, Lebeau, Mercier-Bodard, Millgram, Raynaud, Raynaud-Jammet, Rochefort, Truong & Robel 1971, O'Malley & Schrader 1976, Gorski & Gannon 1976, Whalen & Olsen 1978).

Hence the effects are potentially long-lasting and cumulative. Although recent studies suggest additional non-genomic steroid effects at the level of the cell membrane (Dufy, Vincent, Fleury, Du Pasquier, Gourdji & Tixier-Vadil 1979, Kelly 1979, Szego 1977), it is generally held that the magnitude of a cell's response to E_2 is an, as yet unknown, function of the intracellular concentration of steroid-receptor proteins. It is significant, however, that the effects of E_2 are not expressed identically in each neuron responding to this steroid and hence some understanding of the neuroanatomy of estrogen action is necessary.

Neuroanatomy of Lordosis: Certain neurons synthesize proteins which act as estrogen receptors. Identification of the sites for such neurons helps to define more precisely the relevant functional and electrophysiological studies in the literature.

In the neural tissue of adult female rats and of females of many other species, estrogen receptors are concentrated within cells of the medial pre-optic area, the tuberal region of the medial hypothalamus, the limbic system and in the mesencephalon (Krieger, Morrell & Pfaff 1978). Although systems involved in the mediation of lordosis can be identified in the brain stem and spinal cord, these neural systems take up less estrogen than the forebrain structures implicated in lordosis (Pfaff et al 1973). Furthermore, Kow, Grill & Pfaff (1978) have shown using decerebrate ovariectomized rats, that a net facilitatory influence from the telencephalon or diencephalon is required for mediating the hormone-dependent mating reflex.

Consistent with this is the earlier finding that lesions of the ventromedial nucleus prevent or reduce induction of receptivity by estrogen alone but permit sufficient "conditioning" of the neural substrate that lordosis may be observed after P administration (Mathews & Edwards 1977, Law & Meagher 1958). The deficit associated with this lesion has a long latency (Pfaff & Sakuma 1979b) which may reflect a temporal property of the mechanisms by which the ventromedial nucleus controls lordosis since similar lesions in the midbrain central grey or medullary reticulospinal neurons, nuclei that are known to lie within the lordosis reflex arc result in an immediate loss of lordosis (Sakuma & Pfaff 1979). On the other hand, electrical stimulation in the ventromedial nucleus, in ovariectomized rats primed with E_2 has a facilitatory effect on lordosis (Pfaff & Sakuma 1979a). However, a relatively long period of such stimulation is required before the effect is observed. Since ventromedial nucleus neurons do not respond to somatosensory stimuli adequate for triggering lordosis and hypothalamic neurons, in general, fire too slowly to account for the latencies observed for the lordosis reflex (Bueno & Pfaff 1976), it seems unlikely that the ventromedial nucleus is involved directly in the reflex arc but rather it may exert a tonic, E_2 -induced bias on the reflex arc which is completed in the lower brainstem or midbrain. This net facilitatory influence of the diencephalon and telencephalon may have inhibitory components since preoptic-lesions reduce the amount of estrogen required to evoke sexual behavior from ovariectomized rats (Powers & Valenstein 1972, Law & Meagher 1958). Similarly, female ovariectomized rats with

septal lesions show an increased sensitivity to estrogen and this effect can be attenuated by simultaneous lesions in the amygdala (McGinnis, Nance & Gorski 1978). Whereas lesions of the dorsal and ventral noradrenergic bundles in the midbrain drastically reduce receptivity in estrogen plus progesterone primed ovariectomized rats (Herndon 1976), bulbectomy has a central facilitatory effect (Edwards & Warner 1972). These uptake and functional studies support the suggestion that the sensitivity of lordosis to E_2 should be attributed to supraspinal structures.

Although it is possible to interpret these studies of the behavioral effects of lesions of estrogen-concentrating areas in terms of the elimination of possible sites for the action of estrogen at the level of the genome, each lesion dramatically alters the neurotransmitter balance in sites previously receiving afferents from the lesioned nuclei and hence the observed behavioral effects may be due to more than the simple elimination of estrogen-specific receptors. Furthermore lesion studies do not elucidate the ways in which estradiol's genomic effects are expressed. Nevertheless it seems clear that E_2 acts primarily via supraspinal structures and that within these structures there is a complex interaction of inhibitory and facilitatory E_2 -sensitive substructures.

Role of CNS Amines in Lordosis: Until very recently, it was believed that the number of substances in the brain that could function as neurotransmitters was a mere fraction of the almost thirty substances thought today to have that functional potential.

Monoamines and acetylcholine were the classic neurotransmitters. Several lines of research have repeatedly demonstrated the monoaminergic mediation of female sexual behavior (Rodriguez - Sierra, Naggar & Komisaruk 1976, Ward, Crowley, Zemlan & Margules 1975 and for a review see Carter & Davis 1977) as indicated by changes in amine levels and turnover rates in discrete estrogen concentrating brain nuclei in both intact (Crowley, O'Donohue & Jacobowitz 1978) and estrogen primed ovariectomized rats (Crowley, O'Donohue, Wachslicht & Jacobowitz 1978). Estrogens have effects at the level of amine catabolism, synthesis and reuptake. It is known that degradation of catecholamines occurs primarily either via non-neuronal uptake of the amine or via the direct actions of the enzymes monoamine oxidase and catechol-O-methyl transferase. In general, estrogens act to reduce these activities. Iverson and Salt (1970) have demonstrated in vitro that both E_2 and corticosterone are fairly potent inhibitors of non-neuronal uptake of catecholamines. Luine, Khylichevskaya & McEwen (1975) demonstrated that central monoamine oxidase activity in the corticomedial amygdala and basomedial hypothalamus was decreased by estrogen administration. Inhibition of amine degradation can occur via a liver metabolite of 17 beta-estradiol called 2-hydroxy-estradiol which is a potent inhibitor of catechol-O-methyl transferase in mouse neural tissue and rat liver (as cited in Brownstein 1975). In addition catechol-estrogens, which are the major metabolites of estrogens in the hypothalamus (Fishman & Norton 1975, Paul & Axelrod 1977, Ball et al 1978), also compete with catecholamines for catechol-O-methyl transferase (Breuer & Koster 1974) and thereby reduce the rate of amine degradation. The main point of these studies is that

estrogens inhibit amine catabolism via distinct but complementary mechanisms.

Monoamine reuptake mechanisms reduce the amount of effective neurotransmitter available at the post-synaptic receptor without, at the same time, degrading the amine. Estradiol in combination with progesterone inhibits dopamine reuptake mechanisms in the rat cortex, septum and pre-optic areas whereas E_2 alone has that effect in the thalamus. Progesterone alone inhibits reuptake of serotonin in the pre-optic and septal areas (Wirz-Justice, Hackmann & Lichtsteiner 1974). Estrogens may also affect the enzymes which are the rate limiting steps in amine synthesis. For example, choline acetyltransferase activity levels in the pre-optic area, amygdala and hippocampus are increased by daily EB administration (Lüdtke et al 1975). One could speculate that this may be particularly significant since female rats have higher hypothalamic levels of this enzyme than males (Libertum, Timiras & Kragt 1973) and acetylcholine stimulates luteinizing hormone releasing hormone release (Justo, Motta & Martini 1975) a peptide which facilitates receptivity in the hypophysectomized female rat (Moss & McCann 1973, Pfaff 1973).

Several studies have been done to correlate changes in monoamine levels and turnover with the changes in estrogen and progesterone levels during the cycle. In general, absolute amine levels are less informative than turnover rates since only the latter are a measure of the utilization of the amine. Jori & Dolfini (1976) reported that, in the mouse, diestrus is associated with high levels of striatal dopamine and low turnover rates whereas estrus was

characterized by low levels and high turnover in this area. Using the combination of a synthetic estrogen (mestranol) and progestin (lynestrenol) it has been shown that intact female rats (Algeri, Ponsio, Dolfini & Jori 1976), mice (Jori & Dolfini 1976), and guinea pigs (Ponsio, Achilli & Algeri 1977) lose striatal dopamine more quickly than controls after treatment with alpha-methyl-p-tyrosine, an inhibitor of monoamine synthesis. It was also reported that in the brainstem but not in the limbic area of rats, this treatment resulted in elevated levels of serotonin and its metabolite 5-hydroxyindoleacetic acid as well as a decrease in the level of the tryptophan precursor. This is particularly significant since serotonin is thought to have an inhibitory effect on receptivity (Everitt, Fuxe & Hokfelt 1974, Meyerson, Carrer & Eliasson 1974, Davis & Kohl 1978).

Estrogens' effects on amine turnover are not limited to the striatum and brainstem. For example one study reported increased dopamine turnover in the median eminence of rats given E_2 doses sufficient to block ovulation (Fuxe, Hokfelt, Jonsson & Lofstrom 1973). Another study reported no changes in dopamine or norepinephrine levels in the median eminence based on samples taken the morning of each day of the cycle (Gudelsky, Annunziato & Moore 1977), whereas a third study showed no changes in norepinephrine and a significant increase in dopamine levels from the afternoon of proestrus to estrus (Crowley, O'Donahue & Jacobowitz 1978). Similar changes in catecholamine content have been reported in many other discrete limbic nuclei. Further support for the role of estrogens in

modulating amine levels comes from studies that remove the primary endogenous source of estrogens: the ovaries. Several days after ovariectomy there is a permanent increase in dopamine levels in the median eminence which is reversible by short-term EB treatment (Gudelsky et al 1977). Another study with a comparable post ovariectomy interval found that in the lateral septum, interstitial nucleus of the stria terminalis and central gray catecholamine area a single 5 µg EB injection reduced the depletion of norepinephrine produced by alpha-methyl-p-tyrosine. Under the same conditions there was a reduction in the depletion of dopamine in the nucleus of the tractus diagonalis and an enhancement of norepinephrine depletion in the periventricular and anterior hypothalamic nuclei. It is worthy of note that 1.5 mg of progesterone given subcutaneously 48 hours after the EB and six hours prior to decapitation was sufficient to reverse all the effects of EB only (Crowley, O'Donohue, Wachslight & Jacobowitz 1978). In conclusion, the significance of all these changes is certainly not understood, yet since almost all of these areas exhibiting changes in catecholamine content over the estrus cycle or after ovariectomy are target tissues for estrogen (Stumpf, Sar & Keefer 1975), it seems reasonable to suggest that the observed fluctuations are due to circulating gonadal steroids, particularly estrogen. Although the dimensions of monoaminergic mediation of estrogens' effects on lordosis are already complex, it seems likely that further mechanisms of E_2 action will become apparent as the mediating roles in lordosis of the more recently accepted neurotransmitters are unravelled.

Peripheral Actions of Estrogens: Estradiol acts simultaneously on several other systems that may have indirect effects on the display of lordosis. Estrogens are known to affect cholesterol catabolism, synthesis, absorption, excretion and the shifting of cholesterol between body pools. Since cholesterol is the ultimate precursor of all steroid synthesis (Burstein, Kimball & Gut 1970), estradiol may modulate the bioavailability of other steroids such as progesterone. For example, estradiol enhances cholesterol synthesis by a 1.7 and 2.5 fold increase in beta-hydroxy - beta-methylglutaryl coenzyme A (HMG-CoA) reductase activity in normal and ovariectomized rats, respectively. Estradiol injections increased plasma cholesterol levels by 18% in normal rats and 38% in ovariectomized rats (Abul-Hajj 1978). Adrenal steroid synthesis is modulated by pituitary ACTH and E_2 increases the activity of glucose-6-phosphate dehydrogenase, a key enzyme in ACTH synthesis. Lactic dehydrogenase is necessary for the conversion of pregnenolone to progesterone and its activity is also increased by E_2 (Oaknin, Alonso, Prieto & Mas 1979). These studies indicate a role for E_2 in modulating the synthesis of endogenous steroids known to affect the display of lordosis.

Estrogens are known to affect the functional characteristics of individual primary sensory neurons via mechanisms which may or may not be distinct from those underlying the central effects of estrogens. Since inputs from certain skin areas are sufficient peripheral stimulation for lordosis (Pfaff, Montgomery, & Lewis 1977), it was possible to determine that pressure alone on the skin of the perineum and tail base within a certain range is

sufficient to elicit lordosis. Neither painful stimulation nor hair deflection alone was sufficient or necessary for the reflex (Kow, Montgomery & Pfaff 1979). This study also established that for a given pressure on this area the probability of lordosis can be increased either by increasing the area stimulated or by increasing the dose of estrogen. Since electrophysiological studies have shown that E_2 increases the receptive fields both of the pudendal nerve and of the trigeminal ganglion neurons (Bereiter & Barker 1975), it seems that E_2 increases the probability of lordosis for a given pressure via two distinct peripheral mechanisms. One involves increasing the size of the receptive fields of particular sensory neurons, the other involves lowering the threshold for effective sensory stimuli.

Given these widespread central and peripheral effects of estrogens in the adult animal, it is remarkable that greater attention had not been given to the long-term behavioral and physiological effects of chronic administration of estrogens. This would seem to be an area of special importance in view of the fact that women are being treated with admittedly low but chronic doses of exogenous estrogens in the form of birth control pill throughout a large part of their adult lives. Interest in the effects of estrogens throughout the life cycle has been sparked by the finding that estradiol plays a key role in perinatal life in sexual differentiation. The main point to be taken from these studies for the purpose of the present review is that estrogens appear to have certain growth promoting properties as demonstrated in the neonate and possibly also in the adult rodent.

Estrogens in Sexual Differentiation: Although estrogens are popularly categorized as "female" hormone and androgens as "male" hormones, recent studies suggest that for both genetic males and genetic females certain key aspects of sexual differentiation and, in particular, of adult sexual behavior are mediated by estrogens in early ontogeny.

At birth various rat and mouse hypothalamic nuclei are undifferentiated morphologically and the formation of adult structures for both sexes of these species occurs during the first two weeks of postnatal life. The critical role of E_2 in this process is suggested by the finding that with explanted neonatal mouse hypothalamic tissue, the addition of E_2 or testosterone (T) to the in vitro culture resulted in accelerated and intense proliferation of neuronal processes as compared to tissue at the same hypothalamic level cultured in a control medium (Toran-Allerand 1976, 1978). Given, therefore, a source of endogenous estradiol, those undifferentiated neural tissues that contain E_2 receptors may grow and branch more rapidly than neurons that are indifferent to the steroid. Estrogen-sensitive neurons may thus establish synaptic connections on recipient neurons before the other unaffected axonal systems. In this way they could monopolize the synaptic space of the recipient cells. Therefore in the absence of E_2 , the competition for synaptic space would be more balanced and thus the behavior of recipient neurons would be determined by afferents which otherwise would have had little influence, in steroid-exposed brains (Naftolin & Brawer 1977). This line of reasoning suggests a possible explanation for the different

patterns of dendritic concentration in the preoptic area of male and female hamsters (Greenough, Carter, Steerman & De Voogd 1977); of gross sex differences in the size (Gorski, Harlan & Christensen 1977) and type of terminations (Raisman & Field 1973, Gregory 1975) in the adult rat medial preoptic area, and amygdala (Staudt & Dorner 1976).

These differences in neural growth patterns or schedules may also mediate E_2 's paradoxical effects on both masculinization and defeminization of adult sexual behavior. Masculinization refers to an increased incidence of mounting behavior and defeminization refers to either a reduced incidence of lordosis or to an elimination of the cyclic gonadotropin pattern which is characteristic of the normal female. In a study in which neonatal female rats were given intracerebral implants of T or E_2 , it was found that at any neural site where T implants produced a defeminization of adult gonadotropin release or an increase in male-like adult mounting behavior or a decreased adult behavioral responsiveness to EB as measured by lordosis, estradiol implants were functionally equivalent (Christensen & Gorski 1978). Evidence is also accumulating that during perinatal development, the presence of E_2 may be important in establishing the sensitivity or "feminizing" the neural system involved in lordosis behavior so that as an adult the animal will respond to E_2 with appropriate levels of sexual behavior. Sodersten (1976) showed that rats ovariectomized postpuberally were more sensitive to E_2 when tested as adults than neonatally ovariectomized females. In another study, among male and female rats that had been androgenized prenatally and gonadectomized at birth, those with

ovarian implants from birth to thirty-five days of age had significantly greater receptivity scores when tested as adults compared with similar groups with no ovarian implants (Dunlap, Gerall & McLean 1973, Gerall, Dunlap & Hendricks 1973, Dunlap, Gerall & Carlton 1978). In other words, not only does a single steroid mediate the ontogeny of both male and female forms of adult sexual behavior, but, in addition, the frequency and intensity of lordosis displayed by both genetic male and female adults may depend on the extent of pre-adult exposure to estrogens.

