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Sex difference in estrogen and progestin effects on food intake, body weight and running wheel activity in rats.

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SEX DIFFERENCES
IN ESTROGEN AND PROGESTIN EFFECTS ON FOOD INTAKE,
BODY WEIGHT AND RUNNING WHEEL ACTIVITY
IN RATS

A Thesis Presented
By
R. THOMAS GENTRY

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SEX DIFFERENCES
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BODY WEIGHT AND RUNNING WHEEL ACTIVITY
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Abstract

The effects of sex and experimental androgenization on the responsiveness of food intake and running wheel activity to exogenously administered ovarian hormones was investigated in adult, gonadectomized rats. Food intake was found to be analogous to previous reports of lordotic behavior in that males and androgenized females were relatively insensitive to the influences of either estradiol benzoate (EB) or progesterone (P). Running wheel activity, however, could be differentiated from food intake in that males responded the same as females to the effects of EB and P. Androgenized females (500 ug testosterone propionate on the third day of life) showed a response to EB quantitatively equivalent to that of non-androgenized females, but they had a longer latency to respond. The estrogen antagonistic effects of P were confirmed for both of these behaviors, and there was a positive correlation between the magnitude of the responses to EB and to P.
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Androgenization is the perinatal process that permanently changes the morphology, neuroendocrinology and behavior of mammals to a male-like pattern. This series of experiments investigates the interactive effects of perinatal androgenization and ovarian hormones on two nonreproductive behaviors—food intake and wheel running—in adult, gonadectomized rats.

With few exceptions (e.g. the golden hamster) male mammals are heavier than females of the same species (Aschkenasy-Lelu & Aschkenasy, 1959; Tanner, 1962). Experiments with laboratory rats indicate that these differences may be completely accounted for by the actions of gonadal steroid hormones since, for example, gonadectomy on the first day of life eliminates the sex difference in body weight through adulthood (Slob & van der Werff ten Bosch, 1975).

Gonadal hormones have three types of actions on the sex difference in body weight (Wade, 1975b). First, since androgens are anabolic (Aschkenasy-Lelu & Aschkenasy, 1959) and increase body weight (Bell & Zucker, 1971; Gentry & Wade, in press) and estrogens lower body weight (Brobeck, Wheatland & Strominger, 1947; Sullivan & Smith, 1957; Wade & Zucker, 1970) a great deal of the sex difference is due to the "activational" effects of gonadal steroids acting during the postpubertal life of the animal. Adult gonadectomy in either sex produces a weight change
in the direction of the opposite sex (Kakolewski, Cox & Valenstein, 1968). Second, since exposure to androgens during the perinatal period causes an increase in adult weight even without postpubertal exposure to gonadal steroids there is a purely "organizational" component to the sex difference in body weight (Slob & van der Werff ten Bosch, 1975). Finally, the perinatal exposure to androgens may "organize" the sensitivity of physiological and behavioral responses to the "activational" effects of gonadal hormones which are relevant to body weight regulation. That is, even if males and females were exposed to the same hormones as adults the sex difference may still remain.

Direct comparison of males and females has not been made but results of experimental androgenization studies are available. Bell and Zucker (1971), for example, have found that neonatal androgenization makes male rats more sensitive to the stimulatory effect of testosterone propionate (TP) on body weight. Similarly, experimental androgenization has been found to attenuate the weight suppressing effects of either estradiol benzoate (EB) alone (Beatty, Powley & Keesey, 1970) or of EB and progesterone (P) combined (Bell & Zucker, 1971).

Injected estrogens decrease food intake and increase voluntary exercise (measured by wheel running) producing a negative caloric balance and a body weight loss (see
Wade, 1972, 1975b for reviews). Again, direct male-female comparisons have not yet been made for these behaviors to determine if there is a sex difference in sensitivity to estrogens. Bell and Zucker (1971), however, have looked at the food intake response to EB+P in androgenized and nonandrogenized spayed female rats and found that androgenized females were less sensitive to the anorexic effect of these two hormones in combination. Similarly, Gerall (1967) and Gerall, Stone and Hitt (1973) have found that a high (1250 ug) but not a low (10 ug) dose of TP on the fifth day of life significantly lowered the running response of spayed female rats to exogenous EB. One of the purposes of the present series of experiments then was to determine whether neonatal androgenization of female rats mimics the natural sex differences for weight regulatory behaviors as it does for reproductive behaviors. In other words, does an interaction between organizational and activational effects on eating and running wheel activity contribute to the sex difference in body weight in rats?

