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This is to certify that the thesis prepared under my supervision by

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is approved by me as fulfilling this part of the requirements for the degree of Bachelor of Science in Liberal Arts and Sciences.

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Abstract

Adult female rats show cyclic variations in running wheel activity that correspond to the estrous cycle. Peak amounts of running occur at estrous when estrogen levels are highest. Various brain areas have been implicated as the sites of estrogen action responsible for the estrogen action. The effect of estradiol in the preoptic area, striatum, and nucleus accumbens are examined here.

In the first experiment, female rats were housed individually in activity wheel cages. Daily activity was recorded during the light portion of the light-dark cycle. Animals were implanted bilaterally with cannula in the preoptic area or striatum, and later ovariectomized. After recovering, estradiol (30% in cholesterol) or vehicle treatments began. All animals received both drug treatments. Non-implanted controls received estrogen benzoate in oil (2.5-5 ug) and oil treatments. No differences were found between estrogen and vehicle response among the three groups. All groups ran significantly more with estrogen treatment compared to vehicle. Radio-immuno assay showed that low levels of estrogen diffused into the nucleus accumbens in both preoptic and striatum groups. The second experiment was conducted to account for the possible estrogen effects in the accumbens.

Female rats were put in activity wheel cages, and activity was recorded as in the implant experiment. The rats were ovariectomized and later received electrolytic or sham lesions in the accumbens. After recovery, treatment with estrogen benzoate (2 ug) in oil or oil began. All animals received both drug treatments. Animals in both groups ran significantly more with estrogen compared to oil. However, controls increased significantly more than lesioned animals.

The results indicate that estrogen acts in the preoptic area and the striatum to increase running activity. The increase with estrogen in the preoptic area is consistent with other studies. Estrogen action in the striatum on running activity is an exciting new avenue for further research.
Estrogen action on behaviors such as lordosis, circling, stereotypy has been widely studied. In addition to the behavioral measures, estrogen affects food intake and body weight. This diversity of effects makes estrogen a good hormone to study.

The first experiment was done to determine the brain site of action on running wheel activity. The striatum and preoptic areas were tested as possible sites of action. Because of estrogen diffusing into the nucleus accumbens in the first study the lesion study was conducted.

The two techniques used here to determine the brain site of action are an implant study and a lesion study. The strengths and weaknesses of each technique will be discussed before discussing estrogen effects on various behavioral and physiological measures.

Although the focus here is on rats, a study of heart rate and the menstrual cycle in humans showed similar effects of estrogen (M. Roy, unpublished preliminary data).
Experimental manipulations

**Implant studies**

Implant studies involve the application of drugs directly to the desired group of neurons. The size of the cannula determines in part how many neurons get stimulated by the drug. The other variable that determines the number of neurons affected is the amount of diffusion to other regions of the brain. Controlling diffusion is difficult, but one way to minimize diffusion is to use small diameter cannulas and low drug doses. In the case of estrogen, estradiol to cholesterol concentrations of 1:100 or greater help keep diffusion to a minimum. Cholesterol is used for dilution and as a control because of its structural similarity to estrogen and the lack of behavioral affect due to cholesterol. Another way to examine estrogen function is through antiestrogen implants. Various antiestrogens can be used, and each has its own diffusion pattern.

**Lesion Studies**

A lesion study can help to examine areas that affect a behavior, and can be useful in conjunction with implant studies that exhibit the problem of diffusion. When contemplating a lesion experiment however, consideration must be given to the possible side effects of the lesion. For
instance, when looking at estrogen effects on activity, the area to be lesioned must not affect locomotor activity. Lesions to the preoptic area decrease running wheel behavior, and consequently any effect that estrogen might have will not be observed. Therefore, even though estrogen may act in the preoptic area, lesioning this region will give inconclusive results.

A drug can be administered after the lesion to see if the drug acts in the lesioned area. Estrogen is typically administered systemically through subcutaneous injection. Again, the concentration and dosage of the agent can be manipulated.

Numerous ways are used to lesion neurons. One way is to destroy the tissue chemically. Many different toxic chemicals can be used, such as 6-hydroxydopamine or kainic acid. Just as with implant studies, each toxin spreads in different patterns and has different effects. Amount, concentration, and rate of infusion determine the size of the lesion.

A second way of producing lesions is electrolytically. The amount and duration of current can be varied to control the size of the lesion. Chemical and electrolytic lesions do not always produce the same results. For example, chemical
lesions to the nucleus accumbens decrease running wheel activity, making this type of lesion useless in studying estrogen effects (Kubos, Moran, & Robinson, 1987). On the other hand, electrolytic lesions to the nucleus accumbens increase running activity (Kubos, Moran, & Robinson, 1987). The explanation for the opposite effects is that each type of lesion destroys different cells (see figure 1). Chemical lesions kill the A10 dopamine neurons, while electrolytic lesions destroy cells in the nucleus accumbens. The pathway that controls locomotor activity is from A10 to accumbens to a locomotor region. Each successive region is inhibitory on the next region. Thus, the running activity effect can be explained because chemical lesions allow more inhibition of the locomotor region, while electrolytic lesions disrupt the inhibition process.

Estrous Cycle

Description of cycle

The rat has, on the average, four day estrous cycles which can vary between three and five days. The first day of the cycle is called metestrus. This day has low levels of estrogen and progesterone, although both are on the rise. The second day is called diestrus which is characterized by low estrogen and minimal progesterone levels. Proestrus is
Lordosis responses are typically measured by a score called the lordosis quotient (LQ). This score is obtained by observing a female's behavior when put into a cage with a sexually active male. The number of lordosis responses divided by the number of mounts by the male gives the LQ.

In order to study the effects of gonadal hormones, the endogenous hormones must be removed. This is done quite easily by removing the ovaries in females or castrating males. After approximately one week, which corresponds to approximately two estrous cycles, most of the estrogen and progesterone is gone from the body (Beach & Orndoff, 1974). Once the endogenous hormones are gone, replacement by known amounts of hormones can be administered.

As with some other behavioral responses, lordosis is controlled by estrogen. Beach and Orndoff (1974) found that estrogen deprivation decreases lordosis responses significantly within four weeks from the beginning of deprivation. Replacing the estrogen does not cause a return to normal lordosis responses until 14 days of therapy.