The neonatal brain is considered to be intrinsically organized to support the female pattern of reproductive activity. It is generally held that due to the absence of testicular androgens in the perinatal female rat, the female pattern of sexual behavior and of cyclical gonadotropin release becomes established. Neonatal androgenization of the rodent brain via endogenous sources in the genetic male, or via exogenous sources in the genetic female, results in permanent post-pubertal alterations in the direction of male-like reproductive functioning. Since androgenization is a central and not a peripheral effect (Harris & Jacobson 1952), and since brains of neonatal male rats contain at best very low levels of androgen receptors compared to estrogen receptors (Fox, Vito & Wieland 1978, Westley & Salaman 1977) alternative mechanisms for early androgen action were sought. Since androgens are the immediate metabolic precursors of estrogens in the mammalian brain (Callard, Petro & Ryan 1979) it was considered significant that the neuroanatomical distribution of the aromatase complex which regulates this steroid

conversion essentially paralleled the areas that control adult sexual behavior and concentrated sex steroids i.e. the phylogenetically ancient limbic brain (Naftolin, Ryan, Davies, Reddy, Flores, Petro, White, Takaoka & Wolin 1975). The suggestion was made that androgens exert some of their neonatal effects via aromatization to estradiol followed by binding to that steroid's receptor. The existence of estrogen receptors in the embryonic rat and mouse brain (Vito & Fox 1979, Lieberburg, Maclusky, Roy & McEwen 1978) indicates the biochemical potential exists for response to the sex steroid environment (McEwen, Lieberburg, Chaptal & Krey 1977). The neonatal female brain is protected from masculinization by the high levels of maternal estrogens circulating during the perinatal period (Pang, Caggiula, Gay, Goodman & Pang 1979) through functional inactivation by binding to the fetoneonatal extracellular-binding-protein called alpha-fetoprotein (McEwen, Flapinger, Chaptal, Gerlack & Wallach 1975). Testosterone, which is not bound by alpha-fetoprotein, has free access to the brain where it is aromatized to E_2 and in this form, leads to masculinization. This sensitivity of the brain to testosterone is limited to a "critical period" which, in the rat, extends from the 18th day of gestation to the fifth postnatal day (Barraclough 1971, Lobl & Gorski 1974). During this critical period serum concentrations of E_2 are almost identical in male and females but compared to males females have significantly lower, yet measurable, concentrations of serum testosterone (Pang et al 1979, Hacik 1978, Bieglmayer, Jettman, Adamkier & Spona 1978, Dohler & Wuttke 1975). The extent to which intact female rat neonates will be

masculinized and defeminized by endogenous androgens and estrogens depends both on the ontogeny of the corresponding steroid receptors (Fox et al 1978) and on the extent of functional inactivation of E_2 by alpha-fetoprotein during the critical period (Germain, Campbell & Anderson, 1978). Alpha-fetoprotein decreases linearly, in male and female neonates, from its maximum concentration at birth to levels of zero at weaning (Vannier & Raynaud 1975). Serum E_2 levels, on the other hand, in both male and female neonates are high due to maternal E_2 during the first two postnatal days. After maternal estrogens disappear, estrogens from the adrenals and ovaries appear and rise to a peak concentration between days 10-15. E_2 levels between day 9 and 19 are higher than levels found in the adult proestrous female (Dohler & Wuttke 1975). These findings suggest that some measure of masculinization and/or defeminization of female neonates may take place. The existence of detectable levels of T in neonatal female rats suggests that there exists a threshold androgen concentration below which masculinization does not occur. In a test of this hypothesis it was found that prenatal treatment of male and female rats with an aromatase inhibitor (Clemens & Gladue 1978) or an antiandrogen (Gladue & Clemens 1978) reduced defeminization as measured by a higher frequency of lordosis in both sexes in response to E_2 compared to normal gonadectomized hormone primed male and female rats. Since the Clemens & Gladue (1978) study found that males pretreated with aromatase inhibitors had higher lordosis scores than control males with equal E_2 injections, this was taken as evidence that androgenization decreases sensitivity to E_2 rather than causing a

disruption of the neural organization underlying the lordosis motor pattern. Furthermore there is some evidence that female rats normally undergo some behavioral masculinization since they show a higher probability of mounting behavior when compared to females exposed prenatally to the anti-androgens flutamide (Clemens, Gladue & Coniglio 1978) or cyproterone acetate (Ward & Renz 1972; Stewart, Pottier & Kaczender-Henrik 1971). The main point of these studies is that it is possible to construct a consistent framework to explain how E_2 can mediate both behavioral masculinization and estradiol sensitization or feminization in terms of its nuclear actions at different times during early ontogeny.

Estrogen and Neural Growth in the Adult Rat: The effects of E_2 on neural growth patterns and subsequent defeminization of sexual behavior may not be limited to the neonatal or perinatal period. Adult male rats, whether gonadectomized or not, respond to exogenous E_2 and P with very low levels of lordosis. Chronic exposure to E_2 alone or septal lesions alone do not change their response to the exogenous steroids. However, chronic exposure to E_2 immediately after septal lesioning results in an increased lordosis response to a priming dose of E_2 when tested several weeks after the cessation of daily E_2 treatment (Nance, Phelps, Shryne & Gorski 1977). This basic finding has been extended to male rats lesioned as weanlings, implanted chronically with an E_2 pellet and tested for lordosis twenty days later (Stewart & Atkinson 1977). Female rats receiving septal lesions as adults but no exogenous estradiol also exhibit an increased behavioral sensitivity to estrogen when tested several weeks later

(Nance, Shryne & Gorski 1975). Chronic doses of testosterone beginning immediately after the lesion attenuate the increase in sensitivity (Nance, Shryne, Gordon & Gorski 1977). It seems therefore that endogenous T in the septal lesioned male (SLM) has the same behavioral effect as exogenous T in the septal lesion female (SLF). Similarly endogenous E_2 in the SLF has the same behavioral effect as exogenous E_2 on the SLM. Since the critical period of E_2 exposure coincides with post-lesion neural regrowth events, it seems plausible that in the rat chronic estrogen exposure following the septal lesion may modify the recovery process in a way that increases the probability that subsequent hormone treatment will facilitate lordosis. In other words, in the adult male rat disruption of neural circuitry may permit estrogens to act to alter neural regrowth patterns to favour the development of structures required to mediate lordosis.

Distinct neural mechanisms may underly hormonal cyclicity and female reproductive behavior. There is some evidence to suggest that the neural mechanisms controlling the cyclical pattern of luteinizing hormone secretion in the female rat are more sensitive to the masculinizing effects of androgens and estrogens than is the neural control of lordosis. An early study employing a selected sample of normal young adult female rats who showed persistent vaginal cornification (constant estrus) demonstrated that there is no necessary link between the display of lordosis and the hormonal condition as indicated by vaginal status (Adler & Bell 1969).

When genetically female rats are given low doses of androgens perinatally, they are called lightly androgenized, since they show estrous cycles after puberty but become anovulatory at an early age with persistent vaginal estrus and polyfollicular ovaries (Gorski 1968). When ovariectomized as adults and primed with EB only, lightly androgenized anovulatory females had lordosis scores that were higher than those of a non-androgenized control group (Harlan & Gorski 1978a). Even beyond puberty, endogenous estradiol may have a defeminizing action which manifests itself in the more estrogen sensitive luteinizing hormone control structures rather than in the structures underlying lordosis. Aging female rats progress from regular to irregular estrous cycles, then to persistent vaginal cornification or pseudopregnancies of irregular length and finally to an anestrus state (Huang & Meites 1975). This final anestrus state is characterized by very low plasma luteinizing hormone values and no cyclical changes in E_2 , progesterone, luteinizing hormone, or follicle stimulating hormone (Huang, Steger, Bruni & Meites 1978). The ovaries of the old rats were still capable of near normal function under appropriate gonadotropic stimulation and this suggests that the major cause for cessation of regular estrous cycles in old rats lies in altered hypothalamic-pituitary function. In a study with rats of the same age and strain, lordosis scores, after priming, were reported to be comparable to those of ovariectomized primed young adult rats (Peng, Chuong & Peng 1977). Another very similar study reported an increased behavioral sensitivity to estradiol in old versus young adult rats (Cooper 1977). This finding is suggestive of the

previously discussed role of E_2 in "sensitizing" the neural substrate to subsequent E_2 exposure (Dunlap et al 1978). These studies point to an independence of neural mechanisms underlying different components of the overall female pattern and suggest that defeminization is not a unitary phenomenon.

Chronic versus acute estrogen exposure: Recently several studies have indicated that some of the physiological and behavioral effects of long-term uninterrupted estrogen treatment may be distinct from and in the opposite direction to the effects produced by acute estrogen exposure. For example, one study measured changes in several organ weights and corresponding biochemical parameters based on values taken at diestrus (low plasma E_2) and estrus (high plasma E_2). These values correspond to the short-term changes in estrogen levels associated with the estrus cycle. A similar group of intact females was treated for five months with up to 5 ug of daily estradiol after which organ weights were taken and the biochemical analysis performed. As may have been expected, the hormone treated animals had vagina, thyroid and pituitary weights that were higher and ovarian weights that were lower than their estrus and diestrus controls. Protein, glycogen, lactic acid and alkaline phosphatase concentrations in the uterus, cervix and vagina revealed patterns in which E_2 treatment generally accentuated the normal increase in concentrations from diestrus to estrus. However, notable exceptions were found in the uterus where glycogen and alkaline phosphatase showed sharp drops in concentration below diestrus levels and in the vagina where lactic acid showed a similar drop (Mehrotra & Kamboj

1977). Other lines of research have shown that whereas acute increases in E_2 in intact female rats are associated with a heightening of the stimulating effects of norepinephrine on gonadotropin release, chronic estrogen exposure reduces beta-adrenergic responsiveness (Wagner, Crutcher & Davis 1979, Thrasher & Fregly 1978). Taken together, these studies suggest that long-term estrogen exposure may have biochemical and behavioral effects that could not be predicted from studies involving acute estrogen exposure or endogenous estrogen rhythms.

With these somewhat unrelated findings of distinct short versus long term effects of estrogen exposure in mind, it was decided to pursue a serendipitous finding from an unpublished lesion study done in this laboratory. Female rats, ovariectomized as adults and serving as control group subjects in a study of the effects of brain lesions, were given daily EB and were tested for receptivity once a week over a twelve week period. All animals showed a significant decrease in receptivity over time. The purpose of the first experiment to be reported in this thesis was to study further this diminished behavioral effectiveness of long-term estradiol exposure and to explore potential mechanisms of action.

EXPERIMENT 1

In Experiment 1 three groups of ovariectomized rats were given chronic estrogen exposure via either daily or weekly subcutaneous injections of EB in oil. Weekly receptivity tests took place with half of each group receiving an appropriately timed dose of P. Eventually half of the animals in each hormone treatment group underwent a four week period with no exogenous steroid. The remaining half continued on their hormone schedule. At the end of this period all animals reverted to the pre-interruption steroid schedule and receptivity tests were conducted. Table 1 summarizes the plan of Experiment 1.

Method

Subjects

Fifty-two adult Sprague-Dawley female rats weighing 190-240 g when obtained from Canadian Breeding Farms were used as subjects in this experiment. Animals were housed in pairs and were maintained on a reversed 14 hour light/10 hour dark reversed cycle (lights were off between 10h-20h). Purina Lab Chow and water were available ad libitum in the home cages. The weight of each animal was recorded upon arrival, prior to each behavioral test, and finally, just prior to sacrifice.

Table 1
Treatment of Animals in Experiment 1

Days →	1	2-14	15-78	79-110	111-119	120	121-140	141	Second Interruption of Hormone Treatment	Receptivity Test II	Hormone Resumption	First Interruption of Hormone Treatment	Receptivity Test I	Pretest Treatment	Ovariectomy
Weekly 10ug EB															
Daily 0.5ug EB															
Daily 2ug EB															

□ EB only

▨ EB + P

▨ No Exogenous Hormone

In order to establish a normal range for certain physiological variables, another sixteen adult Sprague-Dawley females were obtained from the same supplier and were housed under identical conditions. Upon arrival their body weight was 200-230 g.

Procedures

Hormone treatments: All fifty-two animals were ovariectomized under sodium pentobarbital anesthesia (Nembutal: 60mg/ml 30 mg/kg) with 0.1 ml atropine sulphate (0.6 mg/ml) on the eighth day after arrival. All hormone injections were subcutaneous and utilized a constant volume of 0.5 ml peanut oil as vehicle. All estradiol benzoate injections were administered between 9h-11h.

Sixteen animals were assigned to a weekly hormone condition. They received 10 ug EB on the seventh day post-ovariectomy. Subsequently, they received a similar injection forty-four hours prior to each test for sexual receptivity. Eighteen animals received daily injections of 0.5 ug EB starting the day after ovariectomy. Another eighteen animals received daily injections of 2 ug EB starting the day after ovariectomy.

Each of these three groups were divided in half: one subgroup received 0.5 mg progesterone on the eighth day post-ovariectomy and subsequently four hours before each test for sexual receptivity.

There were ten weekly tests of receptivity beginning on

the fourteenth day after ovariectomy. After the last of these weekly tests, groups were further subdivided into those that continued to receive hormone and those that received no exogenous hormone for thirty-two days. During this period all fifty-two animals were given weekly exposure to the male studs in the test cages for at least five minutes. This was scheduled at the time of day during which receptivity testing had occurred during previous weeks. At the end of this period, the previous schedule of hormone administration was reinstated for those animals that had been withdrawn from exogenous hormone treatment for thirty-two days. An additional exposure to the stud took place as described above and on the tenth day of hormone re-exposure, a final receptivity test was conducted. This was followed by a further three-week period of either continued hormone administration or no exogenous hormone prior to sacrifice.

Testing procedure for sexual receptivity: Test sessions began approximately three and one half hours into the dark period in a room illuminated by a 25w red bulb. Each animal was placed in a semi-circular test box (63 x 39 x 36 cm) containing a stud male rat that demonstrated vigorous mounting. The test required that the female be mounted ten times with palpitation and thrusting by the male. If a stud failed to mount, the female was moved to another box. Up to four different studs were used to obtain ten mounts. If no mounts were attempted or successful after exposure to a maximum of four studs, the female was given a receptivity score of zero.

The quality of the lordosis response to each mount was

rated on a scale of 0-3: 0 - no response, 1 - stiffening of the body, flattening of the back, and slight raising of the head; 2 - slight curvature of the back, raising of the head; 3 - full curvature of the back, raising of the head, rump and tailbase, lowering of the chest region. Additional points were allocated for darting prior to the mount (1 point) and for holding the lordosis response after the male's dismount (1 point per second). Animals were identified as to group membership at the end of the behavioral test.

Physiological measures: All animals were sacrificed by decapitation. Trunk blood was collected immediately into ice-cold heparinized tubes. Within thirty minutes of collection, the blood samples were centrifuged and the supernatant was frozen at -75°C . The whole brain, including olfactory bulbs, was removed within ninety seconds and was quickly frozen and stored on dry ice. The sella turcica was removed and the whole pituitary was immediately weighed. Immediately after blood collection, both adrenal glands were removed, carefully cleaned of connective tissue and weighed.

Pituitary and pooled adrenal weights were recorded using a precision balance (Federal Pacific Electric Co.). Frozen whole brain weights were recorded using a Mettler H54 scale (Fisher Scientific). Levels of serotonin, norepinephrine and dopamine were determined using the procedure of Barchas, Erdelyi & Angwin (1972) with fluorescence readings taken on an Amincobowman spectrophotofluorimeter.

The steroid and luteinizing hormone assays were done at the Royal Victoria Hospital, Montreal, Canada under the generous sponsorship of Dr. K. Ruf.

In brief, the steroid assay procedure involved incubation of known amounts of the steroid with its specific antisera and tracer doses of the steroid in order to generate the standard curve. Subsequently the samples of unknown steroid concentration, in 0.005 molar phosphate buffer, were similarly incubated. The total assay volume was 0.6 ml. Incubation time varied from two hours to overnight at 4°C. Bound steroid was separated from the free form by a dextran - charcoal (Norit A) Solution, prepared in the buffer mentioned above. Radioactivity was determined with 30% efficiency on a Packard Tricard scintillation counter, model 3330. Quantification of samples was done with a Hewlett-Packard Model 9866A program based on a linear regression of the standard values in semilogarithmic coordinates. Further information on the procedure used is available in Jaffe & Behrman (1974). Supplier and specificity information is available in Appendix E.

Luteinizing hormone (LH) assays were done in duplicate using NIAMDD kits and results were expressed in nanograms per milliliter compared to the LH-RP-1 standard. Variability of less than 15% between assays and less than 5% within assays was considered sufficient for the purposes of this study. Animals were identified as to group membership only after all physiological data had been collected.

Results

Three aspects of the results from Experiment 1 will be considered separately. The first is the analysis of the effects of hormone treatment on the weekly receptivity Tests 1 to 10. The second is the analysis of the change in receptivity scores that occurred between Test 10 and Test 11 following the period of interruption of hormone treatment. Finally the results of the biological assays and organ weights completed after autopsy will be considered.

