Administration of ovarian steroid hormones does not change the reward effectiveness of lateral hypothalamic stimulation in ovariectomized rats

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The effect of two schedules of steroid hormone administration on the reward effectiveness of lateral hypothalamic stimulation was examined in ovariectomized Long-Evans female rats. In Experiment 1, estrogen and progesterone were administered on a 4-day schedule so as to mimic the hormonal profile of the estrous cycle; in Experiment 2, silastic implants of progesterone and estrogen were used to induce steroid hormone levels similar to those observed in mid- and late pregnancy. In both experiments, hormone administration was sufficient to induce characteristic behaviors: lordosis in Experiment 1 and a change in sucrose preference and body weight in Experiment 2. In contrast, hormone administration did not change the reward effectiveness of the electrical stimulation in either Experiment 1 or Experiment 2.

In order to maintain homeostasis and satisfy reproductive imperatives, animals must select among a variety of goal objects available in the environment and then show the appropriate behaviors toward those goal objects. The requirements for particular goal objects change depending on the stage of the life cycle and contingencies imposed by the external environment.

In female rats, changes in reproductive state dramatically alter choice of goal objects. Proceptivity toward males and the appropriate display of maternal behavior toward young both occur at clearly delineated phases of the reproductive cycle (e.g., see Feder, 1981; Numan, 1994). Overall food intake and nutrient choice have also been shown to vary across reproductive states: salt and carbohydrate intake change with estrus cycle stage (Danielsen & Buggy, 1980; Wurtman & Baum, 1980); pregnant female rats increase their intake of sodium and protein, and lactating females increase their intake of protein, fat, calcium, and sodium (Cohen & Woodside, 1989; Leshner, Siegal, & Collier, 1972; Millelire & Woodside, 1989; Richter & Barelare, 1938).

Ovarian steroids clearly play a critical role in controlling changes in goal choice that correlate with sexual and maternal behaviors (Bridges, 1984; Feder, 1981), and there is also considerable evidence to suggest that steroids are important in the control of how much and what female rats eat (Wade, 1976; Wurtman & Baum, 1980). For example, pseudopregnant rats show the same changes in sucrose and saccharin preferences that are seen in pregnant animals

(Wade & Zucker, 1969), and daily estrogen administration is sufficient to mimic the reduction of ethanol intake seen in the latter half of pregnancy (Sandberg & Stewart, 1982). Such findings raise two issues: one is that of identifying the specificity of changes in ingestive behavior induced by changes in ovarian steroid levels; the second, how steroid hormones actually bring about such changes.

Typically, changes in nutrient preference have been studied by giving animals a choice between diet fractions containing varying amounts of a specific nutrient or, in the case of minerals, allowing them to choose between water and a solution containing the mineral under question. Although such paradigms provide reliable information about changes in intake across a reproductive state, they give rise to difficulties in interpretation. For example, the results of a number of studies have shown a relative increase in protein consumption at the end of pregnancy (Cohen & Woodside, 1989; Leshner et al., 1972). It is not clear, however, whether such changes should be attributed to an increase in protein appetite or a decrease in carbohydrate appetite. Difficulties in interpreting the results of two-bottle/two-diet choice paradigms are not limited to studies of changes in diet selection induced by changes in reproductive state. Warwick and Weingarten (1994) recently pointed out similar difficulties in the interpretation of traditional flavor/calorie pairing paradigms.

In order to determine the precise nature of the change in evaluation of nutrients that underlies these changes in behavior, it is necessary to move away from the diet/bottle choice paradigm. An experimental paradigm based on brain stimulation reward (BSR) has proved to be a useful tool for assessing the relative reward value of different stimuli (Conover & Shizgal, 1994). These researchers have developed a competition paradigm in which BSR is used as a standard of measurement for fluctuations in the perceived values of foods or tastants. A rat can choose to gain access to a desired tastant (e.g., sucrose or saline solution)

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or to rewarding brain stimulation. With the tastant held constant, preference for the electrical reward varies as a function of its strength (e.g., the number of pulses in the stimulation train). This paradigm has been used to assess precisely the value of a tastant by determining how much brain stimulation the animal is willing to forego in order to gain access to that tastant. For example, rats will forego suprathreshold trains of brain stimulation to obtain a 0.9% NaCl solution when they are depleted of sodium but not if they are sodium replete (Conover, Woodside, & Shizgal, 1994). Such a paradigm would be useful in assessing the influence of different hormonal states on the reward value of nutrients (Conover et al., 1994). However, before one can use such a paradigm, it is necessary to determine whether changes in hormonal state modulate BSR.

