STRAIN DIFFERENCES IN 8-OH-DPAT-INDUCED HYPERPHAGIA: MODULATION BY FEMALE GONADAL HORMONES.

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To the Dean of the Graduate Studies and Research:

I am submitting herewith a dissertation written by Farzana Shaheen entitled "STRAIN DIFFERENCES IN 8-OH-DPAT-INDUCED HYPERPHAGIA: MODULATION BY FEMALE GONADAL HORMONES." I have examined this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Molecular Biology.

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ABSTRACT

STRAIN DIFFERENCES IN 8-OH-DPAT-INDUCED HYPERPHAGIA: MODULATION BY FEMALE GONADAL HORMONES.

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In a prior experiment, Sprague-Dawley females were more likely than Fischer females to show inhibition of lordosis behavior and to show flattened body posture after subcutaneous (s.c.) treatment with the 5-HT1A receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). Both of these behaviors are thought to be mediated by 8-OH-DPAT's action at postsynaptic 5-HT1A receptors. In the current experiment, 8-OH-DPAT-induced hyperphagia, believed to reflect activation of somatodendritic 5-HT1A receptors, and the ability of female gonadal hormones to modulate 8-OH-DPAT-induced hyperphagia were examined. Ovariectomized age-matched Fischer and Sprague-Dawley rats were used. On the day of the experiment, 1 1/2 hr after lights out, the food was removed from the cages and a premeasured quantity of food was placed in the cage. Rats were injected s.c. with saline or 0.1, 0.15, 0.2, 0.3 mg/kg 8-OH-DPAT and the amount of food eaten two hr and four hr after injection was examined. 8-OH-DPAT-induced hyperphagia was found to be similar in Fischer and Sprague-Dawley rats.

When the rats of the two strains were hormonally primed with 0.0067, 0.0222, 0.033 or 0.17 μg/g of estradiol benzoate (EB) or oil and forty-eight hr later injected with progesterone (P) or with oil, hormonal treatment significantly decreased food intake of Sprague-Dawley rats as compared to Fischer rats. Still, 8-OH-DPAT produced hyperphagia but to a greater extent in Sprague-Dawley compared to Fischer rats. In Sprague-Dawley rats, although P accentuated the suppressing effect of EB on food intake after saline or 8-OH-DPAT treatment, 8-OH-DPAT-induced hyperphagia was present under both EB and EB /P condition. On the other hand, P did not have a significant effect on the food intake of Fischer rats and hyperphagia was present only after administration of the lower EB dose. These results are interpreted to reflect a strain difference in the modulation of food intake by female gonadal hormones.

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CHAPTER I

INTRODUCTION

Serotonin (5-HT) is a neurotransmitter involved in the regulation of feeding behavior [13,24,79]. Activation of brain serotonin mechanisms induces satiety in animals and humans [12,26]. 5-HT releasing drugs or selective 5-HT reuptake inhibitors such as fenfluramine, fluoxetine, and sibutramine inhibit appetite and cause weight loss in humans and animals [24,27,41,61,67]. Administration of the general 5-HT receptor antagonist, methysergide, stimulates food intake in well-satiated rats [28,35].

Serotonin exerts its effect through a variety of receptors. There are seven different families of 5-HT receptors: 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, 5-HT$_4$, 5-HT$_5$, 5-HT$_6$ and 5-HT$_7$ [86]. Several 5-HT receptor families contain multiple receptor subtypes. For example, the 5-HT$_1$ family, which is the largest family of 5-HT receptors, contains at least seven subtypes: 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1D\beta}$, 5-HT$_{1E}$, 5-HT$_{1F}$ [86]. The 5-HT$_2$ family contains 3 subtypes: 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$. With the exception of the 5-HT$_3$ receptor family, 5-HT receptors contain seven transmembrane domains and are members of the G protein coupled receptor superfamily [86]. Agonist binding to these G protein coupled receptors leads to the modulation of second messenger systems. For example, activation of 5-HT$_1$ receptors leads to the reduction of adenylyl cyclase activity and increases the opening of K^+ channels [86]. Activation of 5-HT$_2$ receptors increases phospholipase C activity which leads to the production of inositol triphosphate (IP3) and diacylglycerol (DAG) [86]. IP3

releases Ca^{2+} from endogenous stores and DAG activates protein kinase C [86]. The 5-HT1A, 5-HT1B, 5-HT2A and 5-HT2C receptors are thought to be the most important for the control of feeding [25,27]. 5-HT1A receptor agonists stimulate food intake [29,49,50]; 5-HT1B and 5-HT2 receptor agonists inhibit food intake [48,56,77,78].