The lordosis, darting, and holding scores for the three estrogen treatment conditions (with and without P treatment) obtained from the ten weekly tests were assessed by analysis of variance and the raw data is presented in Appendix A. Body weights associated with each test were similarly analyzed. Tukey tests, modified for use with unequal sample sizes (Keppel, 1973, p. 354) were used with a significance level of $p < .05$.

Lordosis: The analyses of variance carried out on the lordosis scores yielded three significant main effects (Estrogen treatment, Progesterone treatment and Test, see Table 2). It can be seen from Figures 1 and 2 that regardless of treatment all groups showed a general decline in responding across the ten tests. The fact that the EB x Test and P x Test interactions are also significant, indicates that this trend was not uniform across all conditions. Despite the trend of decreasing lordosis scores, the animals receiving

TABLE 2

Summary Table for the ANOVA of Lordosis scores on Ten Weekly

Receptivity Tests

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	1456.4	13.8	0.0
P Treatment	1	6579.1	62.6	0.0
EB x P	2	304.8	2.9	0.066
Subjects	45	105.5		
Test	9	878.4	26.1	0.0
EB x Test	18	68.9	2.0	0.007
P x Test	9	85.6	2.5	0.007
EB X P X Test	17	14.0	0.4	0.98
Subjects x Test	405	33.7		

EB only

Mean Lordosis Score

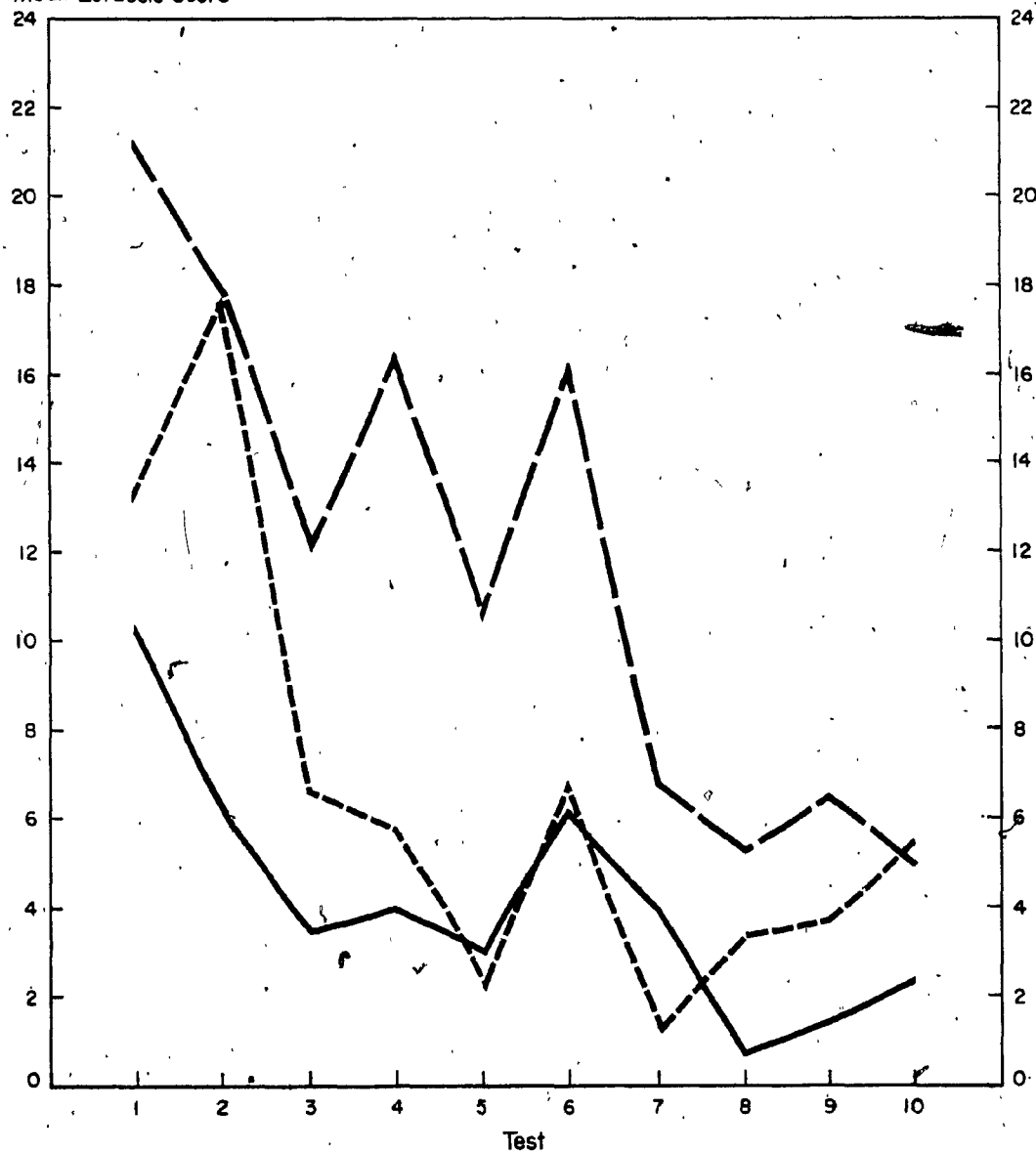


Figure 1. Mean lordosis scores in Experiment 1 on weekly receptivity tests of ovariectomized female rats given only estradiol benzoate: WEEKLY 10 ug (—), DAILY 0.5 ug (---) or DAILY 2 ug (-.-).

EB+P

Mean Lordosis Score

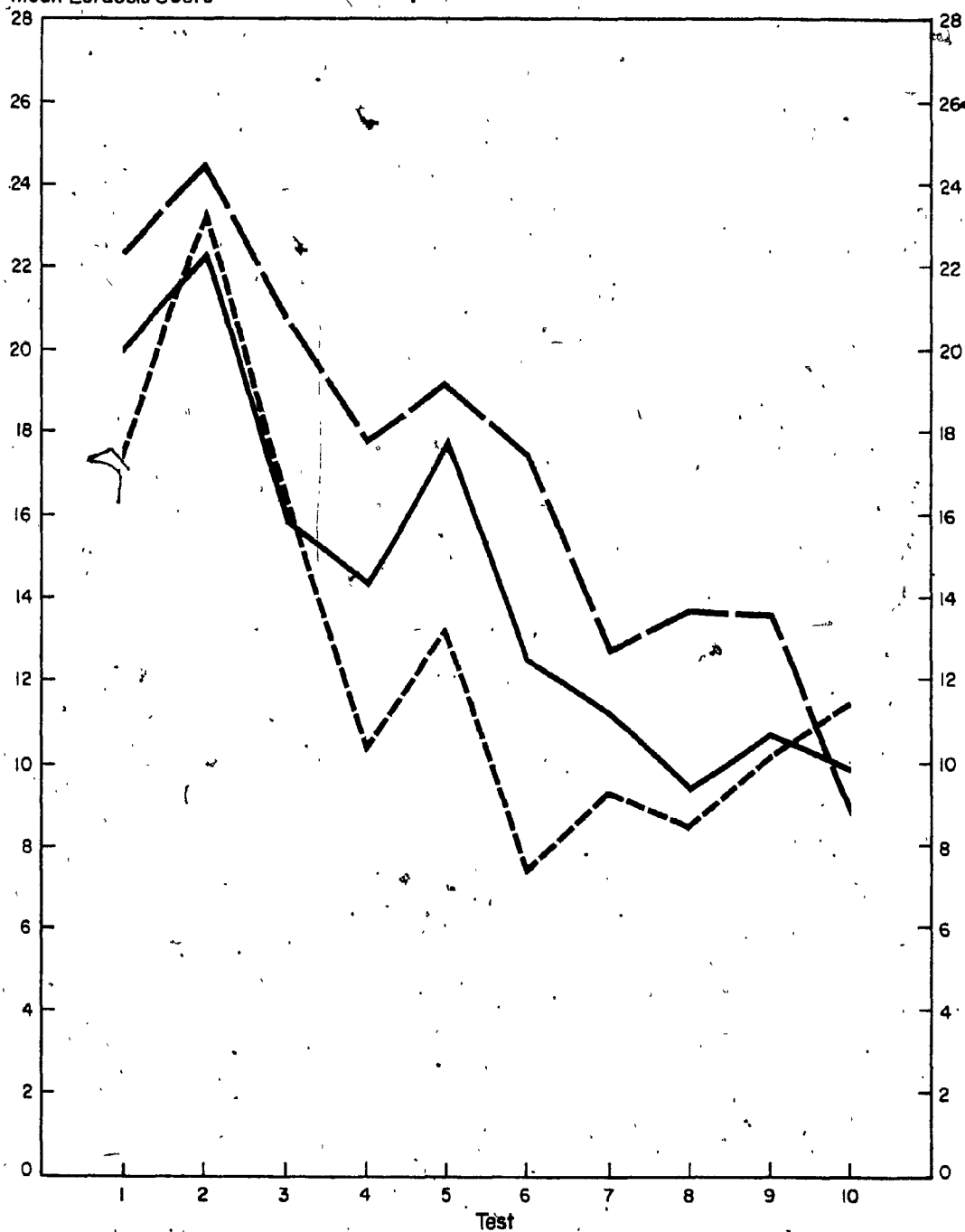


Figure 2. Mean lordosis scores in Experiment 1 on weekly receptivity tests of ovariectomized female rats given progesterone (0.5 mg per week) and estradiol benzoate: WEEKLY 10 ug (————), DAILY 0.5 ug (-----) or DAILY 2 ug (— · — · —).

the highest amounts of EB (DAILY 2 ug) had consistently higher scores than those of the other groups.

The P effect reflects the overall higher scores of animals receiving this steroid. There is some indication from Figures 1 and 2, however, that P had different effects depending on the EB treatment condition. More specifically, it appeared that P was having a relatively greater facilitatory effect in the WEEKLY 10 ug. condition than in any other. This effect is reflected in the considerable, but nonsignificant EB x P interaction and is summarized in Figure 3. In order to investigate this effect further, it was decided to carry out an ANOVA comparing only two estrogen conditions at a time. The results using data from the WEEKLY 10 ug. and the DAILY 0.5 ug. groups are summarized in Table 3. Note that the Progesterone and Test main effects as well as the EB x P interaction were all significant whereas the Estrogen effect was not. It can be seen from the summary in Figure 3 that P had a much greater effect in the WEEKLY condition than in the DAILY 0.5 ug. condition. A similarly significant interaction was found when the WEEKLY condition was compared to the DAILY 2 ug. condition ($F(1, 30) = 5.3$; $p < .03$, see Table 4). No such effect was found when the two daily conditions were compared ($F(1, 31) = 0.07$; $p < .1$, see Table 5).

Darting: Figure 4 illustrates for each of the ten receptivity tests, the number of animals in each group that demonstrated darting behavior as well as the total frequency

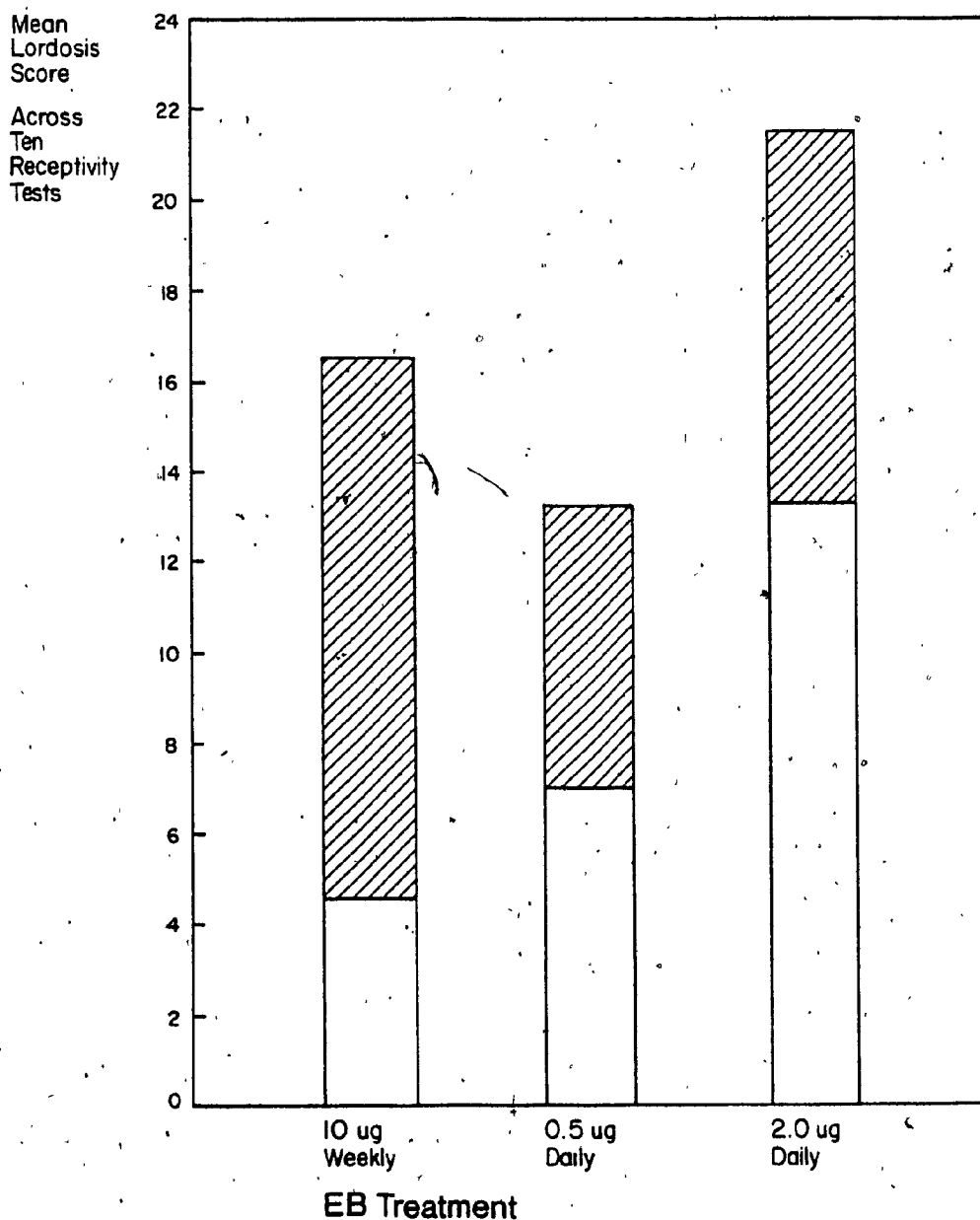


Figure 3. Bar graph depicting the differential facilitatory effects of 0.5 mg progesterone in the three treatment conditions of Experiment 1. Columns represent the mean lordosis score obtained by animals in each treatment group on all ten receptivity tests (☐ represents scores for animals receiving only estradiol benzoate and ☒ represents scores for animals also receiving progesterone).

TABLE 3

Summary Table for the ANOVA of Lordosis Scores on Ten Weekly Receptivity Tests Using Data from WEEKLY Estrogen Treated Animals and 0.5 ug. EB DAILY Animals.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	1	0.966	0.01	0.92
P Treatment	1	5271.8	59.4	0.0
EB x P	1	410.4	4.62	0.040
Subjects	29	88.79		
Test	9	536.3	18.5	0.0
EB x Test	9	54.1	1.87	0.0569
P x Test	9	67.1	2.32	0.01
EB x P x Test	9	15.9	0.55	0.83
Subjects x Test	261	28.9		

TABLE 4

Summary Table for the ANOVA of Lordosis Scores on Ten Weekly
 Receptivity Tests Using Data from WEEKLY Estrogen Treated Animals and
 DAILY 2 ug. EB DAILY Animals.