The action of P was investigated to determine whether androgenization would influence sensitivity to P independent from its influence on sensitivity to estradiol. This was of interest in light of the debate as to whether the refractoriness of lordosis behavior in males and androgenized females to the stimulation of estradiol
and P is due to a neural insensitivity to estradiol (Edwards & Thompson, 1970) or to P (Clemens, Shryne & Gorski, 1970) or to both. Progesterone has no effect on either food intake or wheel running in spayed female rats, but it does antagonize the estrogenic activation of these behaviors (Rodier, 1971; Wade, 1975a). Thus it is actually the antiestrogenic action of P which was investigated here.

The nature of the sexual differentiation process itself was also of interest. Androgenization might cause a generalized insensitivity to estrogens and progestins or there could be examples of behaviors whose response to ovarian hormones remains unaffected.

**Experiment 1: Food Intake and Body Weight**

**Procedure**

Forty female Sprague-Dawley rats, born in our lab from Charles River Breeding Laboratories stock, were raised in litters of eight animals. On the third day of life half of these were injected subcutaneously with .025 cc sesame oil and half were injected with 500 ug TP in .025 cc sesame oil vehicle. Twenty adult males of the same strain were purchased directly from Charles River.

All animals were housed individually in wire bottom cages in the same room which was illuminated for twelve hours per day and maintained between 22°C and 26°C. Each
animal was supplied with Purina laboratory chow and tap water *ad libitum*. Body weight was measured every third day to the nearest gram and food intake was measured at approximately the same time each day to the nearest 0.1 g beginning at 130 days of age.

The two female groups were ovariectomized when they were 140 days old and the adult males were castrated upon arrival from the breeder. Surgery was carried out under Nembutal anesthesia (40 mg/kg). Four to five weeks after gonadectomy 16 males, 16 females and 18 androgenized females had survived in good health. Each of these three groups was divided into three treatment groups matched for body weight and food intake.

The animals in the control groups received vehicle (oil) injections daily throughout the experiment. The EB and the EB+P treatment groups were injected with 2 ug EB daily for an initial period of 19 days. Then while the EB treatment groups continued to receive 2 ug EB, the EB+P treatment groups were injected with a combination of 2 ug EB and 1 mg P each day. Body weight and food intake were measured through the twelfth day of P treatment. All statistical analyses were performed on the change in daily food intake from the appropriate baseline period to the treatment period.

Results

*Estradiol Benzoate.* The most prominent EB suppression
of eating occurred over the first third of the 36-day treatment period (Figure 1). Analysis of relative potency of EB for each neonatal treatment group was therefore calculated on the change of mean daily food intake from the 6-day baseline to the first 12 days of treatment. The EB treatment significantly lowered food intake relative to controls in the females, \( t(9) = 5.762, \ p = .001 \), and androgenized females, \( t(10) = 2.691, \ p = .025 \), but not in males, \( t(9) = 1.215, \ p = .25 \). One-way analysis of variance indicated that the magnitude of the food intake suppression (intake of EB treated animals minus the mean intake of controls) differed across groups, \( F(2, 15) = 11.209, \ p = .002 \). The mean food intake suppression for females was 4.9 g per day which was significantly greater than that of either males (1.1 g per day), \( t(10) = 4.334, \ p = .002 \), or that of androgenized females (2.5 g per day), \( t(10) = 2.843, \ p = .02 \).

The effect of EB on body weight is summarized in Table 1. Relative to control groups, 12 days of EB treatment significantly lowered body weight increment in each of the neonatal treatment groups: \( t(9) = 3.081, \ p = .020 \) for
males; \( t(9) = 6.217, p < .001 \) for females; and \( t(10) = 4.769, p < .01 \) for androgenized females. Analysis of variance, however, indicated that there was no statistically reliable interaction between EB treatment and neonatal group, \( F(2, 28) = 0.658, p < .5 \). On Day 36 of EB treatment there was a significant interaction between EB and neonatal treatment group, \( F(2, 28) = 5.430, p < .02 \), with females showing a greater relative weight loss than either males, \( t(10) = 5.793, p < .001 \), or androgenized females, \( t(10) = 2.793, p < .02 \).