The finding that deprivation of estrogen for an extended period changes the response to estrogen must be considered when doing studies that involve such deprivation. Possibly the deprivation only affects lordosis responses.
However, the deprivation may also affect other responses such as open field activity and running wheel activity. Therefore it is important when designing and analyzing experiments in which animals do not have circulating hormone for extended periods to be aware of the possible confound.

In the same study, Beach and Orndoff (1974) examined the relationship between progesterone and estrogen on lordosis. Progesterone alone did not induce lordosis, but progesterone given along with estrogen enhanced the lordotic response to estrogen. The progesterone effect not only increases the lordosis response, but also can diminish the lordotic response to estrogen, depending upon the time of administration in relation to estrogen administration (Roy & Wade, 1976). When estrogen is given before progesterone, the enhancing effect occurs. Progesterone given before estrogen causes lordosis behavior, which is normally induced by estrogen, to be inhibited (Gerall, Napoli, & Cooper, 1973).

**Site of action**

Determining the brain site of estrogen action is useful to understand the mechanism that causes the response as well as to understanding mechanisms underlying other estrogen responses. The hypothalamic ventromedial nucleus is the site of estrogen action in stimulating the lordosis
response (Fahrbach, Meisel, & Pfaff, 1985). The same study did not find any evidence for estrogen action in the preoptic area or the anterior hypothalamic area. The lack of response in the preoptic area is interesting because this area is responsible for sex behavior in males. Contradictory findings have also been reported. Lesions to the medial preoptic area both facilitate and inhibit sex behavior (King, 1979). Anterior hypothalamus lesions can either decrease or have no effect on sex behavior (King, 1979). The anomalous findings may be due to the different yet related areas within the brain structures that were examined. Fahrbach et al. found that the exact placement of estrogen stimulation greatly affected response. The lesions may have destroyed pathways important to the control of sex behavior that are not mediated by estrogen. Although these two areas may not be important to the lordosis response, the possibility that these areas play a role in other estrogen responses is still open.

Secondary effects of the estrous cycle

**Energy balance theory**

The energy balance theory was proposed to account for the effects of estrogen on such measures and behaviors as food intake, body weight, and activity levels (Wade, 1972).
The theory states that there is a setpoint for body weight. Body weight is affected by the energy input and output of the animal. Alterations in the setpoint occur because of a sensitivity to gonadal hormones. Many studies have examined the effect of ovarian hormones on energy related behaviors. **Food intake and body weight**

Food intake and body weight are two closely correlated measures (Gentry, Wade, & Roy, 1975). Prepubertal rats are not sensitive to estrogen for changing body weight or food intake even though they have estrogen circulating in the body (Zucker, 1972). Is age or body weight the important factor in determining when estrogen begins exhibiting its influence? Examining ovariectomized animals helps to get a fuller picture of the effects of estrogen, progesterone, and the pituitary on food intake and body weight.

It has long been known that rats lose weight at estrus. This suggests that ovarian hormones somehow regulate food intake and body weight. Ovariectomizing rats increases both food intake and body weight (Rodier, 1971). Giving estrogen to the ovariectomized rats decreases the body weight again. The food intake decreases temporarily at first and then returns to control levels (Rodier, 1971). Since the final result of treatment is decreased body weight and
unchanged food intake, something must have happened to the setpoint for body weight. The change in weight could be due to altered metabolism or change in activity levels. Even though all rats show a response to estrogen, there are differences among individual rats (Gentry et al., 1976). If no treatment is given to the ovariectomized rats, 3.5 months pass before the eating returns to lower levels (Gentry et al., 1976).

Another way to examine the effects of estrogen is to give a drug that should block estrogen action. One such antiestrogen is MER-25. Roy and Wade (1976) studied antiestrogen alterations of food intake and body weight in both males and females. Castrated males were used to determine if estrogen sensitivity is the same as in females for food intake and body weight measures. Initially the antiestrogen decreased food intake in females, and body weight did not increase as much as for controls. The change in food intake was not permanent however, and eating returned to normal levels during the MER-25 treatment. Thus, although MER-25 is an antiestrogen, it has the same effects as estrogen on body weight and food intake. Males were affected to the same degree as females for body weight, but food intake was affected more in females than in males. The
differences may be due to the sensitivity of males and females to estrogen. Females are more sensitive to estrogen which may explain why they are also more sensitive to MER-25. Since males are estrogen sensitive, studies using males may be feasible depending upon the measures and manipulations used.

Individual differences among animals suggest that initially heavy rats lose more weight after ovariectomy and subsequent estrogen replacement than lighter rats (Gentry et al., 1976). These data indicate that estrogen lowers the setpoint for body weight, and that food intake changes are secondary to body weight regulation (Gentry et al., 1976 Roy & Wade, 1976, Zucker, 1972). Although the setpoint for body weight is changed by estrogen, excess estrogen cannot cause chronically lower weight. Treatment of obese rats with estrogen does not cause permanent weight loss (Zucker, 1972). The setpoint for body weight may only be altered transiently during the estrous cycle. Other ovarian hormones given alone do not alter the setpoint.

Progesterone alone does not affect either food intake or body weight. When progesterone is given to ovariectomized rats, neither the food intake nor the body weight is changed (Rodier, 1971). However, progesterone attenuates the effects
of estrogen on the two measures (Roy & Wade, 1976; Wade & Zucker, 1970b). Food intake suppression that was brought on by estrogen is inhibited by progesterone. This lack of effect by progesterone alone is characteristic. Progesterone alone did not alter lordosis, and it does not alter food intake or body weight.

Pre-pubertal regulation. Up to this point only mature animals have been considered. The question still remains as to the necessary conditions for initiating sensitivity to estrogen in females. There are two possibilities. First and most obvious, is that a predetermined age is required for sensitivity to estrogen. Second is the possibility that a weight setpoint exists for responsiveness to estrogen. Of course, both hypotheses could be true at the same time.