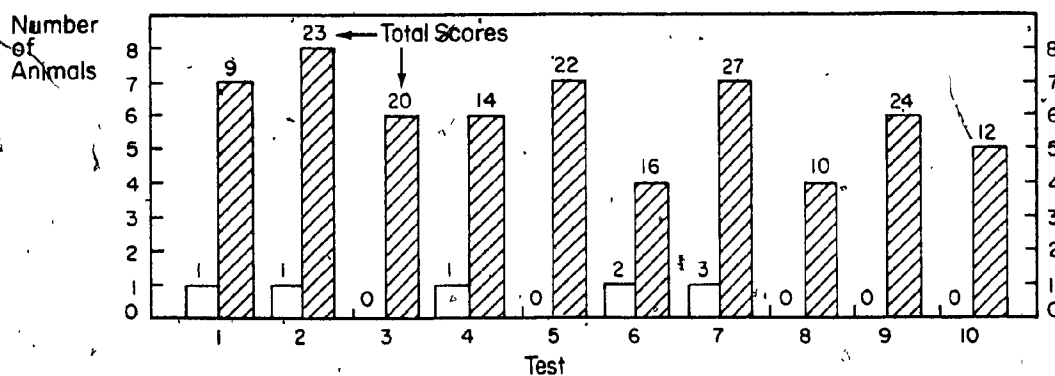
<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	1	2315.6	22.4	0.0
P Treatment	1	4848.9	46.9	0.0
EB x P	1	544.5	5.3	0.0288
Subjects	30	103.3		
Test	9	594.9	19.1	0.0
EB x Test	9	43.3	1.4	0.19
P x Test	9	61.0	1.95	0.0445
EB x P x Test	9	17.6	0.56	0.82
Subjects x Test	270	31.2		

TABLE 5

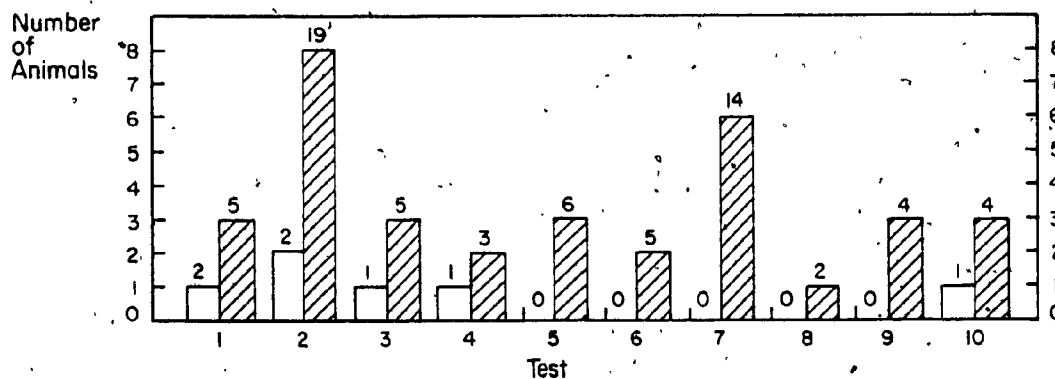
Summary Table for the ANOVA of Lordosis Scores on Ten Weekly
 Receptivity Tests Using Data from DAILY 0.5 vs 2 ug Estrogen Treated
 Animals.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	1	2287.9	19.79	0.00
P Treatment	1	2610.1	22.58	0.00
EB x P	1	7.96	0.07	0.79
Subjects	31	115.6		
Test	9	775.5	20.39	0.00
EB x Test	9	84.3	2.22	0.02
P x Test	9	71.4	1.88	0.055
EB x P x Test	9	7.18	0.189	0.99
Subjects x Test	279	38.0		

Weekly 10 ug EB



Daily 0.5 ug EB



Daily 2 ug EB

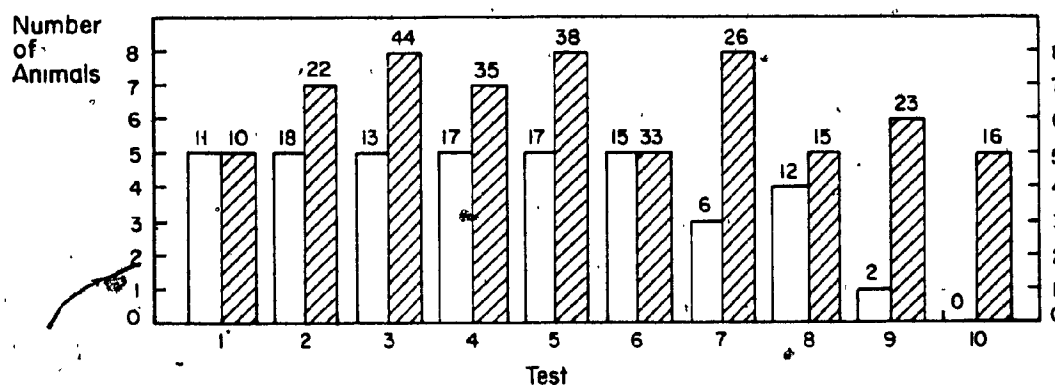


Figure 4. Number of animals displaying darting/hopping behavior ($n = 8$) on each receptivity test in Experiment 1.



represents groups receiving estradiol benzoate only.



represents groups receiving both estradiol benzoate and progesterone. Numbers above each column represent the total score obtained per group.

of darting for each group. A summary of the ANOVA for the frequency data is presented in Table 6. The Estrogen and the Progesterone main effects as well as the Estrogen x Test interaction were significant. The source of the significant Estrogen effect was the overall higher darting scores in the DAILY 2 ug. EB condition compared to both remaining estrogen conditions. The significant Progesterone effect reflects the consistently higher scores obtained by animals receiving this steroid. Although the Test effect was not significant, there was a difference in the pattern of scores across the ten tests depending on the EB condition. The darting scores for the WEEKLY group alternately increase and decrease over the ten tests. The scores of the DAILY 2 ug. group rise to a peak by the third test and then show a steady decline. The DAILY 0.5 ug. group shows a relatively consistent pattern of low scores over all tests. (See Figure 4). This effect is reflected in the significant Estrogen x Test interaction (see Table 6).

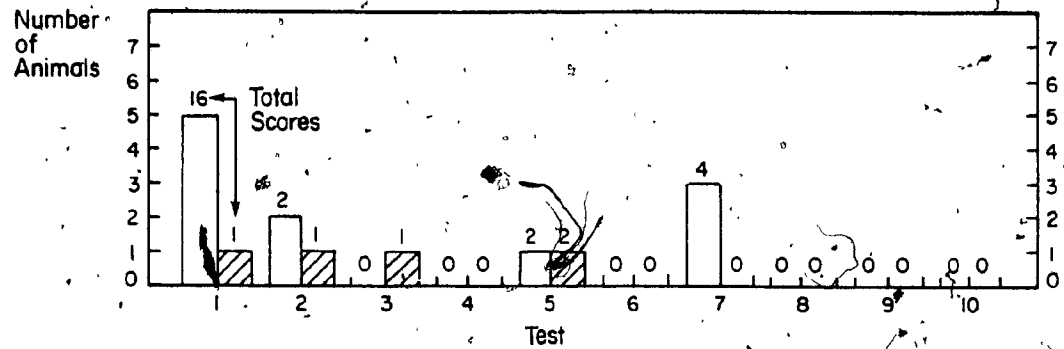
Holding: For each of the ten receptivity tests, the number of animals in each condition that demonstrated holding behavior as well as the total holding frequency score for each group are illustrated in Figure 5. Table 7 presents the summary of the ANOVA for the frequency data. None of the main effects was significant, however, there was a significant P x Tests interaction. This interaction seems to reflect the fact that the scores of the animals receiving EB only treatment were high initially but dropped off sharply (frequency range: 4-40) whereas animals also receiving P had generally lower but constant holding scores (frequency range: 6-12).

TABLE 6

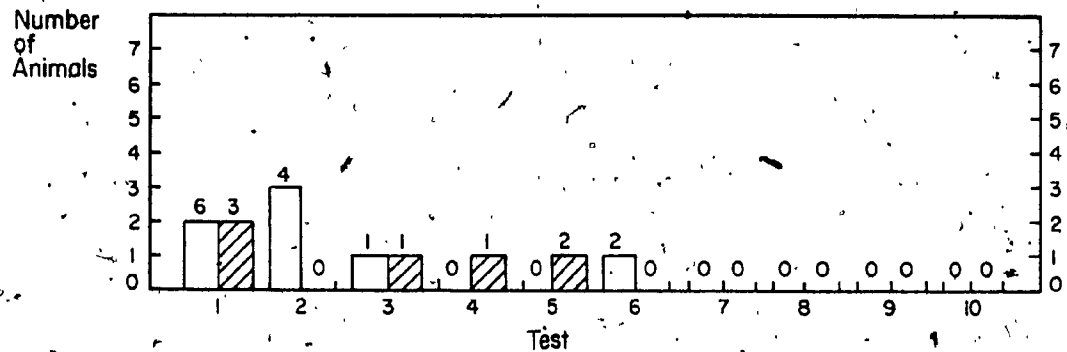
Summary Table for the ANOVA of Darting/Hopping Scores on Ten Weekly
Receptivity Tests.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	87.2	7.8	0.001
P Treatment	1	345.9	31.1	0.0
EB x P	2	9.6	0.9	0.43
Subjects	45	11.1		
Test	9	6.1	1.5	0.16
EB x Test	18	8.5	2.0	0.007
P x Test	9	6.5	1.6	0.12
EB x P x Test	18	4.4	1.0	0.39
Subjects x Test	405	4.2		

Weekly 10 ug EB



Daily 0.5 ug EB



Daily 2 ug EB

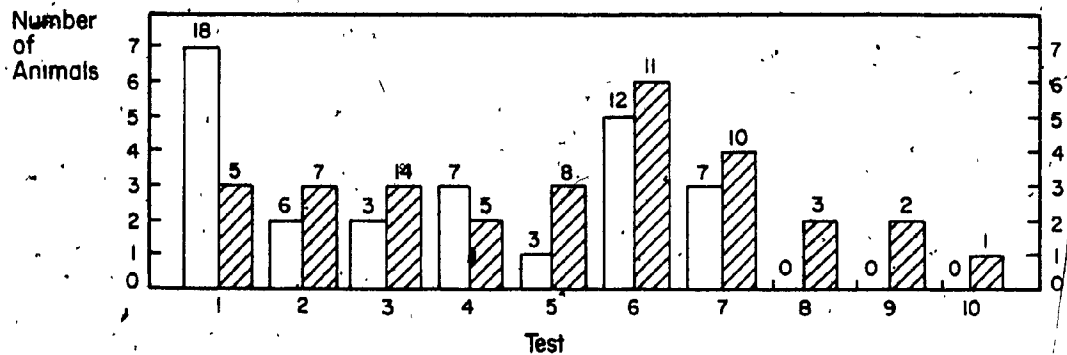


Figure 5. Number of animals displaying holding behavior (n = 8) on each receptivity test in Experiment 1. □

represents groups receiving estradiol benzoate only.

▨ represents groups receiving both estradiol benzoate and progesterone. Numbers above each column represent the

total score obtained per group.

TABLE 7

Summary Table for the ANOVA Holding Scores on Ten Weekly Receptivity

Tests.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	10.6	2.58	0.0867
P Treatment	1	0.75	0.18	0.67
EB x P	2	3.6	0.89	0.41
Subjects	45	4.1		
Test	9	3.9	1.41	0.18
EB x Test	18	3.9	1.41	0.12
P x Test	9	5.5	2.0	0.0374
EB x P x Test	18	3.1	1.12	0.33
Subjects x Test	405	2.8		

Body Weight: Table 8 presents the summary of the ANOVA based on the weight of the animals at the time of each of the ten receptivity tests. The Estrogen and Test main effects were significant as well as the Estrogen x Test interaction. There was no effect of P on body weight. The weights of animals in the three EB treatment conditions are presented in Figure 6.

It can be seen that the animals receiving DAILY 2 ug. EB had the lowest body weights throughout the study. Although initially the animals receiving DAILY 0.5 ug. and WEEKLY 10 ug. were quite similar, as the weeks progressed, the mean weights of animals receiving DAILY 0.5 ug. EB diverged from those of the WEEKLY condition and approached those of the DAILY 2 ug condition.

Effect of Hormone Interruption: Test 11

Between Test 10 and 11 half of the animals in each treatment condition had hormone treatment interrupted. Figure 7 presents the lordosis scores for all groups on Tests 10 and 11 and includes Test 1 data for comparison.

Figures 8 and 9 present the data for darting and holding respectively. The effects of interruption of hormone treatment were tested by comparing scores from Test 10 to scores from Test 11. Because the number of animals in each condition was so reduced after subdividing for the interruption condition, it was decided to combine animals across EB treatment conditions and then to test for the effect of interruption in the EB only and the EB & P

TABLE 8

Summary Table for the ANOVA of Body Weights Prior to Each of Ten Weekly Receptivity Tests.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	23617.6	8.6	0.0007
P Treatment	1	0.0	0.0	0.9996
EB x P	2	2086.1	0.76	0.47
Subjects	45	2744.2		
Test	9	28306.5	336.4	0.0
EB x Test	18	335.6	3.98	0.0
P x Test	9	145.7	1.73	0.0790
EB x P x Test	18	73.1	0.868	0.62
Subjects x Test	405	84.1		

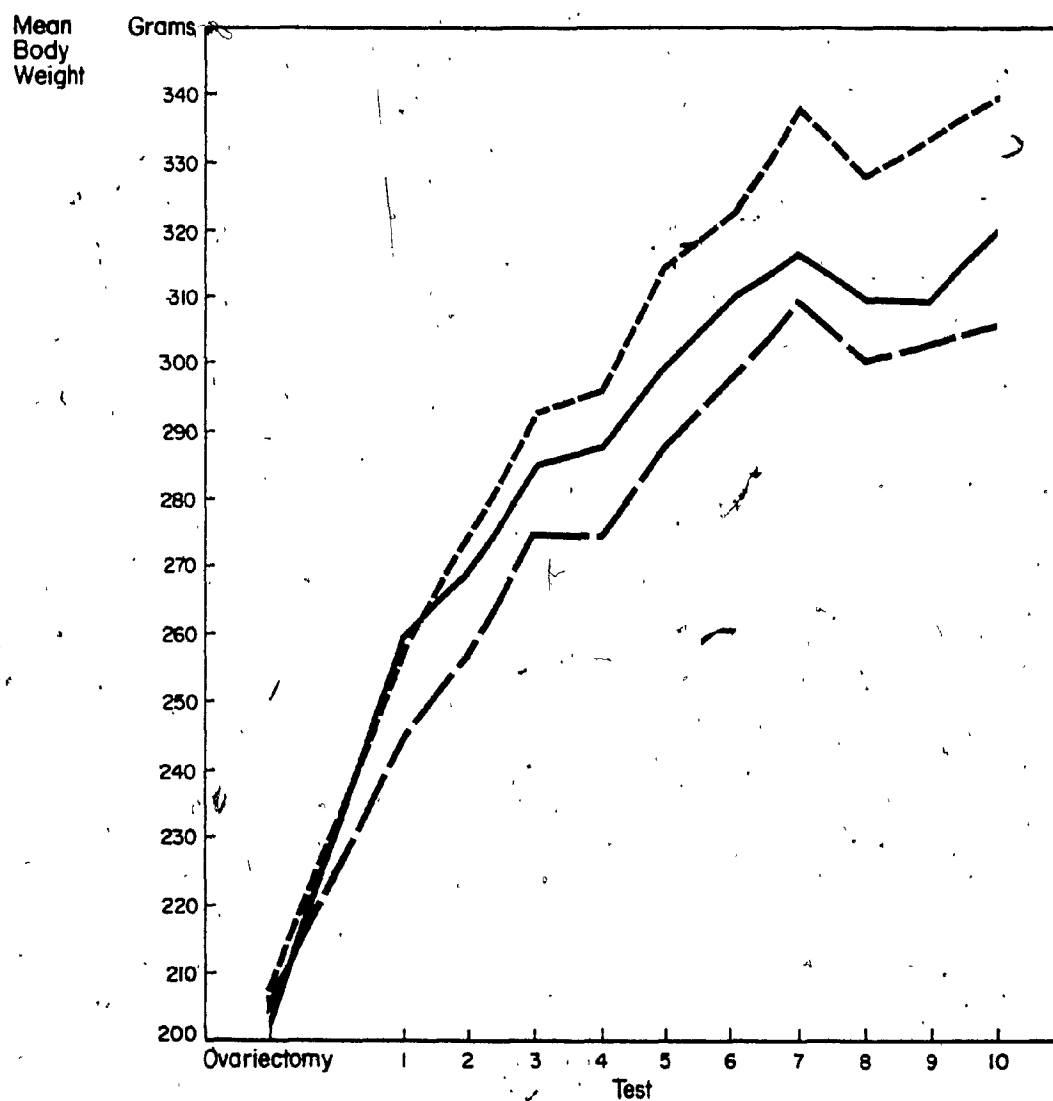
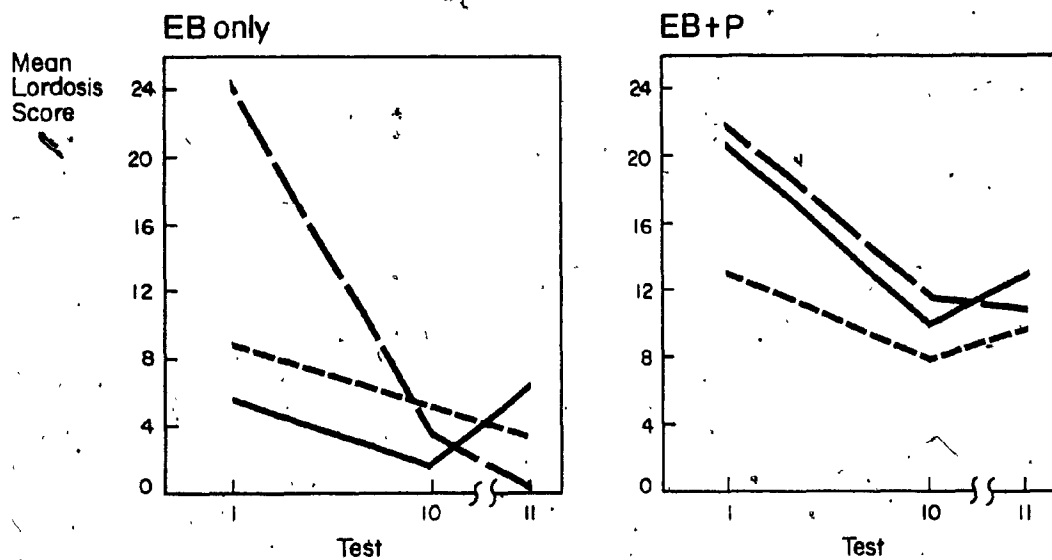


Figure 6. Mean body weights in Experiment 1 on weekly receptivity tests of ovariectomized female rats given only estradiol benzoate: WEEKLY 10 ug (—), DAILY 0.5 ug (---) or DAILY 2 ug (-.-).