**Progesterone.** Figure 2 shows the effect of P on the food intake of animals given daily injections of EB at a time after the anorexic effects of EB have ceased and the intake matches oil-injected controls. In all three neonatal treatment groups the food intake of the two control treatments (EB alone and oil alone) do not differ from each other during either the 4-day baseline or after the addition of P to the EB+P group. Since there was a delay in the action of P on eating statistical analyses compared the baseline and the second 6-day period of treatment (Day 7-Day 12). The addition of P significantly stimulated eating in the females, \( t(9) = 2.339, p < .05 \) compared to controls but was ineffective in both males, \( t(9) = 0.594, \)
and the androgenized females, \( t(10) = 0.505, p = .5 \). Progesterone treatment significantly stimulated body weight gain in each of the three groups (Table 2). Analysis of variance revealed no statistically reliable interaction between adult hormone treatment and neonatal treatment groups, \( F(2, 28) = 2.209, p = .10 \).

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**Experiment 2: Running Wheel Activity**

**Procedure**

Twenty-four female and 16 male rats of Sprague-Dawley (Charles River) stock born in our laboratory were raised in litters of eight. On the third day of life 12 females and the 16 males were injected with the oil vehicle, and the other 12 females were injected with 500 ug TP in .025 cc oil. All animals were gonadectomized at four weeks of age. Prepubertal castration was used to avoid the possible sensitization of the activity response by pre-exposure to estradiol (Asdell, Doornenbal & Sperling, 1962; Asdell & Sperling, 1963).

At eight weeks of age testing was begun by placing half the animals from each group into Wahmann LC-34 activity cages and recording revolutions each day at the middle of the 12-hour light period. At 10 weeks of age, when running activity had stabilized, injections of .1 cc sesame oil
were begun for a five-day baseline period. For the next 25 days all animals in running wheels were given 2 ug EB/animal/day. At the end of the 25 day period each group was divided into two subgroups approximately matched for running activity. One subgroup continued to get the daily 2 ug EB while the other subgroup received 2 ug EB + 1 mg P/day. Fifteen days later all of these animals were removed from the running wheels and the second half of each neonatal treatment group was put in (age: 115 days). These animals were treated exactly like the first half and had completed all stages at 165 days old. The data of animals in each of the treatment groups were combined. All statistical analyses were performed on the change in the daily running activity from the appropriate baseline period to the treatment period.

Results

**Estradiol Benzoate.** Each of the three neonatal treatment groups showed a gradual increase in daily running wheel activity during the 25 days of EB treatment (Figure 3); the data of a male who died during treatment were eliminated. One-way analysis of variance indicated there was no statistically reliable difference between any of the groups for either the entire 25-day treatment period.

Insert Figure 3 about here
\[ F(2, 36) = 0.405, p = 0.5, \] or the last 5 days of treatment, \[ F(2, 36) = 0.611, p = 0.5. \] If, however, the groups were compared over the first 15 days of treatment there was a significant difference between the females and the androgenized females, \[ t(22) = 2.212, p = 0.05, \] but not between males and females, \[ t(25) = 1.295, p = 0.2, \] or between males and androgenized females, \[ t(25) = 1.262, p = 0.2. \]

**Progesterone.** The EB + P treated males decreased their wheel running to 72% baseline over the 15-day treatment; females decreased to 65%; and androgenized females decreased to 67%. Meanwhile, control, EB-alone groups decreased their activity to 99%, 95% and 99% respectively (Figure 4). Analysis of variance revealed a highly significant effect of P, \[ F(1,33) = 22.116, p = 0.0001, \] and the lack of a reliable interaction effect between P treatment and group, \[ F(2, 33) = 0.126, p = 0.5. \]

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**Discussion**

**Sex difference in body weight**

In the introduction it was suggested that an interaction between organizational and activational effects of gonadal hormones on specific behaviors could contribute to the sex difference in body weight in rats. The results of Experiment 1 support this hypothesis for eating behavior,
since EB suppressed eating and body weight gain more in nonandrogenized females than in males or androgenized females. The direct contribution of the depressed food intake displayed by the nonandrogenized females to their decreased body weight over the 36-day period (Table 1) must be viewed cautiously, however, since the food intake difference was no longer present after about Day 12 (Figure 1), and the body weight difference had not yet developed by Day 12 (Table 1).