Rats must have some mechanism to enable estrogen regulation because ovarian hormones have no influence over food intake before maturity (Wade & Zucker, 1970a). Even though no behavior is affected by estrogen before maturity, this does not imply that brain regions are not sensitive to the hormone. In fact, the ventromedial hypothalamus is sensitive and functional to estrogen in prepubertal rats (Wade & Zucker, 1970a). Wade and Zucker (1970a) suggest that growth factors block estrogen action. They also hypothesize
that eating is controlled by temperature in immature rats. In low temperatures immature rats increase their eating, while adults are not affected by temperature. More evidence for the possibility that estrogen during development has an affect on later life comes from another study by Wade and Zucker (1970b). They found that hormonal stimulation early in the neonatal development influences body weight and activity levels after maturity. When do the growth factors cease to cause a block, and what are the necessary conditions for elimination of the block?

Lesions to the ventromedial hypothalamus in adults increase food intake and body weight. This increase does not occur in prepubertal females (Wade & Zucker, 1970a). This suggests that although the ventromedial hypothalamus plays a role in adult energy regulation, other systems must be responsible for regulating energy balance in immature females. Since growth takes up a large proportion of the life cycle, growth factors may be responsible for regulating eating and weight before adulthood. A thorough examination of age and weight by Zucker (1972) showed that both age and body weight are important for estrogen response. However, age alone is not sufficient to induce the estrogen response. An unspecified minimum body weight must be attained in order
for a response to be observed. This idea makes sense because at some point growth ends and growth factors subside. The cessation of growth factor control could correspond to a particular weight. Body weight is a key factor in initiating puberty in both rats and humans. Heavier animals reach sexual maturity at an earlier age (Zucker, 1972). This is another piece of evidence for the body weight theory of estrogen response.

**Site of action.** With the question of estrogen action on eating and weight apparently resolved, it is time to address the question of sites of action in the brain in more detail. Ovarian hormones affect different physiological effects and behaviors in different areas of the brain (Gentry et al., 1976). The hypothalamus has been implicated in the regulation of the estrogen response on eating and weight. This is not surprising, since there are estrogen receptors in the hypothalamus (Pfaff & Keiner, 1973; Wade & Zucker, 1970b). Rats with lesions to this area increase food intake (Wade & Zucker, 1970b). Therefore the hypothalamus must be responsible for inhibiting food intake to some degree. Intracerebral administration of estrogen in the hypothalamus also have revealed that the hypothalamus takes up and binds estrogen (Gentry et al., 1976). This estrogen that is taken
up by the hypothalamus decreases food intake. The most effective way of getting estrogen to affect food intake is to implant estrogen directly in the ventromedial hypothalamus (Wade & Zucker, 1970b). Estrogen action is mimicked by the antiestrogen MER-25 on food intake and body weight measures, and, as expected, food intake decreases with intracerebral antiestrogen administration (Roy & Wade, 1976). The evidence overwhelmingly supports the proposal that estrogen affects food intake by action in the hypothalamus. The evidence for body weight regulation by the hypothalamus comes more indirectly but is still convincing.

Another area of interest is the pituitary. Rats that have had the pituitary removed maintain a reduced body weight. Food intake is also reduced in these animals, and the animals are less sensitive to estrogen's effect of decreasing eating (Wade & Zucker, 1970a; Zucker, 1972). Indications are therefore that the pituitary plays some role. Apparently the pituitary is more important in regulating body weight than is the hypothalamus. Finding more than one area of action on a behavior or physiological process is not uncommon, nor is it too surprising. The brain is a complex network of interconnecting pathways. These pathways are what make the brain both interesting and frustrating to study.
**Open field and running wheel activity**

Open field activity is a measure of the amount of ambulation in an environment in which the rat is free to move around. A cage is marked off in a grid, and the number of grids entered in a given time period is measured. Along with the number of grids, other measures such as rearing, grooming, and sniffing may also be counted.

On the other hand, running wheel activity is the amount of a particular activity—namely running. The running wheel approach only takes into account the number of revolutions a rat runs in a given amount of time. Unfortunately, other behaviors are not counted. The animals are usually measured on running activity for extended periods of time which does not enable researchers to count other behaviors. Whether or not to count revolutions made by an animal standing outside the wheel and turning it is a question since the same neural mechanisms may not be involved in this type of activity. Without constant observation the number of revolutions made in this way cannot be measured. Nevertheless both open field and running wheel activity are commonly accepted methods of determining the amount of activity in a given time period.
Estrogen effects on open field activity have not been studied as extensively as effects on running wheel activity. Estrogen administration decreases the amount of open field activity in rats (Fahrbach et al., 1985). The most obvious explanation is that the decreased activity enables mating behavior. An apparently anomalous finding is that the running activity increases with estrogen. Since the running measure has been more thoroughly studied, this measure will be examined more closely here too.

In addition to being affected by gonadal hormones, running is affected by temperature, the light-dark cycle, and food and water intake. Just as with weight and eating, rats respond differently when put in a running wheel before puberty. They show a gradual increase in running that is similar to a learning curve before 50-60 days of age (Colvin & Sawyer, 1969). In a similar manner to the increase of lordosis behavior, rats also increase their running rates when they are food deprived (King, 1979). Determining the causal relationship between food deprivation and running may help shed light on the validity of the energy balance theory.

Running wheel activity is a good measure for studying estrogen effects because of some interesting differences between males and females when put in the running wheel.
Females run twice as much as males. Implanting males subcutaneously with estrogen pellets increases their running activity, but still not to the level of females' activity. Another way to increase males' running is by administering testosterone (Colvin & Sawyer, 1969). Since testosterone is converted to estrogen, excess testosterone could very well cause an increase in the amount of intracerebral estrogen. If instead of receiving testosterone or estrogen, males receive ovary transplants, they exhibit running activity comparable to females (Colvin & Sawyer, 1969). Typically females are used to study estrogen effects, although some studies use males so the extra procedure of ovariectomizing the animals can be eliminated.

Evidence that estrogen affects running comes from the fact that intact females show a cyclicity in running that is dependent upon the time in the estrous cycle. The revolutions peak at proestrus which correlates with the time estrogen levels are at a maximum. Estrogen levels do not fluctuate widely during pregnancy, lactation, before sexual maturity, and after ovariectomy, and during these times running activity is reduced (Colvin & Sawyer, 1969).
Because of the correlation with estrogen levels, it has been proposed that estrogen is responsible for the cyclicity of running. This idea is erroneous as demonstrated by Gerall, Napoli, and Cooper (1973). They implanted estrone pellets into ovariectomized rats. The estrone pellets release a constant amount of estrogen into the body. If the presence of estrogen causes cycles in running activity, then these implanted animals should show running cycles. The animals did not show cycles. Running varied with the implants, but no regular cycle appeared. The estrone pellets did increase the running. This study suggests that variations in estrogen levels cause cyclic running. The small variations seen when examining graphs of running activity can be attributed to other factors such as temperature and amount of food and water intake immediately prior to running. Rats during the estrus cycle show large variations in the number of revolutions run per day.