Uninterrupted Hormone Treatment



Interrupted Hormone Treatment

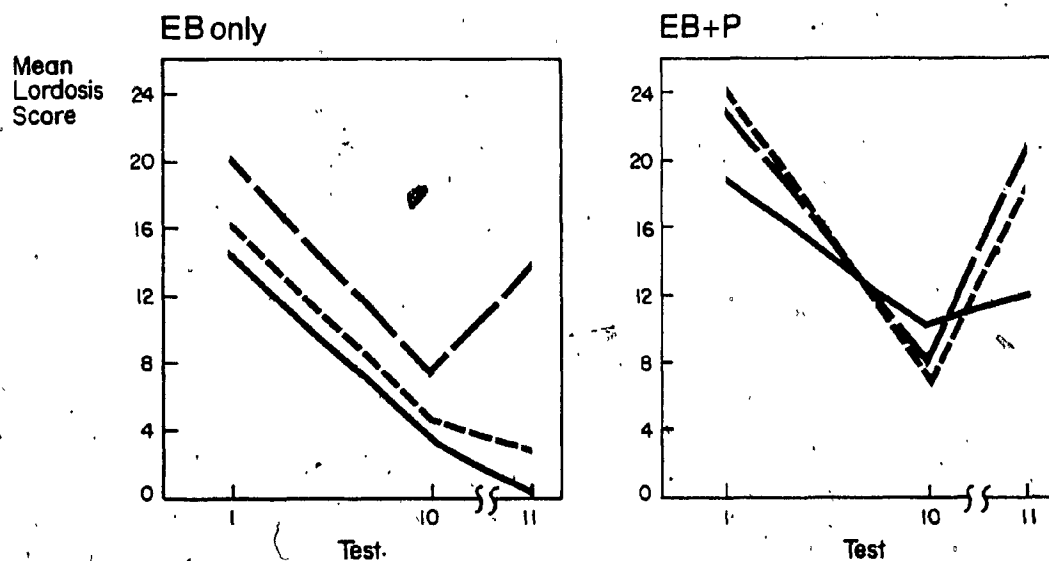
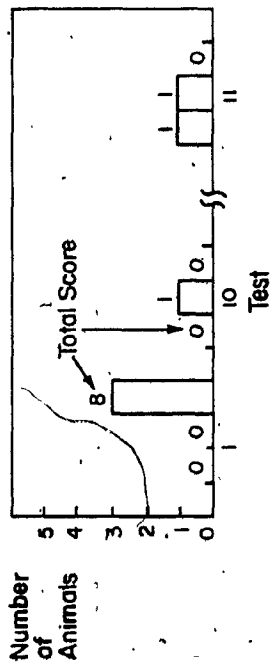


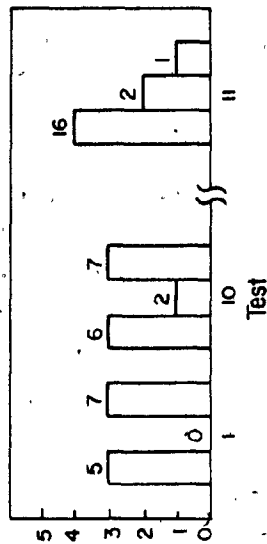
Figure 7. Mean lordosis scores for receptivity tests 1 and 10 conducted prior to interruption of steroid treatment in Experiment 1, and test 11 conducted after resumption of administration of exogenous steroid: WEEKLY 10 ug (—), DAILY 0.5 ug (-----) or DAILY 2 ug (———).

Uninterrupted Hormone Treatment

EB only
n=4,4,4

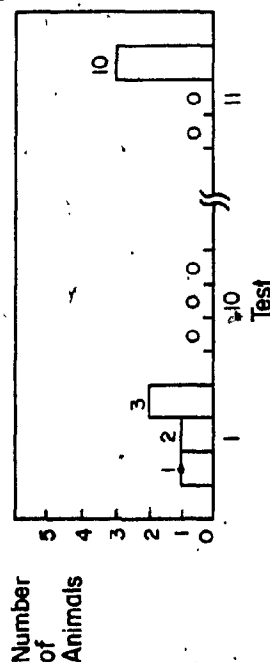


EB+P
n=4,4,4



Interrupted Hormone Treatment

EB only
n=4,5,5



EB+P
n=4,4,5

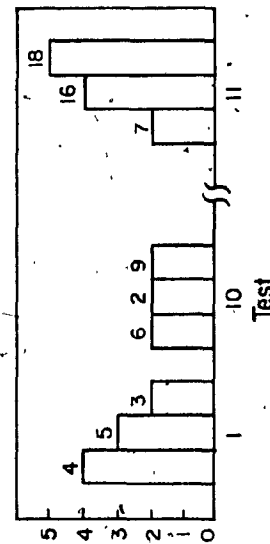
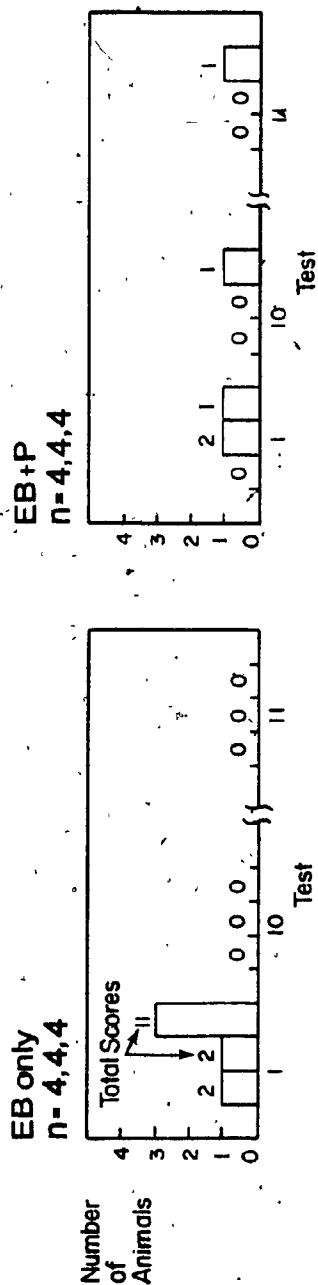


Figure 8. Number of animals displaying darting/hopping behavior on receptivity tests 1, 10 and 11. Total scores received by each group are indicated above the columns. For each condition (EB only or EB&P) and for each receptivity test, three EB conditions from Experiment 1 are represented: in order from left to right WEEKLY 10 ug, DAILY 0.5 ug and DAILY 2 ug.

Uninterrupted Hormone Treatment



Interrupted Hormone Treatment

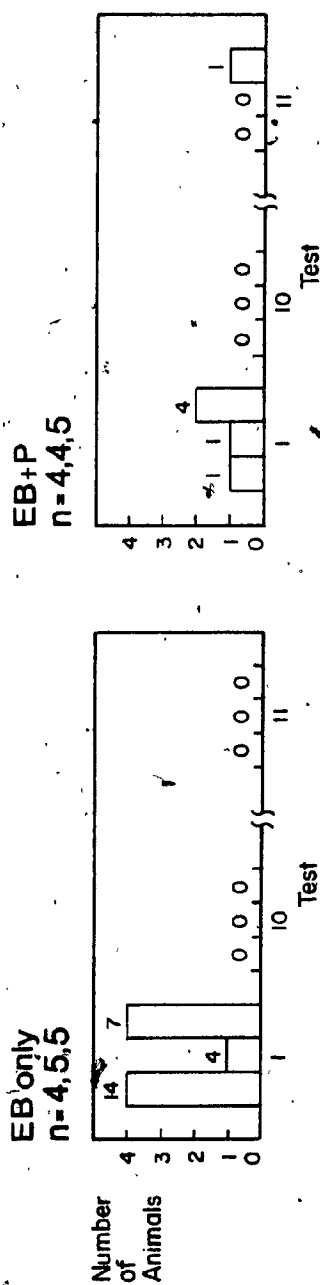


Figure 9. Number of animals displaying holding behavior on receptivity tests 1, 10 and 11. Total scores received by each group are indicated above the columns. For each condition (EB only or EB+P) and for each receptivity test, three EB conditions from Experiment 1 are represented: in order from left to right WEEKLY 10 ug, DAILY 0.5 ug and DAILY 2 ug.

conditions separately. When this was done, using the Wilcoxon Matched Pairs Test, it was found that in the EB & P condition lordosis and darting scores increased significantly after interruption. There was no overall increase in scores for animals in the EB only condition (see Figure 7).

Physiological variables: At the time of sacrifice adrenal, pituitary, brain and body weights were recorded. For the purpose of analysis these data were expressed as a percentage of body weight. Monoamine levels were determined for the whole brain, including the olfactory bulbs, and were expressed in nanograms of amine per gram of whole brain. Serum levels of corticosterone, progesterone and luteinizing hormone were also determined and the raw data are presented in Appendix B.

These data were subjected to two different analyses. In the first, the data from the experimental animals that had been ovariectomized and exposed to the three different estrogen treatment were compared in an analysis of variance for EB treatment x P treatment x Interruption. Separate analyses were done for each dependent measure. The second set of analyses of variance included the data from the intact control group. This group was considered as a fourth EB treatment group in simple one-way analyses of variance. The results of these analyses can be found in Appendix C and D. A summarized presentation is shown in Table 9. Upon inspection of this table, two points are immediately evident. First, EB treatment and

TABLE 9

Chart of Significant Main Effects (Estradiol treatment: EB; Progesterone treatment: P; Interruption: I; and Interactions for Physiological Variables)

	POSSIBLE MAIN EFFECTS AND INTERACTIONS	
	Data From OVX Animals	Data From Intact and OVX Animals
<u>PHYSIOLOGICAL VARIABLES:</u>	E B	E B
	E E P X	E E P X
	B B P	B B P
	E P I x x x x	E P I x x x x
<u>TISSUE WEIGHTS:</u>	B P I P I I	B P I P I I
	X - X - - -	X - X - - -
	X - X - X -	X - X - X -
	- - X - - -	- - X - - -
<u>MONOAMINES:</u>	- - X - - -	- - X - - -
	- - - - - -	- - - - - -
	- - - - - -	- - - - - -
	- - - - - -	- - - - - -
<u>HORMONES:</u>	X X - X - -	X X - X - -
	- - - - - -	- - - - - -
	- - - - - -	- - - - - -
	- - - - - -	- - - - - -

interruption condition account for all but two of the significant effects. Second, monoamine levels as measured in this experiment did not vary significantly as a function of treatment conditions. The nature of each of the significant effects will be considered below. The means associated with each of them are presented in Table 10.

Ovariectomized Animals

Adrenals: Post hoc analysis of the effect of EB treatment on adrenal weight indicated that animals in the WEEKLY condition had weights significantly below those in the DAILY 0.5 ug. condition and they in turn had weights significantly lower than those in the DAILY 2 ug. condition (Table 10 Section A-1). Furthermore, interruption of hormone treatment resulted in significantly lower adrenal weights (Table 10 Section A-3).

Pituitary: The effects of EB treatment and interruption on the pituitary mirror those found for the adrenals. The effect of interruption, however, was much larger in the case of animals on DAILY EB treatment than in the case of animals in the WEEKLY condition. This is reflected in the significant EB x I interaction (Table 10 Sections A-1, A-3 and A-5).

Brain: The significant effect of interruption on brain weight appears to be an artifact due to the expression of brain weights as a percentage of body weight and the changes observed in body weights as discussed below (Table 10 Section A-3).

TABLE 10

Group Means for Statistically Significant Main Effects and Interactions presented in TABLE 9.

Section A: Data From Ovariectomized Animals

A-1: EB Main Effect:

	<u>GROUPS</u>		
	WEEKLY 10 ug	DAILY 0.5 ug	DAILY 2 ug
Adrenals (% Body Weight $\times 10^{-3}$)	144	179	213
Pituitary (% Body Weight $\times 10^{-3}$)	45	55	66
Corticosterone (ng/ml of serum)	838	628	625

A-2: Progesterone Main Effect

	<u>GROUPS</u>	
	No Progesterone	0.5 mg Progesterone Per Week
Corticosterone (ng/ml of serum)	772	622
Luteinizing Hormone (ng/ml of serum)	97	226

A-3: Interruption Main Effect

	<u>GROUPS</u>	
	Interrupted	Not Interrupted
Adrenals (% Body Weight $\times 10^{-3}$)	156	202
Pituitary (% Body Weight $\times 10^{-3}$)	43	67
Brain (% Body Weight)	0.54	0.59
Body (grams)	388	356

A-4: EB x P Interaction

	<u>GROUPS</u> <u>NO PROGESTERONE</u>		
	WEEKLY 10 ug EB	DAILY 0.5 ug EB	DAILY 2 ug EB
Corticosterone (ng/ml of serum)	1077	683	557

	<u>0.5 mg PROGESTERONE WEEKLY</u>		
	WEEKLY 10 ug EB	DAILY 0.5 ug EB	DAILY 2 ug EB
Corticosterone, (ng/ml of serum)	599	574	694

A-5: EB x I Interaction

	<u>GROUPS</u> <u>WEEKLY 10 ug EB</u>	
	Interrupted	Not Interrupted
Pituitary (% Body Weight $\times 10^{-3}$)	39	51

	<u>DAILY 0.5 ug EB</u>	
	Interrupted	Not Interrupted
Pituitary (% Body Weight $\times 10^{-3}$)	41	68

	<u>WEEKLY 2 ug EB</u>	
	Interrupted	Not Interrupted
Pituitary (% Body Weight $\times 10^{-3}$)	50	82

A-6: P x I Interaction

	<u>GROUPS</u> <u>NO PROGESTERONE</u>	
	Interrupted	Not Interrupted
Corticosterone (ng/ml of serum)	745	799

	<u>0.5 ug PROGESTERONE WEEKLY</u>	
	Interrupted	Not Interrupted
Corticosterone (ng/ml of serum)	723	521

Section BData From Intact and Ovariectomized Animals

	<u>GROUPS</u>			<u>INTACT</u>
	<u>WEEKLY</u> 10 ug	<u>DAILY</u> 0.5 ug	<u>DAILY</u> 2 ug	
Adrenals (% Body Wt x 10 ⁻³)	144	179	213	204
Pituitary (% Body Weight x 10 ⁻³)	45	55	66	62
Body (grams)	367	388	359	287
Progesterone (pg/ml of serum)	4997	4398	5652	11863
Luteinizing Hormone (ng/ml of serum)	146	268	79	58

Body: Interruption of hormone treatment resulted in significantly higher body weights for each of the three Estrogen treatment conditions (Table 10 Section A-3). Estrogen treatment alone did not result in significant differences between groups at the time of sacrifice although a consistent pattern of differences was observed (see Figure 6) over the duration of receptivity testing.

Corticosterone: Although there was a significant effect of EB treatment in corticosterone levels (EB main effects Table 10 Section A-1) it can be seen that this was true only in the no P condition in which the WEEKLY animals had significantly higher scores than animals in the DAILY conditions (EB x P interaction, Table 10 Section A-4).

There appeared to be an interaction between the P condition and the interruption condition, although it was not significant at the .05 level ($p = .06$, Table 10 Section A-6). It may be important to note here that this reflects the fact that animals not receiving P had comparable levels of B whether or not they had interruption of hormone treatment, whereas among animals in the P condition interruption resulted in significantly higher B levels.

Although the EB x P x I interaction reached a statistically significant level, the group sizes are too small for a meaningful interpretation of this result.

Progesterone: The absence of any significant effects of P treatment suggests that exogenous P administration had negligible effects on plasma concentration of P at the time of sacrifice.

Luteinizing Hormone: Groups receiving P had significantly higher luteinizing hormone levels than those not receiving P (Table 10 Section A-2).

Finally the data from the one-way analyses in which the intact animals were included as a fourth EB treatment condition can be considered. The means for those measures that yielded significant effects are shown in Table 10 Section B. The data served to allow a comparison between the values found in ovariectomized EB treated groups and normal intact females. Note first that in all cases the values found for intact animals resemble most those found in the Daily 2 ug group. Adrenal and pituitary weight of intact animals did not differ from that of the DAILY 2 ug group. Intact animals did, however, have lower body weights, higher progesterone levels and lower luteinizing hormone levels than animals in the DAILY 2 ug condition. While the lower body weights may reflect in part, the fact that these animals were somewhat younger than the ovariectomized animals, they, like the higher P levels and lower luteinizing hormone levels, most probably reflect the continuous presence of estrogens from the intact ovary (Table 10 Section B).