Determining whether changes in hormonal state modulate BSR would also provide information about how ovarian steroids change nutrient choice. Wade and Gray (1979) suggested that one mechanism by which hormones might modulate food choices would be by modifying metabolic pathways so as to create or eliminate a "need" for nutrients. Since gonadal steroids modulate not only preferences for nutrients such as sucrose but also preferences for saccharin solutions, another possibility is that ovarian steroids modulate taste preferences independent of any effects on metabolism (Wade & Zucker, 1969). In either case the evaluation of the nutrient is changed. Is this change in evaluation achieved by some global effect on the neural substrate for evaluating natural rewards, or is it achieved by modulating the evaluation of a specific taste or postgestional event (Conover et al., 1994)?

Earlier studies of fluctuations of BSR across the estrus cycle showed that bar pressing for BSR increased on the night of estrous (Drewett & Herberg, 1975; Steiner, Kantz, & Carroll, 1982). However, the measure used in these studies was simply the rate of leverpressing. Given that female rats are typically more active at this stage of the estrus cycle, this change in rate could be due to potentiation of motor activity rather than to changes in the reward value of the stimulation.

The goal of the present experiments was to determine whether administering steroid hormones to ovariectomized rats to mimic either estrus cycle fluctuations (Experiment 1) or the hormonal state of pregnancy (Experiment 2) would result in fluctuations in BSR as measured by the curve-shift paradigm. By adopting the curve-shift paradigm (Edmonds & Gallistel, 1974; Miliareisis, Rompré, Laviolette, Philippe, & Coulombe, 1986), we hoped to distinguish changes in the reward value of stimulation from changes in response capacity. Lordosis was assessed in Experiment 1 to provide an independent index of whether the hormone replacement regimen was effective.

EXPERIMENT 1

Method

Subjects

Six female Long-Evans rats obtained from Charles River Breeding Farms (St. Constant, Quebec) and weighing 320–360 g served

as subjects in this experiment. The animals were housed in plastic cages ($19.5 \times 19.5 \times 41.0$ cm) with beta chip bedding on a reverse light:dark cycle (lights off at 700 h; lights on at 1900 h) and given ad-lib access to water, lab chow (Agway, Syracuse, NY), and sunflower seeds throughout the experiment. Testing was carried out in the dark phase of the cycle.

Surgery

Electrode implantation. The animals were given atropine sulfate (0.05 mg/kg) to reduce mucous secretions under anesthesia and to facilitate breathing. They were then anesthetized with sodium pentobarbital (Somnotol, 39 mg/kg, i.p.), with 0.1-cc supplements administered as required. Stimulating electrodes (000 insect pins insulated with Formvar except for the region within 0.5 mm of the tip) were aimed stereotactically at the lateral hypothalamus (LH) using the following coordinates; 2.8 mm posterior to bregma, 1.7 mm lateral to the midsagittal suture, and 7.8 mm below the dura mater. An anode for the stimulation circuit was provided by wrapping an insulated flexible wire around jeweler's screws placed in the skull. The wires attached to the stimulating electrodes and the anode were inserted into a connector, which was then cemented to the skull with dental acrylic. The rats were given buprenorphine hydrochloride (Buprenex, 0.05 mg/kg, s.c.) to relieve postsurgical pain and were allowed to recover for a day on a heating pad before being returned to the animal colony.

Ovariectomy. Ovariectomies were performed after successful training of leverpressing for BSR had been achieved. The rats were anesthetized with a combination of ketamine (85 mg/kg) and xylazine (12 mg/kg) injected intraperitoneally. The ovaries were removed through bilateral dorsal incisions made in the skin and the body wall. The body wall and skin were then sutured, and the animals were allowed to recover under a heat lamp.