Related to the large differences between estrogen action and lack of estrogen action is the idea that extended time in the wheel is not necessary to see changes in running behavior due to estrogen. Hitt, Gerall, and Giontonio (1968) found that as little as one hour sessions in the running wheel can show estrogen effects adequately as compared to 24
hour living in wheels. They point out that the graphs must be examined more carefully, and by using Fourier analysis differences in running levels can be determined. Using less than 24 hours per rat in a wheel can greatly enhance the power of an experiment. When there is only a limited number of wheels, the number of animals per replication can be increased greatly. Increasing the number of animals helps, because running wheel experiments tend to run for long periods of time, and there is often great variability in each animal’s amount of running which leads to ambiguous results.

Regardless of the amount of running the pattern of running activity is similar across rats. Depending upon the time in the estrus cycle, the pattern changes. During pro-, met-, and diestrus running is in a sawtooth pattern when looked at in one hour intervals. During estrus however, animals run in more concentrated time periods with fewer time outs from running (Gerall et al., 1973). One possibility is that the running pattern is affected by differences in amount of feeding. This is plausible because estrogen affects feeding. However, animals in running wheels increase eating even without treatment in order to partially compensate the energy balance. Another account derives from the idea of estrogen as an energy regulator. More energy is used when
activity is constant than when rest periods are inserted between activity sessions. At estrus, body weight decreases. Perhaps this is caused by the fewer time outs and therefore the increase in energy usage.

The fact that estrogen does not act in an environment devoid of other coactors must not be forgotten. Progesterone administration suppresses running in intact but not ovariectomized rats (Rodier, 1971). Just as in the cases of lordosis, eating, and body weight, progesterone cannot act on running activity without estrogen. Progesterone must modulate estrogen effects. Progesterone is not dependent on the mechanism by which estrogen is secreted, because both hormones can be injected and the effect is still seen (Rodier, 1971). Therefore there must be a direct interaction between the hormones. Progesterone may act by interfering with estrogen's promotion of running. This interaction would be similar to progesterone's modulation of estrogen effects on other measures. Of course the determination of progesterone action hinges upon finding the sites and mechanisms of estrogen action alone.

Site of action. The brain region responsible for estrogen effects on open field activity is still not known. Fahrbach, Meisel, and Pfaff (1985) tried to study open field
Estrogen effects

activity. They found estrogen did not increase open field activity in the anterior hypothalamus and ventromedial hypothalamus. The hypothalamus is the same area that affected food intake. No significant effects were found in either the posterior hypothalamus or the preoptic area. The preoptic area, as will be seen later, is one site that has been implicated in running wheel activity. To further complicate matters, open field activity was found to decrease, which is opposite to the effect on running wheel activity (Fahrbach et al., 1985). Open field activity may be mediated by more than one site, in a similar manner to the way body weight is affected by both pituitary and hypothalamus. Another possibility is that the estrogen is partially maintaining the level of open field activity (Fahrbach et al., 1985). Without the estrogen stimulation, the open field activity may actually decrease. The estrogen may not serve to increase open field activity, but rather to keep the open field activity from decreasing substantially.

The data for running wheel activity are also interesting. Running wheel activity increases with estrogenic stimulation of the preoptic area and the anterior hypothalamus (Fahrbach et al., 1985). There is a large number of densely packed estrogen sensitive neurons in both
areas (Pfaff & Keiner, 1973; Wade & Zucker, 1970b). When either of these areas is lesioned, estrogen does not increase the running activity (King, 1979; Fahrbach et al., 1985; Wade & Zucker, 1970b). However, the lack of increase in running with lesions does not necessarily mean that estrogen works in these areas to affect running. Lesioning these areas affects locomotor activity in general. The rats' baseline running without treatment nosedives below post-ovariectomy levels. If destroying these brain regions inhibits the response, then it is unreasonable to infer that estrogen necessarily acts in these regions. Implant studies have so far indicated that estrogen acts in the preoptic area and the anterior hypothalamus (Colvin & Sawyer, 1969). If diffusion did not occur to other brain regions, these studies may have validity. However, Colvin and Sawyer suggest that some diffusion may have taken place, thus making the results ambiguous. Antiestrogen implants may help to alleviate the confusion, because antiestrogens have different diffusion patterns than estrogen. Further support for these two areas comes from the fact that implant sites that affect running activity in the rat are close to areas that are associated with sex behavior in cats (Colvin & Sawyer, 1969).
Another region of interest has been the ventromedial hypothalamus. Results from studies on this area have been variable. Either implants decrease or have no effect on activity levels (Colvin & Sawyer, 1969; Wade & Zucker, 1970b). The decrease cannot be explained yet since estrogen in other brain regions increases activity.

In addition to the hypothalamus and preoptic area, the pituitary has been considered as a site of action. Rats that respond to estrogen have increased adrenal weights at autopsy. However, running activity and not the estrogen may be responsible for the increase in weight (Colvin & Sawyer, 1969).

Radioactively labelled estrogen binds to receptors in all these regions. Gentry, Wade, and Roy (1976) found that the amount of binding is greatest in the pituitary, hypothalamus, and preoptic area respectively.

Not all effects of estrogen are necessarily mediated by the same area. Although a correlation exists between body weight and food intake measures, no correspondence between body weight and running activity exists (Gentry et al., 1976). If running increases, then logically one would assume that body weight should decrease because of an energy balance. This does not happen. Therefore, even though both
measures are regulated by estrogen, the idea that running wheel activity is associated with weight regulation must be questioned. Knowing the response on one measure does not enable the prediction of response on any other measure.

Summary

Estrogen has numerous effects in the body. Implant and lesion techniques are useful for determining the brain site upon which estrogen acts. When designing and interpreting such studies care must be taken to assure that methodological artifacts are not responsible for the observed effects. With implants, replicating effects with more than one drug helps to keep artifacts minimal. With lesions, the choice of lesioning agent is important to ensure that only the desired neurons are lesioned.