Discussion

The principal findings of Experiment 1 were that the receptivity scores of ovariectomized rats exposed to chronic administration of estradiol benzoate decreased steadily over a ten week period and that interruption of the administration of these injections for a period of a month led to a partial recovery of these scores. While this decrease in receptivity scores with chronic treatment occurred in all groups regardless of dose, there were differential effects of treatment dose on several measures. For example, the DAILY 2 ug condition had consistently higher behavioral scores across all tests, whereas animals receiving 0.5 ug EB daily, a dose considered threshold (Davidson, Smith, Rodger & Bloch, 1968), had scores higher than those in the WEEKLY 10 ug EB condition during initial tests but lower scores on subsequent tests. In general, however, although the scores of all groups decreased over tests, the same relative order of group behavioral scores was maintained throughout the study. In other words, although the dose and schedule of EB administration determined the relative positions of group mean scores, some other factor or factors mediated the relatively uniform decreases in scores across time in all groups.

In order to identify potential mediating factors, a closer scrutiny was given to the physiological data. A possible hormonal mediation was suggested by the finding that adrenal and pituitary hypertrophy paralleled the behavioral effects. More

specifically there were also differential effects of treatment dose on the adrenal, pituitary and body weights. The highest adrenal and pituitary weights were found in the DAILY 2 ug condition and the lowest weights were in the WEEKLY condition. Body weights which have been shown to be indicators of estrogen activity (Wade 1972, Wade & Zucker 1970), were lowest in the DAILY 2 ug condition and, by the end of the experiment, highest in the WEEKLY condition. It appears, furthermore, that although the WEEKLY condition received more EB in absolute terms than the DAILY 0.5 ug condition on a per week basis, both behaviorally and physiologically the effects of estrogen administered in small distributed or chronic doses were greater than the effects of estrogen when administered in a single large dose. These physiological results are consistent with other investigations of the effects of estradiol levels on various physiological measures (Bryson & Bischoff 1979, Kitay 1968).

One interpretation of the decreases in receptivity scores found in this experiment is that they are due to non-specific changes associated with aging rather than to estrogen dependant mechanisms, since the percentage of intact rats which can be successfully mated decreases with age (Miller, Wood & Riegle 1979). However, the finding that lordosis scores increased after an extended interruption of steroid administration does not fit with an interpretation based on aging. Rather it appears that the behavioral decreases are brought about, directly or indirectly by chronic estrogen exposure.

Several studies have shown that P and other related steroids can have inhibitory effects on lordosis scores in both ovariectomized and intact rats (Blaustein & Wade 1977, Edwards et al 1968, Feder & Marrone 1977). When ovariectomized rats are given P prior to initial priming with an estrogen, their scores on subsequent receptivity tests are lower than the scores of control groups not receiving P prior to estrogen. Under these conditions the P acts to reduce the effectiveness of the estrogen in inducing receptivity. This has been labelled concurrent inhibition by P due to the presence either in serum or intracellularly of the exogenous P at the time of estrogen administration. In this connection, therefore, it is interesting to note that one effect of exogenous estrogen administration to ovariectomized rats is an increase in adrenal metabolism due to both direct effects of estrogen on the adrenal and indirect effects of estrogen on ACTH release at the level of the pituitary (Kitay 1968, Bartosik, Szarowski & Watson 1971). Although serum corticosterone levels are much better indicators of adrenal activity, significant amounts of progesterone are also synthesized in the adrenal (Shaik & Shaik 1975, Bartosik et al 1971, Feder, Resko & Goy 1968). Therefore it seems reasonable to suggest that chronic administration of exogenous estrogens might produce serum levels of adrenal steroids in ovariectomized rats sufficient to mediate the observed decreases in scores across tests. For example adrenal progesterone may mediate the decreasing receptivity scores by mechanisms analogous to those proposed elsewhere for progesterone's inhibitory effects (Blaustein & Wade 1977, Haug 1979).

Other potential candidates for adrenal steroid mediation of the behavioral decrease are the androgens. Several studies have suggested that these steroids may have a role in inhibiting lordosis in female rats (Baum, de Greef, Kloet & Schretten, 1979, Baum & Vreeburg 1976). Consistent with these speculations are several previously unexplained findings that ADX-ovariectomized rats have higher receptivity scores than ovariectomized rats in response to exogenous estrogen (Eriksson & Sodersten 1973, Davidson, Rodgers, Smith & Block 1968, Larsson, Feder & Komisaruk, 1974).

The findings of Experiment 1, together with those reported in the literature pointed to adrenal mediation of the observed estrogen effects. Experiment 2 was designed to test this idea.

Experiment 2

The primary purpose of this experiment was to evaluate the role of the adrenals in mediating the pattern of decreasing receptivity scores previously observed under conditions of chronic estrogen administration. Furthermore, in order to assess the replicability of the observed pattern of decrease of behavioral scores under other methods of chronic estrogen administration, chronic silastic implants containing the estrogen were used instead of daily injections.

A recent study of estrogen levels in ovariectomized rats receiving exogenous estrogen injections, found that within twelve to twenty-four hours of the injection the serum estradiol concentration had returned to its pre-injection level (Butcher, Inskeep & Pope 1978, Tapper et al 1974). This suggests that in Experiment 1, the procedure of daily estrogen injections did not produce stable serum concentrations of this steroid. In order to assess the relevance of this finding for the behavioral results of Experiment 1, chronically implanted estradiol pellets, with constant release rates, were used in Experiment 2.

In this experiment, receptivity scores and serum levels of adrenal progesterone and corticosterone were monitored in ovariectomized rats over an extended period of exposure to an estradiol implant. To assess the importance of the adrenal in

mediating the behavioral effects observed, half of the animals were ADX and further receptivity tests were conducted. ✓

In order to test for a role of adrenal androgens in inhibiting lordosis, a final behavioral test was held after daily administration of the anti-androgen cyproterone to both sham and ADX animals.

Method

This experiment involved the administration of chronic doses of E_2 to ovariectomized rats by means of silastic implants with known release rates. For the first part of the experiment receptivity tests took place each week and blood samples were collected every other week. Then the animals were split into two groups. One group was ADX and the other had a sham - ADX. The schedule of receptivity tests and blood sampling was maintained to the end of the study.

Treatment of subjects: Twenty-four adult Sprague-Dawley female rats weighing 200-270 gms when obtained from Canadian Breeding Farms were used as subjects in this experiment. Animals were initially housed in pairs for several weeks prior to the start of this experiment under conditions identical to those in Experiment 1. For the duration of this experiment they were housed individually. One week before ovariectomy, daily handling of each animal began and continued throughout the experiment.

In order to eliminate totally unresponsive animals from this experiment, a single receptivity test was held under conditions of conventional priming with 10 ug EB and 0.5 mg P. No animal was eliminated.

At the end of the experiment the animals were decapitated and the whole brains quickly removed. The pituitary and surrounding area was inspected for any obvious abnormalities. Finally the presence of the estradiol implant with remaining steroid was confirmed by visual inspection.

Hormone treatments: All twenty-four animals were ovariectomized on Day 1 following the procedure outlined in Experiment 1. At this time each animal received 10 ug EB in 0.5 ml peanut oil subcutaneously. On Day 8 another 10 ug EB was given followed forty hours later by a subcutaneous injection of 0.5 mg P in 0.5 ml peanut oil. The receptivity "pre-test" took place four hours later. On Day 13 all animals were implanted with silastic tubing containing estradiol. Sodium pentobarbital anesthesia (Nembutal: 60 mg/ml, 30 mg/kg) with 0.1 ml atropine sulphate (0.6 mg/ml) was used. For a fourteen week period beginning day 22, each animal received a weekly injection of 0.5 mg P in 0.5 ml peanut oil four hours before the start of receptivity testing.

After the ninth test under chronic estrogen exposure the animals were split into two groups. On Day 79 one group was adrenalectomized and the other group underwent a sham-adrenalectomy.

involving full exposure of both adrenals. ADX animals were switched to 0.9% saline for the balance of the experiment. The anesthetic procedure used was identical to that used earlier for ovariectomy and implantation. The four receptivity tests after adrenalectomy took place on Days 85, 92, 106 and 115. All animals were given 10 mg of cyproterone (Schering) in 0.2 ml peanut oil at 10h on Days 111-115 inclusive.

Blood sampling: Six blood samples were taken from each animal, four before ADX or sham-ADX and two after. The first sample was taken on Day 34 when the estradiol implant had been in place for twenty days. The last sample was taken on Day 104, two days before the twelfth receptivity test. All blood samples were taken at the same time of day beginning at 15h. Animals were taken in groups of four or five from the animal room to the room where blood collection was to take place. Each animal underwent light ether anesthesia and approx 2.5cc of blood was quickly removed from the jugular vein using 16 gauge needles into heparinized syringes. The blood was kept on ice in glass test-tubes for at most ninety minutes prior to centrifuging at 2400 rpm for ten minutes. The supernatant was transferred to small glass test-tubes which were sealed with Parafilm and frozen at -75 C.

The order in which animals were sampled was recorded since it was felt that within a group of four or five animals those sampled first would have been less stressed by the light, noise etc. of the blood collection room than the animals sampled last.

Furthermore for each animal the elapsed time between the start of ether anesthesia and the withdrawal of the needle from the jugular was recorded in order to be able to assess the importance of duration of anesthesia on steroid levels.

Testing procedure for sexual receptivity: Test sessions began approximately eight hours into the dark period in the same test boxes and room as described for Experiment 1. Lordosis, holding, and darting scores were recorded as outlined in Experiment 1.

Assignment to adrenalectomy (A) or sham (S) group: An attempt was made to match the groups with respect to receptivity scores and the pattern of change of these scores over time.

Each animal was given a total lordosis score which was made up of the sum of its lordosis scores on the pretest and on each of the first eight receptivity tests. Similar scores were calculated for both darting and holding. In addition, individual plots of the scores obtained on each receptivity test were made. The total scores were used together with the plots to assign an equal number of animals to the two groups. Since not all animals showed similar decreases in scores across tests, an attempt was made to assign animals showing similar patterns to each of the groups. The resulting means and standard deviations were: for lordosis 110 ± 33 (A) versus 110 ± 27 (S), for darting 34 ± 17 (A) versus 33 ± 15 (S) and for holding 3 ± 3 (A) versus 4 ± 4 (S).

Steroid Assays: The six sets of blood samples were analyzed by radioimmunoassay for levels of estradiol, progesterone and corticosterone. Initially, the samples were analyzed in three separate subgroups: sample 1 by itself, samples 2, 3 and 4 together, and then samples 5 and 6 together. Later, estradiol levels were reassessed using samples 1 through 6 inclusive in a single assay. Refer to Experiment 1: Method for an outline of the steroid assay procedure.

Preparation of estradiol implants:

Silastic medical grade tubing (Dow Corning no. 602-305; 0.235 in. outside diameter, 0.078 in. inside diameter) was used. Capsules were packed with 10 mm of crystalline 17-beta - estradiol (Sigma) and stored in peanut oil for twenty-four hours prior to being implanted in the animals. Steroid absorption was not measured directly but was taken to be 2.4 ug/cm/day (Robaire, personal communication).

Results

The behavioral data from this experiment will be considered first. Tests 1 - 9 provide data concerning the changes in sexual behavior over approximately twelve weeks when all animals were similarly treated with the chronically implanted E_2 pellet. Tests 10 - 13, carried out after half of the animals were ADX, provide information about the possible contribution of the adrenal to the observed changes. Test 13 was done after treatment with cyproterone.

Behavioral Tests 1 - 9: The data from these tests were subjected to analyses of variance for Group X Test. For although animals were assigned to the ADX or sham condition only after Test 9, the analyses of variance include a Group variable in order to permit an identification of any pre-adrenalectomy group differences which could have been due only to the process of assignment to groups.

It can be seen from Figures 10, 11 and 12 that there is a general decline in responding across the nine tests. Due to the large number of zero scores for holding, an analysis of variance was not possible. However, for both lordosis scores and darting scores, this decline is reflected in a significant Test effect (see Tables 11, 12). As expected, the Group effect was not significant. This indicates that no significant differences existed between the animals which were assigned to the adrenalectomy or sham condition.

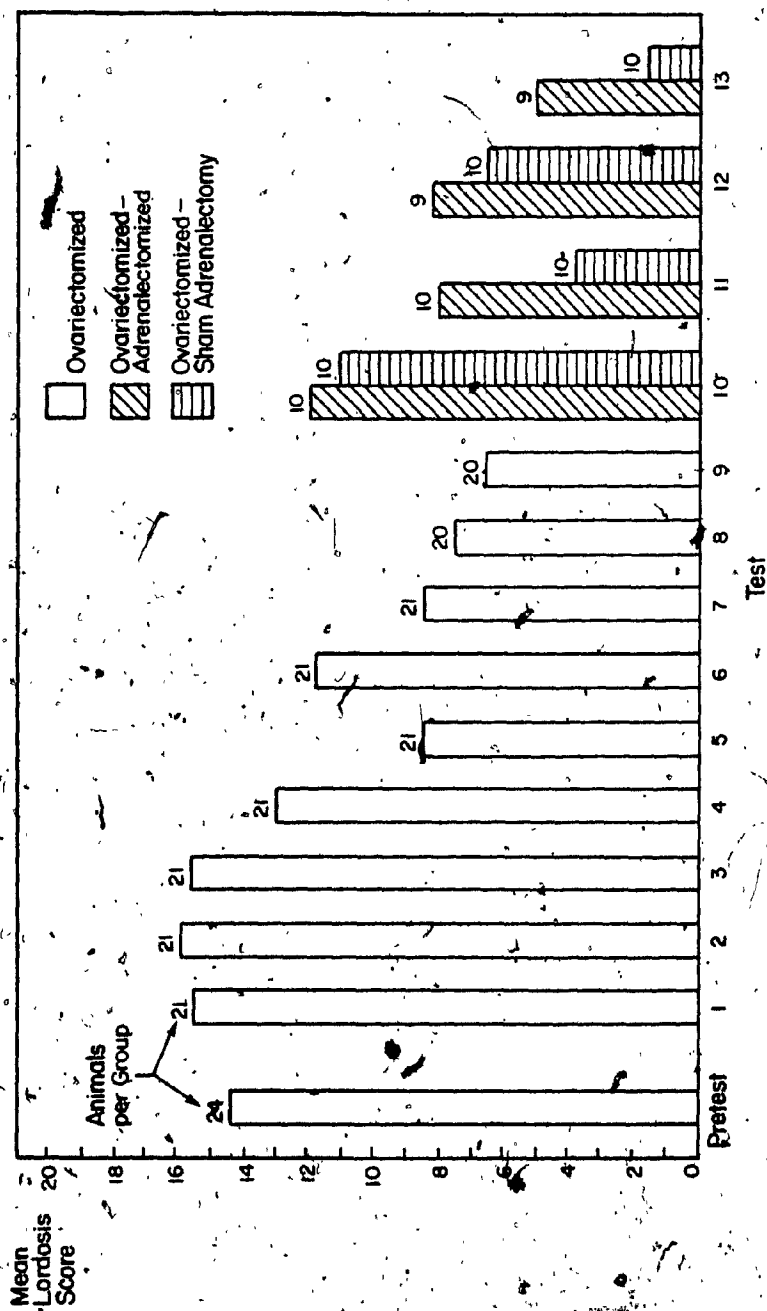


Figure 10. Mean lordosis scores on each receptivity test for animals in

Experiment 2. Numbers above columns indicate the number of animals tested.