Hormone treatment. Hormone replacement was given in a 4-day cycle for 16 cycles beginning approximately 5 weeks after ovariectomy. On Day 1 of the cycle, the rats received a subcutaneous injection of 10 μ g estradiol benzoate in 0.1-cc peanut oil at 9 a.m. Fifty-two hours later (1 p.m. of Day 3 of the cycle), the rats received 500 μ g progesterone in 0.1-cc peanut oil. No injections were given on Days 2 and 4 of the cycle.

Apparatus

Training was carried out in $70 \times 25 \times 25$ cm Plexiglas and wood chambers with a wire mesh floor. Stimulation leads were attached to a slip ring, allowing subjects to move freely. A light situated above a lever 5 cm above the floor of the cage was illuminated each time stimulation was available. Leverpressing resulted in the delivery of a 0.5-sec train of cathodal rectangular pulses, 0.1 msec in duration. The stimulation trains were produced by integrated-circuit pulse generators and dual-channel constant-current amplifiers (Mundl, 1980). To prevent the accumulation of charge at the electrode-brain interface, a switching circuit shorted the outputs of the stimulator through a 1-k Ω resistor during the interpulse interval.

Testing was carried out with computer-controlled stimulators (Macmillan, Simantirakis, & Shizgal, 1985) and chambers similar to those employed for training.

Procedure

Training. Training began 1 week after electrode implantation. The rats were trained to press the lever to trigger the stimulation and to abruptly stop responding when the stimulation was no longer available. For each subject, three currents (0.25 or 0.3 log₁₀ units apart) were chosen so as to yield minimal forced movements and avoidance/escape responses when the number of pulses was set to a value that supported vigorous leverpressing. Testing was carried out at three different currents in order to stimulate neurons at different distances from the electrode tip. If hormone-modulated neurons or their afferents were found only relatively far from the electrode tip, their contribution would be manifest only at the highest

current. Conversely, if such cells were found only near the electrode tip, their contribution would be largest at the lowest current and would be diluted as the current increased. Thus, testing three currents increased the likelihood that the contribution of hormone-modulated neurons would be registered.

Testing. Rats were tested at 2 p.m. on each day of a 4-day cycle and then given a rest cycle. Thus BSR data were obtained for 8 of the 16 hormonal treatment cycles. The animals were approximately 5 months old at the start of the hormonal treatment cycles. To assess the efficacy of the hormonal replacement regimen, lordosis testing was carried out on each day of the cycle for the first three to four cycles and thereafter on Day 3 only. Lordosis testing was carried out at the end of the BSR session. Manual stimulation of the flanks was given 10 times to each animal, and the resulting behavior was scored for lordosis intensity on a scale ranging from 0 to 3 (Hardy & DeBold, 1971).

In each daily self-stimulation session, four rate–number curves were obtained for each of the three currents (200, 400, 800 μ A); the first curve served as a warm-up and was not included in the analysis. Each point on the rate–number curve corresponds to the number of barpresses recorded during a 30-sec trial during which the number of pulses per train was held constant. (Note: with train duration held constant, the stimulation frequency increases linearly with the number of pulses per train.) Immediately prior to the beginning of each trial, five priming trains of stimulation were delivered, with the frequency set to the value that would be available during the subsequent 30 sec. During the first trial of each determination of the rate–number curve, the number of pulses per train was set to a value that supported vigorous leverpressing. The number of pulses per train was then reduced from trial to trial, in 0.05 $\log_{10}$ unit steps, until the subject ceased responding for two consecutive trials. The position along the abscissa of the resulting rate–number curves was measured by determining the number of pulses required to maintain half-maximal performance (the required number). The required number was then averaged over the three curves obtained for each current.

Histology. At the completion of the experiment, the subjects were injected with an overdose of sodium pentobarbital (Somnotol, 65 mg/rat, i.p.) and perfused intracardially with buffered saline followed by 10% formalin. The brains were removed, fixed, and stored in 10% formalin. They were placed in a 20% sucrose/10% formalin solution 24 h prior to slicing. The brains were cut coronally in 30- μ m sections on a freezing microtome, and two separate sets of T sections were mounted on glass slides. One set was stained according to the formol-thionin method (Donovick, 1974) and the other by using the Weil method. Electrode placements were determined through microscopic examination and drawn on plates from the Swanson (1992) atlas of the rat brain (see Figure 1).