During the estrous cycle estrogen and progesterone levels vary which affects many behaviors. Lordosis increases when estrogen levels are high, and is facilitated by progesterone. The site of estrogen action on lordosis is still not known. Estrogen in the medial preoptic area has provided contradictory findings. The anterior hypothalamus either decreases or has no effect on lordosis. The exact placement of the implant in the studies may greatly affect the response.
Food intake and body weight are two more measures influenced by estrogen. When estrogen levels are high food intake decreases while body weight decreases. Estrogen appears to transiently alter the setpoint for body weight. Pre-pubertal rats are not affected by estrogen on body weight or food intake measures. An unspecified minimum body weight and age are necessary before rats respond to estrogen. The hypothalamus and pituitary are two areas that are important to estrogen action on the measures.

Two more measures affected by estrogen are open field and running wheel activity. Estrogen increases running activity but decreases open field activity. The site of action on these measures is not yet known. The anterior and ventromedial hypothalamus does not affect these measures. Running wheel activity may be increased by implants in the anterior hypothalamus and preoptic area. The pituitary also may be a site of action with respect to running wheel activity. The study presented here examines the striatum, preoptic area, and nucleus accumbens as possible sites of action on running wheel activity.
Methods

Implant Experiment

Female Long-Evans rats were housed individually in activity wheel cages starting at approximately 6 weeks of age. Throughout the experiment the previous night's activity was recorded during the light portion of the light-dark cycle. Food and water were available ad lib. After 3-4 weeks the animals were implanted bilaterally in the striatum (n=10) or the preoptic area (n=6) with 22g guide cannula under sodium pentobarbital anesthesia (40-45 mg/kg). (Coordinates: Striatum AP 0.8mm, ML 2.35mm, DV -2.2mm from skull; Replication II preoptic area (POA) AP 0.0mm, ML 1.0mm, DV -7.1mm from skull; Replication III POA AP -0.8mm, ML 1.0mm, DV -6.6mm from skull. All measurements taken from Bregma with level head.) Brevitol and atropine sulfate were also administered as needed. Blank inners, 28g, were inserted into the guide cannulas to keep them open until the time of the drug treatments. Twelve to fifteen days after the implant animals were ovariectomized (ovx). Four to six days after ovx the drug treatments began. Estradiol (30% in cholesterol) or cholesterol was delivered in a 28g inner 2mm longer than the guide cannula. Each animal received both estradiol and cholesterol treatments. The order of treatment
was counterbalanced with some animals receiving estrogen first and the rest of the animals receiving cholesterol first. Control animals (n=7) received systemic estrogen benzoate (EB) and sesame oil instead of the above treatments. In replication I, EB animals received .1cc of EB 25 ug/ml s.c. For replication II EB rats received .1cc of 50 ug/ml EB s.c., and in replication III EB rats received .3cc of 25 ug/ml EB s.c. In the first replication all drug treatments lasted 5 days with 3 days rest between switching drug treatment. In replication II and III treatments lasted 4 days with no rest between treatments.

In order to determine the spread of estradiol from the site of the implant, animals were treated with estradiol for 3 days. The animals were sacrificed, the brains were sectioned coronally, and cuts were made along the cannula track and below the ventral surface. Estradiol was extracted and radio-immuno assayed using the method described by Clark and Roy (1985).

Lesion Experiment

Female Long-Evans rats approximately 4 weeks of age were housed individually in activity wheel cages. As in the implant experiment, the previous night's activity was
recorded during the light portion of the light-dark cycle. Food and water were available ad lib. When the animals showed sufficient amounts of daily running they were ovariectomized under brevital anesthesia. One to two weeks after ovx animals received either electrolytic (n=16) or sham (n=14) lesions to the nucleus accumbens. (Coordinates: AP 1.2mm, ML 1.2mm, DV -6.9mm from skull. Measurements taken from Bregma with level head.). Surgery was performed under sodium pentobarbitol anesthesia (40-45 mg/kg). Additionally brevitol and/or atropine sulfate were administered as needed. The lesions were made by lowering an insulated cannula into the brain and passing a current of 2ma through it. The cannula was uninsulated 0.60-0.69mm at the tip. In the first replication current was passed for 10 seconds, and for the second and third replications the current was passed for 20 seconds. Sham lesions consisted of lowering the cannula and not passing any current. Seven to ten days after surgery drug treatments were started. Animals received .1cc of 20 ug/ml EB or sesame oil. All animals received both treatments. Drug treatments were given for 5 days with 5 days rest between treatments in all replications except replication II which had 7 days rest between treatments. Following drug treatments animals were decapitated and the
brains were put in 30% sucrose formaldehyde solution until sectioning. Alternate 60 um sections were mounted on slides and stained with cresyl violet in order to determine the site and extent of the lesion.

Results

Implant Experiment

Baselines. The baseline means for each group are shown in table 1. Before surgery the animals exhibited four day cycles on the average. The striatum and systemic groups were running approximately the same amount before and after the implant. The preoptic group ran somewhat more before implant than either of the other groups, while after the implant they appeared to run less than the other groups. After ovx the preoptic and systemic groups were running at the same level, while the striatum group was running slightly more than either of the other groups. As expected, all three groups decreased running after ovx. Figure 2 shows graphs of daily running for three individual animals.

The decrease in running in all three groups can be seen clearly in figure 2. The decrease in running seen after implant is partially due to a gradual decline in activity with age. In addition, damage done by the cannulas may have caused the greater decrease observed in the preoptic area.
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Damage to the preoptic area impairs running wheel activity. Because the preoptic group was running at the same level as the systemic group after ovx, the damage was not enough to prevent the drug treatments from showing an effect.

Pre-implant baselines were the average of the 5 days prior to the implant. Post-implant baselines were the average of the 5 days before ovx, and post-ovx baselines were the average of the 4 days immediately following ovx.

Main effects. The means of estrogen and cholesterol treatment effects are shown in table 2 by area. Three means were computed for each group; a ratio of treatment to treatment-plus-baseline-before-treatment \((T/(T+B))\), a ratio of treatment divided by baseline \((T-B)\), and a difference score of treatment minus baseline \((T-B)\). Each measure exhibits similar findings. Estrogen treatment increased the running in each group, while cholesterol treatment did not increase running.