In contrast, energy expenditure as measured by running wheel activity probably does not contribute to the observed interaction of organizational and activational hormone effects on body weight. Results of Experiment 2 indicate that male and female rats cannot be differentiated with respect to their latency to respond to EB, their response to P (Figure 4). It is likely that the reported sex difference in spontaneous running of gonadally intact rats (Hitchcock, 1925; Kennedy, 1964; Wang, Richter, & Guttmacher, 1925) is the result of different levels of available estrogens, rather than a difference in behavioral sensitivity.

That a very high neonatal dose of TP (1250 ug, Gerall et al., 1972) decreases the sensitivity of a behavior not found to be sexually divergent is not completely explicable. It may be due to a slower rate of induction
of the full running response since the present study showed that androgenized females (500 ug TP) were significantly less active than females if compared after 15 days of EB treatment (the maximum time Gerall et al., 1972, allowed) but not if compared after 25 days of treatment. This attenuated acceleration in the androgenized females was also reflected in the latency for individual animals to reach half-maximum response (defined as the day in treatment that activity first exceeded one half the mean activity over the last five days). The mean latency for both males and females was 5.5 days, whereas the latency for androgenized females was 8.3 days, which was significantly greater than either the males, \( t(24) = 2.473, p = .05 \), or the females, \( t(22) = 2.321, p = .05 \). Consequently, the results of Experiment 2 tend to suggest that 500-1250 ug TP may be a supraphysiological dose when compared to the natural androgenization process in males.

**Progesterone as an estrogen antagonist**

Behaviorally P is known to have both synergistic and antagonistic interactions with the activational effects of estradiol. Progesterone, for example, facilitates the estrogenic stimulation of lordosis in rats, guinea pigs and hamsters (Boling & Blandau, 1939; Collins, Boling, Dempsey & Young, 1938; Frank & Fraps, 1945; respectively). Behavioral systems that demonstrate antiestrogenic effects of P include female guinea pig sexual receptivity (Zucker,
1968), rat food intake (Wade, 1975a; Zucker, 1969), and rat running wheel activity (Rodier, 1971).

Although there are some indications that P can inhibit the neural concentration of tritiated estradiol (Anderson & Greenwald, 1969 using autoradiography; Lisk, Ciaccio & Reuter, 1972 using scintillation counting) the precise nature of P's antiestrogenic action is unknown. With respect to eating behavior, the results of Experiment 1 indicate that P only reversed the effect of EB in the females—the group that was the most sensitive to EB. This relationship between the stimulation by EB and the inhibition by P is more clearly seen in Experiment 2 with running wheel activity. Using animals of all three groups the correlation between the increase in running during the 25 days of EB treatment, to the decrease in running during the 15 days of P treatment is $r(17) = .809$.

For both food intake (Drewett, 1973; Wade, 1975a) and running wheel activity (Gerall, et al., 1972; Roy & Wade, 1975) the amplitude of the response is a function of the estrogen dose. Thus the high correlation between estradiol stimulation and P inhibition is consistent with the hypothesis that P interacts directly with estradiol. One possibility is that P could lower plasma levels of estradiol by, for example, induction of hepatic enzymes that metabolize estradiol. Another possibility is that P could compete directly with estradiol for the appropriate
diencephalic receptors controlling food intake and wheel running.

**The process of neonatal androgenization**

There are a number of hormone-stimulated behaviors for which castrated male and female rats are differentially sensitive. These include male and female sex behavior, and some behaviors not directly concerned with reproduction, e.g., saccharin preference (Valenstein, Kakolewski & Cox, 1967). Analogous to the sexual differentiation in neuroendocrinological function, it has been found that neonatal androgen treatment to females mimics the natural androgenization process in males. In general the result of the androgenization process is a relative refractoriness to the activational stimulation of ovarian hormones. Thus, gonadectomized males and androgenized females do not display lordosis (e.g., Barraclough & Gorski, 1962) or saccharin preference (Wade & Zucker, 1969) in response to normally effective doses of EB and P.

Is it possible, then to explain all of the effects of androgenization in terms of decreased sensitivity to estrogens and progestins, and are all the estrogen and progestin sensitive behaviors affected in the same way by androgenization? The results of Experiment (Figures 1 & 2) confirm the suggestion of Bell and Zucker (1971) that eating behavior is analogous to female sexual receptivity in its response to androgenization. In an
extension of their findings the present studies indicate:
(a) the relative insensitivity is to both EB and P, and
(b) the experimental androgenization of females does indeed mimic the refractoriness seen in castrated males.
With running wheel activity, however, the results of Experiment 2 indicate that experimental androgenization with 1250 ug TP (Gerall, 1967; Gerall, et al., 1972) does not mimic the undiminished responsiveness of castrated males compared to females.