Results

Required Number

Figure 2 (upper panel) shows data from an individual animal for all eight testing cycles. Prior to analysis, the required number values were transformed to common logarithms. Within each current, a grand mean was then calculated for each animal over all days and all cycles, and the deviation from that mean was calculated for each data point. This manipulation reduces between-subjects variability and also eliminates the effect of current on the required number, thus rendering any effect of the hormonal manipulation more salient.

A 3-way within subjects ANOVA (cycle $\times$ day $\times$ current) was performed on these data. The inclusion of the current factor in the analysis allowed for the preservation of any possible treatment $\times$ current interactions in spite of the inevitable lack of a current effect. Only the

Figure 1. Coronal sections depicting the locations of the tips of stimulating electrodes for subjects in Experiment 1. From *Brain Maps: Structure of the Rat Brain* (plates 24, 26, 27, 30, and 31), by L. W. Swanson, 1992, Amsterdam: Elsevier. Copyright 1992 by Elsevier Science Publishers B.V. Reprinted with permission.

main effect of cycle reached statistical significance [$F(7,35) = 2.967, p < .05$]. Post hoc pairwise comparisons showed that Cycles 1 and 2 differed from Cycles 5, 6, 7, and 8. No other pairwise comparisons were significantly different. Means and *SEs* for each of the 4 test days collapsed across cycles, current, and animals are shown in Figure 2 (lower panel, light bars).

Maximum Rate

The maximum rates observed in each of the last three required number determinations at each current were averaged and then transformed to common logarithms. Using the same techniques described for required number, the maximum rates were further transformed into deviations from the grand logarithmic mean for each current. Means and *SEs* for each of the 4 test days collapsed across cycles, current, and animals are shown in Figure 2 (lower panel, dark bars). Analysis of these data yielded a statistically significant main effect of day [$F(3,15) = 3.876, p < .05$] and a statistically significant day $\times$ cycle interaction. Post hoc analysis of the main effect showed that overall the maximum rate was higher on Day 1 of the cycle than on any of the other days, and analysis of the interaction using simple main effects analysis showed that this difference reached significance only on Cycles 4 and 8.

Lordosis Scores

Mean lordosis scores for Day 3 of the cycle for all animals over all eight testing sessions was 2.64 ± 0.36 . None of the animals showed a scorable lordosis response on any of the other days tested.

Discussion

The number of pulses required to support a half-maximal rate of self-stimulation was not changed following the administration of estrogen (Day 1 of the cycle) and progesterone (Day 3 of the cycle) even though the rats showed a robust lordosis response on this day. Thus, although the hormone regimen used in this study did restore

Figure 2. Upper panel: the number of pulses necessary to maintain half-maximal performance (the required number) for each current (200, 400, and 800 A) for each test day for Subject R11. Lower panel: deviation from within-current mean for each of the testing days, pooled over cycle, current, and subjects for the required number of pulses (light bars) and maximum rate (dark bars) Means $\pm$ SE are shown.

sexual responsiveness in ovariectomized females, it was ineffective in altering the reward effectiveness of LH stimulation. These results are consistent with the notion that the hormonal treatment was more effective in modulating the sensitivity of the neural circuitry subserving the lordosis reflex than that of the circuitry subserving BSR.

Hormone administration was effective in changing the maximum rate of responding during a session, but as can be seen in Figure 3, this effect was small, about 4%. This effect occurred during the session immediately following estrogen administration, suggesting an acute effect of estrogen on activity which is consistent with earlier reports. There were no effects on rate of responding on Day 3 of the cycle, after progesterone administration when females were showing sexual receptivity. These data contrast with earlier reports (Drewett & Herberg, 1975; Steiner et al., 1982) that showed an increase in responding for BSR at the time of behavioral estrus.

Without repeated measures of circulating hormone levels, it is not possible to know how closely the levels produced by the exogenous hormone administration reg-

imens approximate those seen in the endogenous cycle. Clearly, our behavioral measures show that the hormones administered were effective in inducing the lordosis response; moreover, the fact that females failed to demonstrate this response on other days of the cycle suggests that circulating levels of estrogen must have dropped below 15 pg/ml—the tonic level at which lordosis responding has been shown to decrease (Albert et al., 1991).