There were no significant differences among the three groups in response to drug treatment \((F=1.52, p>.05)\). Estrogen treatment significantly increased running in all of the areas compared to cholesterol \((F=34.06, p<.0001)\). These results indicate that estrogen administration increases running activity in the two brain areas tested and when
administered systemically. The increase in the systemic and preoptic groups is consistent with other reports (Fahrbach, Miesel & Pfaff, 1985; Rodier, 1971; Wade & Zucker, 1970b). There have not been any reports of testing for increase running due to estrogen in the striatum.

Inferential analysis was done using the T/B ratio. The T/(T+B) ratio has an upper limit which could mask estrogen effects, and the difference scores do not adjust for differences between high and low baselines before treatment. Analysis was performed using a repeated measures general linear model. In the first replication baselines were the average of the two days prior to drug treatment, and the treatment average was the third and fourth days of treatment. For the second and third replications, the same treatment averages were used. The baseline averages were the two days prior to drug treatment. However, because there was no rest between treatments in these replications, the baseline of the second treatment was the same as the first treatment average.

**Order effects.** There was a significant effect of treatment order (F=10.93, p<.004). Table 3 is the T/B ratio means shown by area and order. Animals receiving estrogen first ran less than animals receiving estrogen second.
Animals that received cholesterol first ran less than animals that received cholesterol second.

There was also a significant interaction effect between drug treatment and order (F=9.42, p<.007). When the first treatments began, it is possible that the animals were continuing to decrease the amount of running after the ovx. In that case the baseline before the first treatment would not yet have leveled off, which would make the difference between baseline and treatment smaller. Therefore, the estrogen second animals would show a greater increase in running since they had lower baselines because of the extra time before treatment. Similar reasoning explains the order effect with cholesterol. The baseline of the cholesterol second animals was elevated because the estrogen effects were still being seen. Therefore the cholesterol second animals ran more than the cholesterol first animals.

Replication effects. No significant differences between replications were found for the estrogen effect in any area (POA F=0.13, p>.05; striatum F=4.06, p>.05; systemic F=0.42, p>.05), or with cholesterol (POA F=0.10, p>.05; striatum F=1.12, p>.05; systemic F=5.38, p>.05). The tendencies towards a replication effect are probably due to the differences in drug dosage and variations in the amount
of rest time between drug treatments. Table 4 is the T/B ratio means in each area by drug treatment and replication.

**Histology.** Figure 3 shows the average amount of hormone in picograms detected by radio-immuno assay in the striatum and preoptic area. Eight animals were used for the figure in the preoptic area, and five animals were used in the striatum. The other animals in the experiment were used as controls (no intracerebral estradiol). Two of the animals used in the preoptic area assay were not used to collect running wheel activity data. The sensitivity of the assay is slightly above physiological levels of hormone. Thus, although no hormone was detected in some areas, it is possible that undetected physiological levels of hormone were present.

In the preoptic group there was a high concentration of estradiol in the preoptic area, with some diffusion of hormone up the cannula track. Small amounts of hormone were also detected in the striatum and nucleus accumbens.

The highest concentration of hormone in the striatum group was in the middle of the dorsal striatum. A small amount of hormone was also found in the nucleus accumbens. No hormone was detected in the preoptic area.
Lesion Experiment

Baselines. The baselines for the two groups are shown in table 5. Before the ovx the animals in the control group were running somewhat more than the animals in the lesion group. The greater amount of running in the control group persisted after ovx and before the lesions. After the lesions, the lesion group was running slightly more than the control group. Other studies have reported that electrolytic lesions in the nucleus accumbens increases running (Kubos, Moran, & Robinson, 1987). In this study it is not clear if lesions had any effect on running. Both control and lesion groups decreased running from pre-ovx to pre-lesion to post-lesion. However, the control group ran more than the lesion group until after the lesion. Whether the greater amount of running in the lesion group post-lesion is due to the lesion or not is unclear. Had the two groups run the same amount before the lesion, then it would be possible to determine the effect of the lesion. Figure 4 are graphs of daily running of two individual animals. The differences in baselines is not readily apparent from the graphs.

The pre-ovx baseline was calculated as the average of the 10 days preceding ovx. The pre-lesion baseline was the
average of the 5 days before the lesion, and the post-lesion baseline was the average of days 5-10 after the lesion.

**Main effects.** The means of the two groups on estrogen and oil treatment are shown in table 6. As in the implant experiment, means are given for a ratio of treatment to treatment-plus-baseline \((T/(T+B))\), a ratio of treatment divided by baseline \((T/B)\), and a difference score of treatment minus baseline \((T-B)\). Both lesion and control group means were higher with estrogen compared to oil. The means of the lesion group with estrogen administration were lower than the means of the control group with estrogen.

Inferential statistical analysis of the \(T/B\) ratio showed that control and lesion groups responded significantly differently to drug treatment \((F= 8.16, p<.008)\). Estrogen significantly increased running compared to cholesterol \((F=50.17, p<.0001)\). There was also a drug and group interaction \((F=6.98, p<.01)\). The interaction is not surprising because of the differences in response to estrogen depending upon the group as seen in table 6. Post hoc tests using Tukey's HSD showed that both lesion and control groups responded significantly more to estrogen than they did to oil (control \(d=1.88, p<.05\); lesion \(d=1.76, p<.05\)). Comparing groups, the lesion and control groups differed significantly
when given estrogen, but did not differ significantly when given oil (estrogen d=1.66, p<.05; oil d=1.66, p>.05).

Inferential analysis of the ratio was done using a repeated measures general linear model. The T/B ratio was used for the same reasons it was used in the implant experiment. Treatment averages were days 7-9 from the start of the drug treatment. Baseline averages were days 5-7 from the end of the last treatment.

**Order effects.** There were no significant differences between estrogen or oil first compared to estrogen or oil second, respectively, in this experiment (F=2.71, p>.05). Table 7 shows the group means by order.

**Replication effects.** There were no significant differences across the replications in either the control or the lesion group in response to estrogen (control F=3.25, p>.05; lesion F=0.85, p>.05). There were also no significant differences across replications in response to cholesterol (control F=0.44, p>.05; lesion F=0.17, p>.05). Table 8 shows the means of the groups by replication and drug treatment.