Thus, whereas eating behavior is analogous to female receptivity, running wheel activity is not. The results confirm that all behaviors affected by androgenization show a tempered responsiveness to ovarian hormones but there is at least one behavior—running wheel activity which may not normally be affected by either the natural androgenization process or experimental androgenization using a dose that is more than sufficient by other measures of masculinization.

With respect to the interaction with the organizational action of androgens, the effects of P are analogous to those of EB. For eating behavior P was only effective in females and failed to stimulate food intake in either males or androgenized females. In the case of running wheel activity the effects of P were not differentiated by sex or neonatal androgenization with each of the three groups showing a remarkably equal (approximately 30%) depression
in activity.
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Kakolewski, J. W., Cox, V. C., & Valenstein, E. S. Sex differences in body-weight changes following gonadectomy of rats. Psychological Reports, 1968, 22, 547-554.


Table 1

Effect of Oil and Estradiol Benzoate (EB) on Mean Body Weight (g) on the Last Day of Baseline and After 12 or 36 Days of Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 0</th>
<th>Day 12</th>
<th>Day 36</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>499±40*</td>
<td>502±40</td>
<td>512±37</td>
</tr>
<tr>
<td>EB treatment</td>
<td>6</td>
<td>497±22</td>
<td>484±24**</td>
<td>491±24*</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>360±12</td>
<td>372±11</td>
<td>393±13</td>
</tr>
<tr>
<td>EB treatment</td>
<td>6</td>
<td>359±14</td>
<td>349±15***</td>
<td>345±14***</td>
</tr>
<tr>
<td><strong>Androgenized Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>355±12</td>
<td>370±12</td>
<td>387±14</td>
</tr>
<tr>
<td>EB treatment</td>
<td>6</td>
<td>362±9</td>
<td>356±10***</td>
<td>362±9***</td>
</tr>
</tbody>
</table>

a Standard Error

* P < .05
** P < .02
*** P < .01
Table 2
Effect of Estradiol Benzoate (EB) and EB plus Progesterone (P) on Mean Body Weight (g) on the Last Day of Baseline, and after 12 Days of Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 0</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB treatment alone</td>
<td>6</td>
<td>478±24\textsuperscript{a}</td>
<td>478±24</td>
</tr>
<tr>
<td>EB+P treatment</td>
<td>5</td>
<td>472±19</td>
<td>481±21\textsuperscript{*}</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB treatment alone</td>
<td>6</td>
<td>353±16</td>
<td>354±19</td>
</tr>
<tr>
<td>EB+P treatment</td>
<td>5</td>
<td>352±18</td>
<td>364±19\textsuperscript{*}</td>
</tr>
<tr>
<td><strong>Androgenized Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB treatment alone</td>
<td>6</td>
<td>356±8</td>
<td>354±8</td>
</tr>
<tr>
<td>EB+P treatment</td>
<td>6</td>
<td>357±15</td>
<td>362±15\textsuperscript{**}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Standard Error.

\textsuperscript{*}p .02.

\textsuperscript{**}p .01.
Figure Captions

Figure 1. Effect of estradiol benzoate (Eb, 2 ug/day) begun at the time indicated by arrow and oil vehicle (oil) on food intake of gonadectomized males, females and androgenized females.

Figure 2. Effect of progesterone (P, 1 mg/day) begun at the time indicated by arrow on food intake of gonadectomized males, females and androgenized females being given estradiol benzoate (EB, 2 ug/day). Control groups are those continuing to receive EB and those receiving oil vehicle throughout.

Figure 3. Effect of estradiol benzoate (EB, 2 ug/day) on running wheel activity of gonadectomized males, females and androgenized females. Treatment began at the time indicated by the arrow.

Figure 4. Effect of progesterone (P, 1 mg/day) begun at the time indicated by arrow on running wheel activity of gonadectomized males, females and androgenized females being given estradiol benzoate (EB, 2 ug/day). Control groups are those continuing to receive EB.
Figure 1
Figure 2
Figure 3
Figure 4