The lack of effect of ovarian steroid environment on the reward effectiveness of LH stimulation is perhaps surprising, given the many demonstrations that the BSR substrate is sensitive to manipulation of both dopamine and opioid systems and the growing body of evidence that both of these systems are modulated by estrogen and progesterone. For example, estrogen enhances both the behavioral effects of amphetamine and the ability of amphetamine administration to increase the release of dopamine in the striatum (Becker, 1990; Becker & Cha, 1989). Further, estrogen administration either alone or in conjunction with progesterone has been shown to reduce naloxone binding sites in a number of hypothalamic

Figure 3. Coronal sections depicting the locations of the tips of stimulating electrodes for subjects in Experiment 2. From *Brain Maps: Structure of the Rat Brain* (plates 29, 30, 32, and 34), by L. W. Swanson, 1992, Amsterdam: Elsevier. Copyright 1992 by Elsevier Science Publishers B.V. Reprinted with permission.

nuclei, as well as to decrease behavioral responses to morphine administration (Berglund, Derendorf, & Simpkins, 1988; Wieland & Wise, 1990). However, the data presented in this paper together with those showing that a similar regimen of hormone administration does not affect the acquisition or maintenance of heroin self-administration (Stewart, Woodside, & Shaham, 1995) suggest that under some circumstances in which these pathways are already highly activated, such as during intracranial self-stimulation and the self-administration of opiates, the action of ovarian steroids on these pathways does not alter the stimulation- or drug-induced behavior.

EXPERIMENT 2

The results of Experiment 1 suggest that administration of ovarian steroids to ovariectomized female rats that is sufficient to restore the lordosis response has no effect on the reward effectiveness of LH stimulation. However, in interpreting this finding, one should keep in mind that the pattern and timing of steroid exposure is often critical for hormonal effects. For example, estrogen and progesterone replacement such as that used in Experiment 1 is sufficient to induce a reduction in responsiveness to endogenous opiates if these compounds are administered on the afternoon following the progesterone injection; the effect of the steroid administration appears to be due, at least in part, to a reduction in opiate receptor density (Berglund et al., 1988; Wieland & Wise, 1990). In contrast, a replacement schedule designed to instate chronically the progesterone/estrogen ratios typical of pregnancy has been shown to increase mu receptor density in the preoptic area (Hammer & Bridges, 1987; Hammer, Mateo, & Bridges, 1992) as well as to stimulate the onset of maternal behavior in rats (Bridges, 1984).

Given the importance of the pattern and timing of steroid administration, it seemed appropriate to determine whether another ovarian steroid replacement regimen,

one that mimics the estrogen and progesterone levels seen in pregnancy, would alter the reward effectiveness of LH stimulation. This reproductive state seemed particularly pertinent, given that it is associated with well-documented changes in both taste preferences and nutrient selection (Leshner et al., 1972; Wade & Zucker, 1969).

Method

Two cohorts of female Long-Evans rats obtained from Charles River Breeding Farms, St. Constant, Quebec, served as subjects in this experiment. Both cohorts were ovariectomized as described in Experiment 1 and subjected to a hormone replacement treatment that mimicked pregnancy. Animals in one cohort ($n = 5$) were used to assess the effect of this treatment on the threshold for BSR and were subjected to the same surgical, training, and testing procedures as described in Experiment 1. Members of the second cohort ($n = 6$) were tested for changes in sucrose preference as a function of hormonal state.

Hormone Replacement Schedule

Hormone treatment was begun within 2 weeks of ovariectomy and after a week of testing. On Treatment Day 1, the animals were anesthetized (ketamine/xylazine) and given subcutaneous steroid implants. The implants were made of silastic tubing (interior diam .078 in.; external diam 0.125 in. obtained from Dow Corning) and prepared according to the method of Bridges (1984). This hormone replacement regimen has been shown to induce steroid levels similar to those of mid- to late pregnancy and sufficient to stimulate both the rapid onset of maternal behavior (Bridges, 1984) and the changes in the mu opiate system typical of females in the latter part of pregnancy (Hammer et al., 1992).