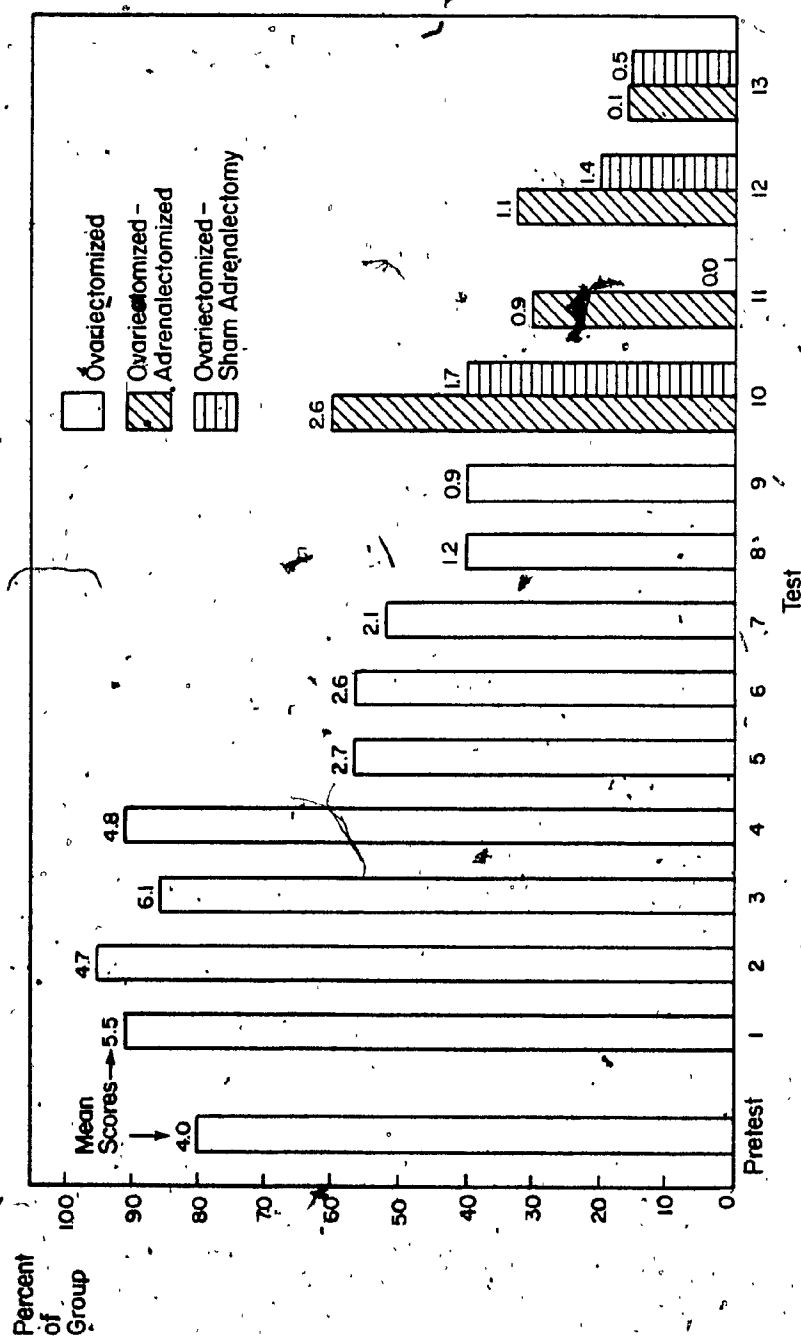


Figure 11. Percent of animals displaying darting/hopping behavior on receptivity tests in Experiment 2. Numbers above columns represent the mean scores on each test.

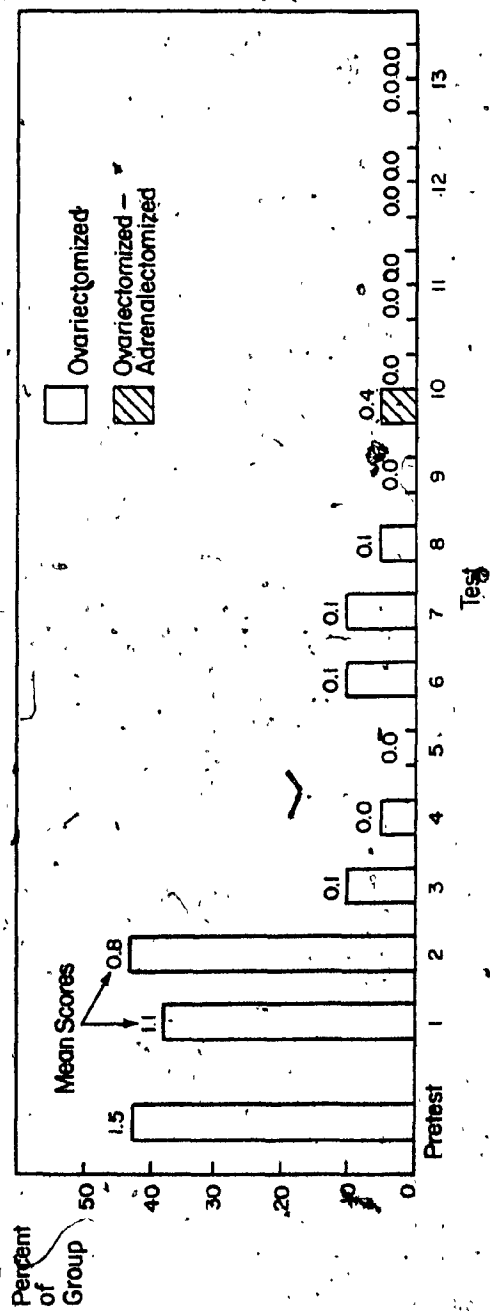


Figure 12. Percent of animals displaying holding behavior on receptivity tests in Experiment 2. Numbers above columns represent the mean scores on each test.

TABLE 11

Summary Table for the ANOVA of Lordosis scores on Test 1 through Test 9 inclusive.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
Treatment	1	11.4	0.45	0.5
Test	8	282.2	11.22	0.001
Treatment X Test	8	29.1	1.16	0.3
Subjects	169	25.1		

TABLE 12

Summary Table for the ANOVA of Darting scores on Test 1 through Test 9 inclusive.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
Treatment	1	0.29	0.04	0.85
Test	8	75.4	9.1	0.001
Treatment X Test	8	6.05	0.7	0.66
Subjects	169	8.24		

Tests 10 - 12 and Test 13 (post-adrenalectomy tests): The significant Group main effect for lordosis scores ($p = 0.05$, see Table 13) reflect the consistently higher scores across the three tests for the ADX group (Figure 10). The significant Test effect for lordosis scores reflects the decline in mean lordosis scores across the three tests as is apparent in Figure 10.

No significant effect of group was found in the analysis of variance carried out on the darting scores (see Table 14). Almost no holding was observed across these three tests.

In order to test for the significance of the change in lordosis scores pre - and post - adrenalectomy, a separate analysis of variance was done using the lordosis scores for the three tests preceding adrenalectomy as one condition and the first three tests following adrenalectomy as the second condition, Table 15 indicates that the scores of ADX animals do not differ significantly from the sham - operated animals across the six tests included in the analysis. Furthermore, there were no significant differences for either group in their pre versus post adrenalectomy scores.

Test 13 took place after five days of treatment with cyproterone. No significant differences in lordosis, darting or holding scores were found between the two groups on this test ($t = 1$, $df = 17$, $p = 0.2$).

TABLE 13

Summary Table for the ANOVA of Lordos's scores on Tests 10, 11 and 12

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
Treatment	1	92.6	3.98	0.0512
Test	2	159.1	6.85	0.0023
Treatment X Test	2	13.0	0.56	0.57
Subjects	52	23.2		

TABLE 14

Summary Table for the ANOVA of Darting scores on Tests 10, 11 and 12

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
Treatment	1	3.62	0.68	0.41
Test	2	14.0	2.64	0.08
Treatment X Test	2	2.25	0.42	0.66
Subjects	52	5.32		

TABLE 15

Summary Table for the ANOVA of Lordosis Scores before and after adrenalectomy.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>R</u>
Treatment	1	78.6	2.7	0.10
Order	1	18.7	0.65	0.42
Treatment x Order	1	28.8	0.99	0.32
Subjects	115	28.9		

RIA for serum steroid levels: In order to interpret the results of Experiment 2 it was important to determine whether the serum estradiol levels remained relatively constant throughout the study. In addition, in order to assess possible changes in adrenal activity throughout the experiment, the serum levels of corticosterone and progesterone were monitored.

In spite of the fact that the assays were not run in a single batch, the intra-assay variabilities for progesterone and corticosterone, as well as the blank and pool values across assays, suggest that inter-assay comparisons are meaningful. Unfortunately, a great deal of intra - and inter-assay variability was found for estradiol in the original runs. In an attempt to overcome this, a final assay was done with all samples in a single batch. There was, however, insufficient serum remaining in many cases by this time, and the data are, therefore, incomplete. Therefore, the estradiol concentrations from the original separate runs were reassessed and converted to standardized scores by prorating in a consistent manner. These data indicated that at least for the first blood sample, taken 20 days after implantation of the pellet, and for the last blood sample, taken 2 days before Test 12, the mean estradiol levels were comparable and in the order of 70 ng/ml.

As can be seen from Figures 13 and 14, both the progesterone and corticosterone levels decreased steadily across the first four blood samples; progesterone levels dropped more steeply.

Progesterone
(pg/ml)

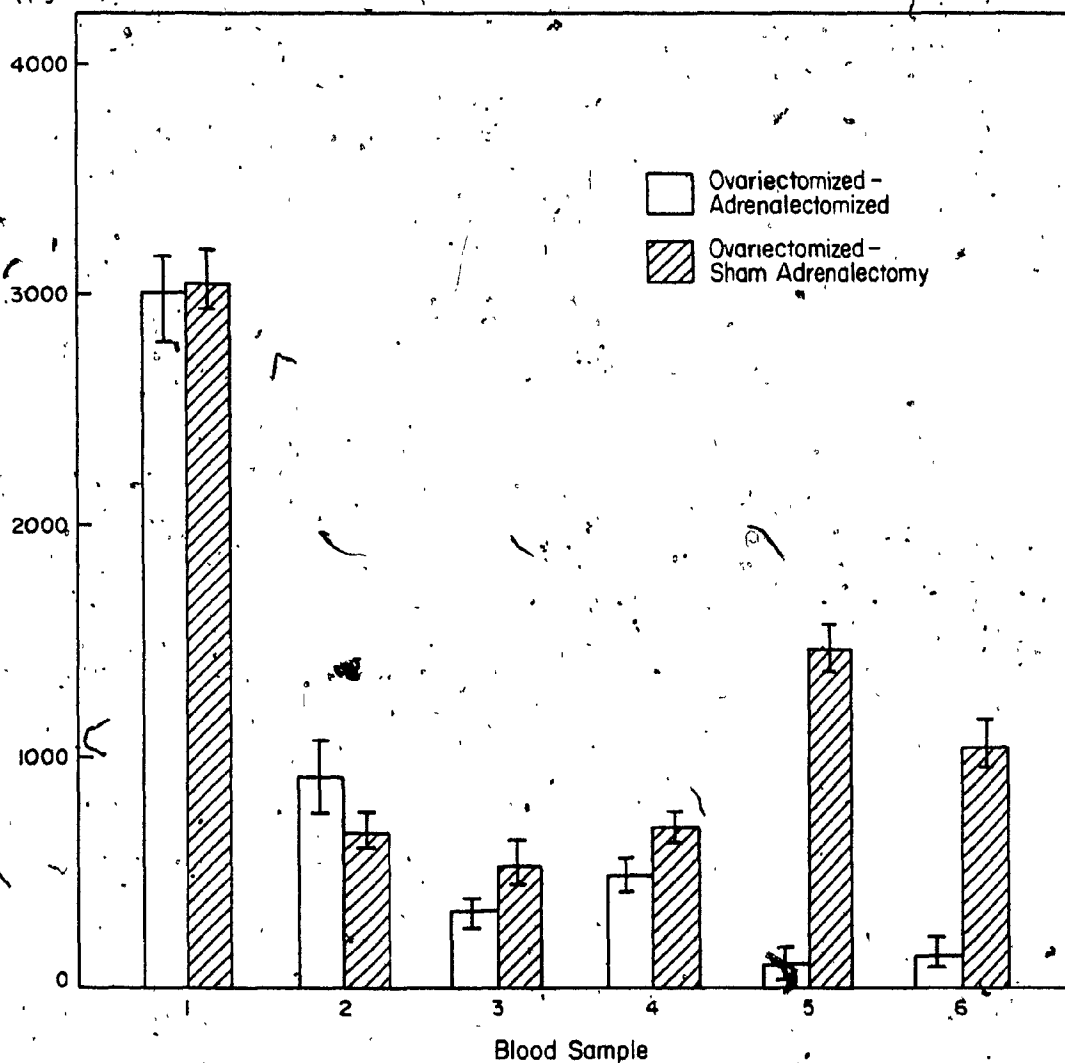


Figure 13. Mean serum progesterone levels obtained from blood samples taken every two weeks during Experiment 2 in ovariectomized and ovariectomized-adrenalectomized groups both before adrenalectomy (Samples 1 - 4) and after (Samples 5 and 6). Bars represent the mean of the 95% confidence intervals associated with concentration readings from individual samples.

If progressively decreasing mean receptivity scores were to be explained by mechanisms similar to those invoked to explain concurrent inhibition by progesterone (Blaustein & Wade 1977), then, at a minimum, a pattern of relatively high progesterone or corticosterone levels would have been expected. Not only was this not found, but in addition the observed values were within ranges reported elsewhere as physiological (Shaikh & Shaikh 1975, Döhler & Wuttke 1975, Huang et al 1978). It appears, therefore, that in spite of stable estradiol levels in the physiological range and in spite of apparent decreasing adrenal steroid output, behavioral measures of receptivity decrease over time.

The results of the final behavioral test, after cyproterone treatment are unclear. If, as was hypothesized, adrenal androgens mediate the decreasing lordosis scores, then the scores of ADX animals that have no adrenal androgen should be unaffected by cyproterone treatment. (For the sham animals, however, cyproterone might have been expected to produce a disinhibition of lordosis resulting in scores equal to or higher than those found in the ADX animals. The fact that the lordosis scores for both groups were lower on this test than on preceding tests yet not significantly different from each other suggests that adrenal androgens are not involved in the pattern of decreasing scores.

GENERAL DISCUSSION

These experiments show that chronic exposure of ovariectomized rats either to estradiol from an implant or to estradiol behzoate via subcutaneous injection is associated with a pattern of decreasing receptivity scores. These decreases were accompanied by decreases in serum corticosterone and progesterone levels. In addition it was found that receptivity scores partially recovered after a four week interruption of the estradiol administration schedule. Since these findings are new and appear to contradict findings reported in the literature, some clarification is necessary.

A great deal of research has been done on the effects of repeated matings on lordosis scores. Although many of these studies were not done with the intention of assessing the effects of repeated exposure to estradiol, their designs are such that certain comparisons with the experiments outlined in this study are possible. Typically in one kind of study each receptivity test in a series is preceded by a "priming" exposure to estrogen and often by a facilitating dose of progesterone. A general finding in such studies is that repeated mating tests either result in successively higher lordosis scores or in constant scores across tests. For the ovariectomized rat, no reports exist of repeated mating tests resulting in progressively lower lordosis scores. Upon closer scrutiny it becomes evident, however that these studies cannot be compared directly to the present one. In many studies successive receptivity tests were held within the same day or on a daily basis

(Larsson, et al 1974, Zemlan & Adler 1977, Hardy & Debold 1973, Beach 1976). These results of increasing lordosis scores are generally interpreted in terms a slowly developing neuroendocrine change at the level of the pituitary-adrenal axis which favours a subsequent display of lordosis.

Several other studies involve weekly receptivity tests and either chronic estrogen implants or injections. Henrik & Gerall (1976) reported increasing lordosis scores over seven weekly tests using priming injections of EB followed by P. However, unlike the present studies, the exposure of their animals to estrogen prior to the first receptivity test was such that lordosis scores were at a minimum for the first test. Furthermore the dose of EB was tripled after the third test in that study whereas, in Experiment 1 all estrogen doses were held constant for the duration of the experiment. In another study utilizing chronic estrogen implants, constant lordosis scores were maintained for a thirty-five day period (Campbell & Baum 1979). A significant difference between that study and Experiment 2 was their use of estradiol cypionate a very long acting esterized form of estradiol while in Experiment 2, the free base was used. Furthermore, whereas Campbell & Baum (1979) reported that by the end of their study serum estradiol levels had dropped to values found in the control group with blank implants, in Experiment 2 initial and final serum estradiol levels were comparable suggesting a truly chronic exposure to the steroid.

Finally, in a study involving repeated estrogen injections to ovariectomized-ADX rats and receptivity testing over a

ten month period, no progressive insensitivity to estradiol was observed (Zemlan and Adler 1977). Rather this study demonstrated the importance of the prior hormonal history in determining both the quality and quantity of the lordosis response to subsequent acute hormone administration. No direct comparison with the present experiments is possible because the EB injections consisted of a set of progressively increasing or decreasing doses. Thus, while at first sight it appeared that the results of the present study were contradicting to these reported in the literature it appears that there really are no results in the literature that can be said to conflict with the findings of Experiments 1 and 2. On the other hand, due to significant differences in experimental design, the results of even superficially similar studies cannot be brought to bear on the questions of mechanisms underlying the findings of this study.

In spite of these limitations, several possibilities are suggested by the data. In Experiment 1, the WEEKLY condition may have differed from the two DAILY conditions in a subtle manner. When P was given to animals receiving WEEKLY estrogen, the facilitating effect of P was much more dramatic than when P was given to animals receiving DAILY estrogen. One could speculate that the mechanisms of P receptor synthesis, that are triggered by exposure to estrogen, were in some sense exhausted by daily exposure to estrogen and this may have resulted in few P receptors being available at the time of P administration.