**Histology.** Since current was passed for a shorter time in the first replication, lesions were smaller in the first replication than in the second and third replications. A coronal section showing the middle of the lesion is in figure
5. All the lesions in the first replication were virtually the same size. The lesions began after the corpus callosum joins, and extended to the bed nucleus in all but one animal which began before the corpus callosum joins. The accumbens medial of the anterior commissure was spared in two of the animals, but the area containing estrogen concentrating cells, according to Pfaff and Keiner (1973), appeared to be destroyed.

The lesions in the second replication were larger than in the first replication and showed greater variability in size. Figure 5 shows coronal sections through the middle of the lesion. The lesions started before the corpus callosum joins and extended to the bed nucleus. In two animals the extreme rostral accumbens was spared. The estrogen concentrating cells in the accumbens appeared to be destroyed.

The lesions in the third replication were made using the same parameters as in the second replication, but tended to be smaller than in the second replication. A coronal section in figure 5 is a section showing the middle of a lesion. The lesions began after the corpus callosum joins, and spared the rostral portion of the accumbens. One animal was not used in the analysis because the lesion did not
destroy the estrogen concentrating cells. In the remaining animals the lesion extended into the preoptic area.

Discussion

Estrogen increased running compared to cholesterol. The response did not differ significantly between groups when analyzed using the T/B ratio. This may indicate that estrogen can increase running activity in more than one brain area. However, the difference in means on estrogen response between the preoptic area and the other two areas, when examining difference scores, is very large (table 2). The difference scores indicate that there is little or no response to estrogen in the preoptic area. The ratio measure accounts for low baseline, while the difference measure accounts for a simple change in the number of revolutions. Because of these differences, the increased running in the preoptic area is ambiguous compared to the results obtained in the other two groups.

Damage to the preoptic area decreases locomotor activity. Some damage was caused by the cannula. Not enough damage was done to block all response to estrogen, however. Other studies have found implants in the preoptic area to be effective for increasing running (Fahrbach, Miesel & Pfaff, 1985, Wade & Zucker, 1970b). Although the increase seen here is small, it is consistent with other findings.
The observed estrogen effect in the systemic and striatum groups are consistent regardless of the measure used. This is because the pre-treatment baselines for both groups were higher than the baselines in the preoptic group.

The order effects may be due to the continued decrease in running when cholesterol was administered first. Since the cholesterol does not increase running, the number of revolutions run each night continues to decrease after ovx. The decreased baseline compared to the estrogen-first animals allows for a larger T/B ratio. Therefore, the effect is probably an artifact.

The interaction between order and area may be caused by the method of administration of the drug. Table 3 shows that the order effect in the systemic group is not as large as in the other two groups. The systemic group appears to have increased running more with estrogen then did the other groups, regardless of order. Intact rats have estrogen circulating systemically, and estrogen may stimulate more than one brain area to increase running. Therefore, the systemic group would show a greater increase compared to the other groups that only received localized estrogen. Systemic injection of estrogen would take more time to be metabolized completely in the body because it is not concentrated in one
area. In contrast, the other two groups have only localized regions of estrogen.

The RIA done in the implant experiment left open the possibility that diffusion to the nucleus accumbens had occurred in both preoptic and striatum groups. Estrogen concentrating cells have been found in the accumbens (Pfaff & Keiner, 1973). In order to ensure that the accumbens was not responsible for the estrogen effects, the lesion experiment was done. Also, estrogen in the preoptic implants diffused up the cannula track into the striatum. Of particular interest was whether the striatum could be responsible for the increased running.

Since some reports indicate that striatal lesions cause waxy flexibility, the accumbens was lesioned. Kubos, Moran, and Robinson (1987) found that electrolytic lesions in the nucleus accumbens increases running activity. Because chemical lesions decrease running activity, electrolytic lesions were used in the present experiment. The increased running in lesioned animals compared to controls was ambiguous in this experiment. The ambiguity was due to differences in baselines between the two groups before the lesion.
Estrogen treatment increased running in both control and lesioned animals. However, there was a significant difference between the control and lesion groups with respect to the amount of the increase. One possibility is that the lesioned animals responded to estrogen because not all of the accumbens was destroyed. The region containing estrogen concentrating cells was destroyed in the lesions, but it is possible that estrogen also acts on non-estrogen concentrating cells (Pfaff & Keiner, 1973). If estrogen acts on non-estrogen concentrating cells, then it is also possible that the striatum is the area responsible for the increase in activity. There was diffusion in the implant experiment preoptic implants up the cannula track into the striatum. In addition, the lesions in the second and third replications of the lesion experiment destroyed a portion of the striatum. If the striatum is the site of action, then it explains why running only decreased compared to controls and did not stop entirely. The animals still had enough striatum intact for estrogen action.

Another possibility is that estrogen does act only on the estrogen concentrating cells. In this case, the nucleus accumbens may be one of several brain areas that contributes to the increased running. Because of these possibilities it
is still not clear whether or not the striatum can increase running.

An experiment to test specifically the striatum would be to lesion the local fibers while leaving fibers of passage intact. This type of lesion is possible using ibotenic acid (Kohler & Schwarz, 1983). Ibotenic acid is a weak neurotoxin which can be used to make small lesions without affecting surrounding tissue. A pilot study is currently being done in our lab using ibotenic acid.

The striatum is not an unlikely site of action because it is important in locomotor activity such as the Ungerstedt turning affect (Ungerstedt, 1974). In addition, estrogen affects many dopamine mediated locomotor activities, such as circling, catalepsy, stereotypy, and dyskinesia (Bedard & Boucher, 1986; Earley & Leonard, 1978; Gordon, 1980; Joyce, Smith, & Van Hartesveldt, 1982).