On the 1st day, each animal received a 2-mm-long estradiol implant. Three 30-mm-long progesterone implants were inserted on Day 3 of treatment, and these were removed on Day 13 of treatment. The estradiol capsules remained in place for the subsequent 10 days. Thus, data on thresholds for BSR were obtained for each of three hormonal states: ovariectomized, estrogen + progesterone, and estrogen alone. To eliminate any effects of surgery, data from the last 6 days of each of these hormone phases was used in the analysis.

Sucrose Preference Test

To provide an independent validation of the behavioral and physiological effectiveness of the hormone regimen used in the BSR study, a second cohort of animals ($n = 6$) were maintained on ad lib access to food, water, and 14% sucrose solution. They received the same hormonal regimen as that described above, with the exception that after the estrogen implant was removed, data were recorded for a posttreatment phase. For these animals, water and sucrose solution intake as well as body weight were recorded daily. The position of the bottles containing sucrose solution and water were switched daily. The sucrose preference ratio was calculated as $\log_{10}$ (sucrose intake/water + sucrose solution intake).

Histology

Electrode placements were localized as described in Experiment 1, with the exception that only the formol-thionin method was used. See Figure 3.

Results

Required Number

As in Experiment 1, the number of pulses necessary to maintain half-maximal performance (the required number) each day was determined for individual animals for each of the three rate-number curves obtained for each current (200, 400, 800 μ A). The required number was then

Figure 4. Upper panel: the number of pulses necessary to maintain half-maximal performance (the required number) for each current (200, 400, and 800 A) for each test day for Subject A. Lower panel: deviation from within-current mean for each hormone condition, pooled over cycle, current, and subjects for the required number of pulses. Means $\pm$ SE are shown.

averaged over the three curves obtained for each current in a given session. The resulting data for an individual animal is shown in Figure 4 (top panel).

For the purposes of data analysis, only data from the middle six sessions of each treatment condition for each animal were used. These data were transformed using the techniques described in Experiment 1 and analyzed with a three-way repeated measures analysis of variance (ANOVA; treatment $\times$ session $\times$ current). As in Experiment 1, the inclusion of the current factor in the analysis allowed for the preservation of any possible treatment $\times$ current interactions in spite of the inevitable lack of a current effect. The ANOVA yielded no significant main effects and no significant interactions. Figure 4 (bottom panel) shows these data grouped across current, sessions, and subjects within a treatment condition.

Sucrose Preference

A mean $\log_{10}$ sucrose preference ratio was obtained for each animal for a 2-week baseline period prior to ovari-

ectomy. $\log_{10}$ preference ratios for the subsequent periods were then expressed as change from baseline and a mean calculated for each treatment condition. As can be seen in Figure 5 (upper panel), the mean preference ratio increased relative to baseline during the ovariectomy condition, decreased during the estrogen + progesterone condition, and increased again during the estrogen and posttreatment conditions. A one-way repeated measures ANOVA performed on these data showed a main effect of condition [$F(3,15) = 6.17$; $p < .05$], and post hoc analysis showed that the change in preference ratio observed during the estrogen + progesterone condition was significantly different from that seen in any other condition.

Body Weight

As with sucrose preference, mean daily weight change also varied as a function of hormonal state (see Figure 5, lower panel). The animals gained weight during the ovariectomy, estrogen + progesterone, and posttreatment phases of the experiment and lost weight during the estrogen alone

Figure 5. Upper panel: $\log_{10}$ sucrose preference (expressed as change from baseline). Lower panel: Daily body weight change for each hormone condition. Means + SE are shown.

phase. A repeated measures ANOVA performed on these data showed a significant effect of hormonal state [$F(2,17) = 56.605, p < .001$], and post hoc analysis showed that all conditions differed significantly with the exception of the estrogen + progesterone and posttreatment conditions.

Discussion

As in Experiment 1, ovarian steroid administration did not produce a statistically significant change in the reward effectiveness of LH stimulation in ovariectomized animals. Both sucrose preference and body weight were, however, altered by the hormonal regimen. The changes in sucrose preference and body weight are, in general, consistent with previous reports and provide evidence for the effectiveness of the hormone replacement regimen.

As expected on the basis of earlier studies (see Wade, 1976, for a review), animals gained weight during the ovariectomy, estrogen + progesterone, and posttreatment phases of the study and lost weight when estrogen was present alone. Similarly, sucrose solution intake was highest during ovariectomy and posttreatment conditions.