Since this possible difference in mechanism between the WEEKLY and DAILY conditions would be in effect across all tests, an

additional separate mechanism may be needed to explain why all groups showed a pattern of decrease across tests. Several studies have shown that after prolonged estradiol deprivation, the ovariectomized rat requires one or more estradiol injections before normal levels of receptive behavior can be observed (Beach 1976, Beach & Orndoff 1974, Zemlan & Adler 1977, Damassa & Davidson 1973, Gerall & Dunlop 1973). In the case of the WEEKLY condition one could speculate that the pattern of decreasing scores is due to a progressive insensitivity to estradiol due to chronically inadequate plasma levels of the steroid. After all, these animals may have had no exogenous serum estradiol for six of the seven days of each week (Butcher et al 1978). If this interpretation is correct then several days of estrogen injections would "resensitize" these animals to estrogen, after which normal levels of receptivity should be observed.

A different mechanism is needed to explain the decreases in the DAILY condition since no prolonged estrogen deprivation was involved. This study has suggested that daily exposure to estrogen may exhaust the mechanisms that synthesize P receptors. One could speculate that in some analogous manner, daily exposure may reduce or "down-regulate" estrogen receptor synthesis or render existing estrogen receptors less sensitive to subsequent estrogen or possibly exhaust the nuclear substrate to which the estrogen-receptor complex binds in order to exert its effects. The behavioral result of any such changes would be decreasing receptivity scores in response to a fixed amount of exogenous estrogen.

With this explanation of the decreases in receptivity scores for the daily conditions, it follows that interrupting the chronic estradiol treatment permits the reestablishment of a neural intracellular environment like that found in rats several weeks after ovariectomy. Subsequent exposure to estrogen for several days would resensitizes the neurons to estrogens and hence result in receptivity scores higher than those found prior to interruption of the steroid. Although the data suggest that interruption improved behavioral scores only in the EB plus P condition, interruption had significant effects on adrenal, pituitary and body weights in both the EB only condition and the EB plus P condition. It may be that only animals in the EB plus P condition showed a recovery in receptivity after interruption because the re-sensitizing procedure was adequate to permit facilitation by P but involved doses of EB too small to permit observation of receptive behavior in the absence of P.

In summary the present set of experiments demonstrate several apparently new behavioral and physiological findings associated with chronic estrogen exposure and its interruption. Furthermore evidence has been presented against a significant mediating role for the adrenals in the behavioral pattern. The relevance of the mechanisms proposed in these latter paragraphs can only be determined by future experiments.

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Appendix A

Raw receptivity scores for tests 1, 10 and 11
in experiment 1.

TABLE 1

Lordosis (L), Darting (D) and Holding (H) Scores for Tests 1, 10 and 11 for animals receiving no progesterone and having uninterrupted treatment.

ESTROGEN TREATMENT	ANIMAL NUMBER	TEST #1			TEST #10			TEST #11		
		L	D	H	L	D	H	L	D	H
WEEKLY 10 ug EB	4	22	0	2	4	0	0	11	0	0
	6	0	0	0	0	0	0	9	0	0
	7	0	0	0	2	0	0	4	1	0
	8	0	0	0	0	0	0	0	0	0
DAILY 0.5 ug EB	19	0	0	0	2	1	0	0	0	0
	21	12	0	0	6	0	0	0	0	0
	22	0	0	0	8	0	0	3	0	0
	24	24	0	2	7	0	0	10	1	0
DAILY 2 ug EB	33	26	0	7	0	0	0	2	0	0
	36	18	2	2	6	0	0	0	0	0
	38	25	4	2	0	0	0	0	0	0
	40	20	0	2	8	0	0	0	0	0

TABLE 2

Lordosis (L), Darting (D) and Holding (H) Scores for Tests 1, 10 and 11 for animals receiving weekly progesterone and having uninterrupted treatment.

ESTROGEN TREATMENT	ANIMAL NUMBER	TEST #1			TEST #10			TEST #11		
		L	D	H	L	D	H	L	D	H
WEEKLY 10 ug EB	9	17	0	0	11	0	0	11	2	0
	10	19	2	0	8	2	0	11	6	0
	11	27	1	0	11	2	0	14	6	0
	13	21	2	0	9	2	0	7	2	0
DAILY 0.5 ug EB	25	22	0	2	7	0	0	9	1	0
	28	14	0	0	5	0	0	7	0	0
	29	4	0	0	8	0	0	8	1	0
	32	11	0	0	12	2	0	12	0	0
DAILY 2 ug EB	42	20	4	0	14	3	0	13	0	0
	44	19	2	0	15	3	0	11	0	0
	46	21	1	0	16	1	1	17	2	0
	47	27	0	1	2	0	0	12	0	0

TABLE 3

Lordosis (L), Darting (D) and Holding (H) Scores for Tests 1, 10 and 11 for animals receiving no progesterone and having interrupted treatment.

ESTROGEN TREATMENT	ANIMAL NUMBER	TEST #1			TEST #10			TEST #11		
		L	D	H	L	D	H	L	D	H
WEEKLY 10 ug EB	1	16	0	6	8	0	0	2	0	0
	2	18	1	3	0	0	0	0	0	0
	3	11	0	4	0	0	0	0	0	0
	5	16	0	1	7	0	0	3	0	0
DAILY 0.5 ug EB	17	20	2	5	2	0	0	0	0	0
	18	17	0	0	0	0	0	7	0	0
	20	13	0	0	12	0	0	11	0	0
	50	11	0	0	1	0	0	0	0	0
	53	21	0	0	10	0	0	0	0	0
DAILY 2 ug EB	34	18	0	1	11	0	0	12	3	0
	35	25	1	0	0	0	0	14	0	0
	37	14	1	0	0	0	0	11	4	0
	39	23	0	3	14	0	0	9	0	0
	51	21	3	1	8	0	0	18	3	0

TABLE 4

Lordosis (L), Darting (D) and Holding (H) Scores for Tests 1, 10 and 11 for animals receiving weekly progesterone and having interrupted treatment.

ESTROGEN TREATMENT	ANIMAL NUMBER	TEST #1			TEST #10			TEST #11		
		L	D	H	L	D	H	L	D	H
WEEKLY 10 ug EB	12	22	1	1	11	2	0	4	0	0
	14	18	1	0	8	0	0	11	0	0
	15	22	1	0	10	0	0	10	3	0
	16	15	1	0	11	4	0	11	4	0
DAILY 0.5 ug EB	26	18	1	0	13	1	0	12	7	0
	27	24	2	1	13	1	0	20	1	0
	30	26	2	0	0	0	0	15	2	0
	31	22	0	0	2	0	0	10	6	0
DAILY 2 ug EB	43	22	2	3	17	3	0	22	3	0
	45	24	0	1	0	0	0	16	3	0
	48	30	0	0	0	0	0	18	2	0
	49	24	1	0	13	6	0	14	7	0
	52	15	0	0	3	0	0	15	4	0

Appendix B

Raw serum concentrations of progesterone,
corticosterone and luteinizing
hormone on day 141 of Experiment 1.

TABLE 1

Serum concentrations of Progesterone (P), corticosterone (B) and Luteinizing Hormone (LH) on Day 141 of Experiment 1 for animals receiving no progesterone and having uninterrupted treatment.

ESTROGEN TREATMENT	ANIMAL NUMBER	P pg/ml	B ng/ml	LH ng/ml
WEEKLY 10 ug EB	4	8566	1048	68
	6	6697	1347	10
	7	4557	1241	10
	8	6628	1608	58
DAILY 0.5 ug EB	19	4315	352	25
	21	3907	660	10
	22			
	24	3846	718	10
DAILY 2 ug EB	33	5356	868	10
	36	8043	575	10
	38	1288	281	10
	40	2152	309	10

TABLE 2

Serum concentrations of Progesterone (P), corticosterone (B) and Luteinizing Hormone (LH) on Day 141 of Experiment 1 for animals receiving weekly progesterone and having uninterrupted treatment.

ESTROGEN TREATMENT	ANIMAL NUMBER	P pg/ml	B ng/ml	LH ng/ml
WEEKLY 10 ug EB	9	4789	978	48
	10	4702	730	148
	11	632	189	10
	13	4255	653	16
DAILY 0.5 ug EB	25	669	35	10
	28	11253	557	N/A
	29	1423	512	N/A
	32	4765	772	10
DAILY 2 ug EB	42	5085	698	10
	44	4519	552	10
	46	7067	704	10
	47	12482	772	10

TABLE 3

Serum concentrations of Progesterone (P), corticosterone (B) and Luteinizing Hormone (LH) on Day 141 of Experiment 1 for animals receiving no progesterone and having interrupted treatment.

ESTROGEN TREATMENT	ANIMAL NUMBER	P pg/ml	B ng/ml	LH ng/ml
WEEKLY 10 ug EB	1	2031	703	427
	2	3757	1021	306
	3	3685	774	280
	5	2859	732	222
DAILY 0.5 ug EB	17	4371	894	201
	18	7201	922	198
	20	2704	605	117
	50	4658	802	133
	53	2395	710	135
DAILY 2 ug. EB	34	5399	382	265
	35	11869	1119	10
	37	3293	323	10
	39	3006	634	34
	51	3616	571	133

TABLE 4

Serum concentrations of Progesterone (P), corticosterone (B) and Luteinizing Hormone (LH) on Day 141 of Experiment 1 for animals receiving weekly progesterone and having interrupted treatment.

ESTROGEN TREATMENT	ANIMAL NUMBER	P pg/ml	B ng/ml	LH ng/ml
WEEKLY 10 ug EB	12	3978	574	332
	14	2983	758	292
	15	6389	834	253
	16	5481	974	135
DAILY 0.5 ug EB	26	4270	431	35
	27	5580	854	N/A
	30	3227	601	201
	31	5782	832	201
DAILY 2 ug EB	43	5220	512	10
	45	4953	710	347
	48	3226	729	66
	49	9977	876	44
	52	5168	704	10

Appendix C

Analysis of variance of physiological
variables from Experiment 1
using data from ovariectomized animals only.

TABLE 1

Summary Table of ANOVA of Adrenal Weights expressed as a percentage of body weight for the ovariectomized animals of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	18516.	24.3	0.00
P Treatment	1	260.0	0.34	0.56
Interruption (I)	1	24690.	32.4	0.00
EB x P	2	917.3	1.2	0.31
EB x I	2	434.4	0.57	0.57
P x I	1	1.8	0.0	0.96
EB x P x I	2	618.5	0.81	0.45
Subjects	38	762.4		

TABLE 2

Summary Table of ANOVA of Pituitary Weights expressed as a percentage of body weight for the ovariectomized animals of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	179734.	19.3	0.00
P Treatment	1	12471.	1.3	0.25
Interruption (I)	1	688507.	73.9	0.00
EB x P	2	6514.	0.68	0.51
EB x I	2	45630.	4.9	0.01
P x I	1	1895.	0.20	0.65
EB x P x I	2	531.9	0.0	0.94
Subjects	38	9310.		

TABLE 3

Summary Table of ANOVA of Brain Weights expressed as a percentage of body weight for the ovariectomized animals of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	7657.5	2.48	0.09
P Treatment	1	307.1	0.0	0.75
Interruption (I)	1	30951.	10.0	0.00
EB x P	2	1495.9	0.48	0.62
EB x I	2	433.9.	0.14	0.86
P x I	1	1057.0	0.34	0.56
EB x P x I	2	2511.7	0.81	0.45
Subjects	38	3091.6		

TABLE 4

Summary Table of ANOVA of Body Weights for the ovariectomized animals
of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	3320.8	2.93	0.06
P Treatment	1	19.17	0.0	0.89
Interruption (I)	1	11985.	10.58	0.00
EB x P	2	451.2	0.40	0.67
EB x I	2	173.8.	0.15	0.85
P x I	1	257.8	0.23	0.63
EB x P x I	2	478.9	0.42	0.65
Subjects	38	1132.8		

TABLE 5

Summary Table of the ANOVA of Serotonin Concentrations for the Ovariectomized Animals of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	379.8	0.27	0.76
P Treatment	1	624.9	0.44	0.50
Interruption (I)	1	2549.8	1.80	0.18
EB x P	2	3802.9	2.69	0.08
EB x I	2	1739.6	1.23	0.30
P x I	1	2.10	0.0	0.96
EB x P x I	2	653.8	0.46	0.63
Subjects	38	1409.6		

TABLE 6

Summary Table of the ANOVA of Norepinephrine Concentrations for the
Ovariectomized Animals of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	90.70	0.9	0.90
P Treatment	1	33.07	0.0	0.84
Interruption (I)	1	1217.0	1.3	0.25
EB x P	2	1220.5	1.3	0.27
EB x I	2	255.4	0.28	0.75
P x I	1	3385.7	3.7	0.06
EB x P x I	2	427.8	0.46	0.62
Subjects	38	911.0		

TABLE 7

Summary Table of the ANOVA of Dopamine Concentrations for the Ovariectomized Animals of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	747.85	0.0	0.98
P Treatment	1	12304.	0.30	0.58
Interruption (I)	1	63941.	1.58	0.21
EB x P	2	91455.	0.22	0.79
EB x I	2	1490.5	0.37	0.96
P x I	1	10763.	0.27	0.60
EB x P x I	2	51773.	1.28	0.28
Subjects	38	40432.		

TABLE 8

Summary Table of the ANOVA of Serum Corticosterone Concentrations for
the Ovariectomized Animals of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	235921.	4.62	0.016
P Treatment	1	266989.	5.23	0.028
Interruption (I)	1	65944.	1.29	0.26
EB x P	2	381436.	7.48	0.002
EB x I	2	66999.	1.31	0.28
P x I	1	195717.	3.84	0.0577
EB x P x I	2	256189.	5.02	0.01
Subjects	37	51014.		

TABLE 9

Summary Table of the ANOVA of Serum Progesterone Concentrations for
the Ovariectomized Animals of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB Treatment	2	6.6×10^6	0.88	0.42
P Treatment	1	4.8×10^6	0.64	0.43
Interruption (I)	1	1.8×10^5	0.02	0.88
EB x P	2	3.7×10^6	0.49	0.61
EB x I	2	3.9×10^5	0.05	0.95
P x I	1	2.4×10^6	0.32	0.58
EB x P x I	2	1.8×10^7	2.40	0.10
Subjects	37	7.5×10^6		

TABLE 10

Summary Table of the ANOVA of Serum Luteinizing Hormone Concentrations
for the Ovariectomized Animals of Experiment 1.

<u>Source</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>P</u>
EB treatment	2	140097.	2.87	0.07
P Treatment	1	200526.	4.11	0.05
Interruption (I)	1	151947.	3.11	0.09
EB x P	2	141616.	2.90	0.07
EB x I	2	47512.3	0.97	0.39
P x I	1	18421.1	0.38	0.54
EB x P x I	2	35604.6	0.73	0.49
Subjects	37	48839.4		

Appendix D

Analyses of variance of physiological
variables from Experiment 1
using data from intact and
ovariectomized animals.

TABLE 1

Summary Table of ANOVA performed on each of ten physiological measures based on four estrogen treatment groups: WEEKLY, DAILY 0.5 microgram, DAILY 2 microgram and intact.

PHYSIOLOGICAL VARIABLES	Source	df	Mean Square	F	P
ADRENALS (% of Body Weight)	E S	3 61	14187.2 1090.5	13.0	0.00
PITUITARY (% of Body Weight)	E S	3 61	830.2 280.9	2.96	0.04
BRAIN WEIGHT (% of Body Weight)	E S	3 61	6.38 0.31	20.5	0.00
BODY WEIGHT (grams) (% of Body Weight)	E S	3 61	30205.2 1248.3	24.2	0.00
SEROTONIN (ug/gram of Whole Brain)	E S	3 61	301.9 1388.8	0.22	0.88
NOREPINEPRINE (ug/gram of Whole Brain)	E S	3 61	1623.4 974.0	1.67	0.18
DOPAMINE (ug/gram of Whole Brain)	E S	3 61	9504.8 46472.0	0.20	0.89
CORTICOSTERONE (ug/ml of serum)	E S	3 61	176687. 97724.9	1.81	0.16
PROGESTERONE (ug/ml of serum)	E S	3 61	1.9×10^8 2.2×10^7	8.72	0.00
LUTEINIZING HORMONE (ug/ml of serum)	E S	3 61	145259. 49189.6	2.95	0.04

Appendix E

Supplies and Sources for Radioimmunoassay

<u>Antiserum To:-</u>	<u>Antigenic Type</u>	<u>Source</u>
Corticosterone	-21-succinyl-BSA	Endocrine Sciences
Progesterone	-11 B-succinyl-BSA	New England Nuclear
Estradiol	-	gift from Dr. John Challis University of Western Ontario, London, Ontario H6A 5C1

<u>3H-Ligand</u>	<u>Specific Activity</u>	<u>Source</u>
Corticosterone [1,2- ³ H(N)]	40-60cc/mmol	New England Nuclear.
Progesterone [1,2- ³ H(N)]	90-115 cc/mmol	" "
Estradiol [6,7 ³ H]	40-60 cc/mmol	" "

Addresses:

Endocrine Sciences, 18418 Oxnard Street
Tarzana, California 91356

New England Nuclear, 15 Harvard St.
Worcester, Massachusetts 01608.