Upon determining the brain site or sites of estrogen action, the mechanism of action is the next step to the puzzle. Estrogen may act alone or it may interact with other neurotransmitters such as dopamine. The dopamine interaction hypothesis holds some promise, since estrogen increases the number of dopamine receptors without affecting the affinity of the receptors in the striatum (Hruska & Pitman, 1982).
Hruska & Silbergeld, 1980). Finding the link between estrogen and dopamine would help in the understanding of how hormones and neurotransmitters interact in the brain.
Bibliography


### Tables

#### Table 1. Implant experiment baseline means before drug treatment

<table>
<thead>
<tr>
<th>Area</th>
<th>Pre-implant</th>
<th>Pre-ovx</th>
<th>Post-ovx</th>
</tr>
</thead>
<tbody>
<tr>
<td>POA</td>
<td>18422 ± 1204</td>
<td>2168 ± 388</td>
<td>3761 ± 1180</td>
</tr>
<tr>
<td>Striatum</td>
<td>9866 ± 1528</td>
<td>7478 ± 1331</td>
<td>5955 ± 976</td>
</tr>
<tr>
<td>Systemic</td>
<td>11353 ± 2733</td>
<td>7861 ± 2204</td>
<td>4481 ± 937</td>
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</table>

#### Table 2. Comparison of implant experiment ratio and difference means

<table>
<thead>
<tr>
<th>Area</th>
<th>Estrogen</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T/(T+B)</td>
<td>T/B</td>
</tr>
<tr>
<td>POA</td>
<td>.61±.10</td>
<td>1.88±.47</td>
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<tr>
<td>Striatum</td>
<td>.62±.04</td>
<td>2.12±.50</td>
</tr>
<tr>
<td>Systemic</td>
<td>.70±.04</td>
<td>2.67±.46</td>
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**Note**: T=Drug treatment, B=Baseline
Table 3. Implant experiment percentage ratio means examining order effect

<table>
<thead>
<tr>
<th>Area</th>
<th>Estrogen</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E 1st(^a)</td>
<td>E 2nd(^b)</td>
</tr>
<tr>
<td>POA</td>
<td>1.50 ± .34</td>
<td>3.77 ±</td>
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<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Striatum</td>
<td>1.09 ± .14</td>
<td>3.16 ± .77</td>
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<tr>
<td>Systemic</td>
<td>2.26 ± .54</td>
<td>3.69 ± .30</td>
</tr>
</tbody>
</table>

\(^a\)Estrogen treatment first
\(^b\)Estrogen treatment second

Table 4. Comparison of implant experiment percentage ratio means across replications

<table>
<thead>
<tr>
<th>Area</th>
<th>Estrogen</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rep 1</td>
<td>Rep 2</td>
</tr>
<tr>
<td>POA</td>
<td>-</td>
<td>2.01±.7</td>
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<tr>
<td>Striatum</td>
<td>1.52±.4</td>
<td>4.33±1.7</td>
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<tr>
<td>Systemic</td>
<td>2.68±.7</td>
<td>3.17±1.1</td>
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### Table 5: Lesion experiment baseline means before drug treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-ovx</th>
<th>Pre-lesion</th>
<th>Post-lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion</td>
<td>6049 ± 647</td>
<td>2586 ± 302</td>
<td>2450 ± 288</td>
</tr>
<tr>
<td>Control</td>
<td>8784 ± 1422</td>
<td>3283 ± 258</td>
<td>2270 ± 262</td>
</tr>
</tbody>
</table>

### Table 6: Comparison of lesion experiment ratio and difference means

<table>
<thead>
<tr>
<th>Group</th>
<th>Estrogen T/(T+B)</th>
<th>T/B</th>
<th>T-B</th>
<th>Oil T/(T+B)</th>
<th>T/B</th>
<th>T-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion</td>
<td>.75±.03</td>
<td>3.84±.65</td>
<td>5749±1196</td>
<td>.45±.03</td>
<td>.94±.14</td>
<td>-1017±530</td>
</tr>
<tr>
<td>Control</td>
<td>.84±.02</td>
<td>6.82±1.1</td>
<td>8839±148</td>
<td>.39±.04</td>
<td>.81±.19</td>
<td>-2954±858</td>
</tr>
</tbody>
</table>

*Note* T=Drug treatment  
B=Baseline
Table 7. Lesion experiment percentage ratio means examining order effects

<table>
<thead>
<tr>
<th>Group</th>
<th>E 1st&lt;sup&gt;a&lt;/sup&gt;</th>
<th>E 2nd&lt;sup&gt;b&lt;/sup&gt;</th>
<th>E 1st&lt;sup&gt;a&lt;/sup&gt;</th>
<th>E 2nd&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Lesion</td>
<td>3.72 ± 1.04</td>
<td>3.67 ± .75</td>
<td>0.74 ± .17</td>
<td>1.06 ± .20</td>
</tr>
<tr>
<td>Control</td>
<td>5.73 ± 1.00</td>
<td>8.27 ± 2.02</td>
<td>0.40 ± .04</td>
<td>1.36 ± .32</td>
</tr>
</tbody>
</table>

<sup>a</sup>Estrogen treatment first  
<sup>b</sup>Estrogen treatment second

Table 8. Comparison of lesion experiment percentage means across replications

<table>
<thead>
<tr>
<th>Group</th>
<th>Rep 1</th>
<th>Rep 2</th>
<th>Rep 3</th>
<th>Rep 1</th>
<th>Rep 2</th>
<th>Rep 3</th>
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</thead>
<tbody>
<tr>
<td>Lesion</td>
<td>2.70±.6</td>
<td>4.49±1.1</td>
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<td>0.90±.2</td>
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<tr>
<td>Control</td>
<td>4.72±1.3</td>
<td>5.42±1.0</td>
<td>9.90±2.1</td>
<td>1.09±.6</td>
<td>0.64±.2</td>
<td>0.75±.2</td>
</tr>
</tbody>
</table>
Figure 1 Destruction of tissue in electrolytic and chemical lesions.
Figure 2. Implant experiment graphs of individual animal's daily activity.
Figure 3. Implant experiment levels of estradiol (in picograms) detected in various brain regions.
Figure 4. Lesion experiment graphs of individual animal's daily activity.
Figure 5. Coronal sections through the middle of the lesions. Top figure is lesions in the first replication. Middle and bottom figures are lesions from the second and third replications.
I would like to thank my advisor Dr. Edward Roy for all his patience and guidance. I would also like to thank the graduate students Michelle Montemayer, Kathy Dwyer, Cheryl Condon, and the lab technician Diane Lynn. Their help and suggestions were invaluable. Mark Stasson and Marti Cohen-Parsons provided help with statistical analysis. Dr. Michael Coles and Dr. Robert Henderson each provided their own brand of inspiration throughout the project.