The latter data are in agreement with those of Kenney and Redick (1980), who showed that ovariectomized rats increase their intake of glucose solution. In the present experiment, sucrose solution intake was lowest in the estrogen + progesterone condition; given that this phase of the hormone replacement regimen was intended to mimic that of mid- to late pregnancy, these data are consistent with the reduction of sucrose intake reported to occur after Day 12 of pregnancy in the rat (Sandberg, Stewart, & Amit, 1982).

It is perhaps surprising that the dramatic reduction in sucrose preference seen in the estrogen + progesterone condition was not extended into the period when the rats were receiving estrogen alone, because estrogen administration to ovariectomized females has been reported to decrease overall food intake and to decrease carbohydrate intake specifically (Wurtman & Baum, 1980). It is possible that the smaller effects seen during the estrogen-alone condition reflected habituation to estrogen administration, as has been demonstrated for estrogen's effects on food intake or an increased tendency for rats to drink

more sucrose solution over time. That the estrogen implants were still operational in this phase of the study is shown by the data on body weight change. Together, the data on body weight change and changes in sucrose preference demonstrate that the lack of effect of ovarian steroid state on thresholds for BSR seen in this study do not reflect a general absence of behavioral effect of the steroids themselves.

Although there was no statistically significant effect of hormone treatment on required number in this experiment, inspection of Figure 4 shows that there was a tendency for the required number to be lower in the estrogen phase of the experiment than in the other two phases. It is possible that with a larger sample size, a statistically significant effect of estrogen on required number would have been obtained. Nonetheless the magnitude of the effect observed in the present experiment is far smaller than that usually considered meaningful in curve-shift analyses.

Although the observed changes in required number were very small and statistically insignificant, it is important to keep in mind recent work by Gallistel and his colleagues (Gallistel & Leon, 1991; Mark & Gallistel, 1993). They used an operant matching technique to measure the growth of BSR, as a function of the number of pulses per train. They showed that relatively small changes in the required number may correspond to relatively large changes in reward. For example, given the estimates of reward growth provided by Gallistel and Leon, the $0.016 \log_{10}$ unit difference between the mean required number values for the estrogen and ovariectomy conditions would correspond to an 8%–50% change in reward value. Thus, in future experiments on hormonal modulation of BSR, it may be best to use a method such as single-operant matching (Heyman, 1988), which can provide estimates of the change in reward value in addition to the change in required number.

GENERAL DISCUSSION

Taken together, the results of Experiments 1 and 2 demonstrate that the administration of ovarian steroid hormones so as to mimic either the hormonal fluctuations of the estrous cycle or the hormonal state of pregnancy were ineffective in changing the reward effectiveness of LH stimulation, given the precision of the measurement method employed. That the hormonal manipulations were effective is implied by the induction of lordosis (Experiment 1) and effects on both sucrose preference and body weight (Experiment 2), changes that have previously been shown to accompany the reproductive states that the hormonal regimens were designed to mimic.

These data are particularly interesting, given that both patterns of estrogen and progesterone administration have been shown to affect opiate and/or dopaminergic substrates and that opiate and dopamine receptors have been implicated in BSR. Rewarding stimulation of the medial forebrain bundle activates the meso-accumbens dopa-

mine system (Gratton, Hoffer, & Gerhardt, 1988; Phillips, Blaha, & Fibiger, 1989), and it has been argued that opiate-induced changes in BSR are mediated by dopaminergic neurons (Rompré & Wise, 1989). If so, perhaps the high level of stimulation-induced activity in the meso-accumbens dopamine system masks any modulatory effects of ovarian hormones.

The results of these two experiments are germane to the question of how steroid hormones bring about changes in goal object choice. If one adopts the notion that BSR reflects an endogenous system for measuring the value of environmental stimuli, then the failure of ovarian steroids to modulate this system suggests that the effect of hormonal state on goal selection may be due to an action upstream from the point where rewarding brain stimulation injects a signal in the evaluative circuitry. By acting at an early stage in the circuitry, hormones could achieve modality- and/or stimulus-specific effects. A similar argument has been put forward to explain the observation that postingestive stimuli and a homeostatic error signal alter gustatory reward while producing little or no change in BSR (Conover & Shizgal, 1994; Conover et al., 1994).

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