Activation of 5-HT1A receptors also produces a variety of behavioral effects including several components of the "serotonin behavioral syndrome" [59,84] and inhibition of female rat lordosis behavior [88]. The flat body posture and forepaw treading induced by the 5-HT1A receptor agonist, 8-OH-DPAT, are thought to be mediated by activation of postsynaptic 5-HT1A receptors [59,84], most likely located in brainstem areas [52]. Inhibition of lordosis results from activation of postsynaptic 5-HT1A receptors located in the mediobasal hypothalamus [1,88]. In contrast, 5-HT1A receptor agonists increase food intake by activation of somatodendritic 5-HT1A autoreceptors at 5-HT cell bodies [24,49]. Infusion of 8-OH-DPAT into dorsal or median raphe nuclei increases eating [49]. Since activation of these autoreceptors reduces firing of 5-HT neurons and thereby decreases release of 5-HT from 5-HT terminals [80], 5-HT1A receptor agonists are thought to increase food intake by decreasing 5-HT's inhibition of food intake. 8-OH-DPAT has been shown to increase feeding in many species [26]. 8-OH-DPAT-induced feeding is elicited by low doses of 8-OH-DPAT (40-100 µg/kg) which activate somatodendritic 5-HT1A receptors (with lesser effects at postsynaptic sites) and is correlated with a decrease in indices of 5-HT metabolism in various brain regions [30,31,49,50]. Higher doses of 8-OH-DPAT (1-5 mg/kg) activate postsynaptic as well as presynaptic 5-HT1A sites and decrease food intake due to the production of

stereotyped behaviors such as forepaw treading, head weaving and flat body posture [29,84].

Female gonadal hormones modulate both presynaptic and postsynaptic 5-HT receptors. Lakoski [58] reported that the ability of 5-HT1A receptor agonists to inhibit firing of 5-HT neurons in the dorsal raphe nucleus (DRN) was reduced by estrogen. Rats treated with EB also showed less eating after 8-OH-DPAT treatment [76]. Proestrous rats (with high estrogen levels) showed less hyperphagia after 8-OH-DPAT than diestrous rats (with low estrogen levels) [90]. It is, therefore, possible that estrogen attenuation of 8-OH-DPAT-induced hyperphagia results from the hormonal modulation of somatodendritic 5-HT1A receptors. At the postsynaptic level, 5-HT1A receptor functioning is also modulated by estrogen. Two consecutive weeks of estrogen plus P priming reduces the ability of 8-OH-DPAT to inhibit lordosis behavior in ovariectomized rats [87]. The density of 5-HT receptors may also be regulated by estrogen but this regulation differs for different 5-HT receptors. For example, for 5-HT1A receptors, most investigators have reported that estrogen does not reduce [H^3]-8-OH-DPAT binding [17,42,68]. On the other hand, 5-HT2 receptor density has been reported to increase after estrogen [9] and the density of 5-HT2A receptors in forebrain was higher in proestrous than in diestrous rats [81].

Estrogen is known to reduce food intake in many species, including rats, primates and ruminants [91] and ovarietomized rats show an increased level of feeding [38] which is reversed by treatment with physiological doses of estradiol [2,69,83,91]. During proestrus, rats increase their activity and sexual receptivity and decrease their food intake [38]. A decrease in food intake occurs in many species, including humans, after and during ovulation [38].

In contrast to the hypophagic effect of estrogen, P has been reported to increase food intake [73,75,91] and attenuate the effects of estradiol on feeding behavior and body weight gain [91]. Less attention has been paid to P's effect on 8-OH-DPAT-induced hyperphagia, but if P decreases the firing of DRN 5-HT neurons, it might also modulate 8-OH-DPAT-induced hyperphagia. However, Salamanca and Uphouse [76] reported that P did not influence 8-OH-DPAT-induced hyperphagia.

Strain differences in components of the 5-HT system and in the response to serotonergic drugs are well documented [5,14,15,16, 23,46,57]. Strain differences have been reported in the behavior produced by 5-HT [54,94]. Such differences include the intensity of the 5-HT syndrome which was significantly correlated with brain 5-HT content in five strains of mice [94]. 5-HT_2 receptor binding with ketanserin in frontal cortex varies among mouse strains [94]. Sprague-Dawley and Fischer rats are of particular relevance to the current research because of their differences in several aspects of serotonergic function. For example, the hippocampus of Fischer rats contains more 5-HT than that of Sprague-Dawley rats, while the hypothalamus of Sprague-Dawley rats contains more 5-HT and its metabolite, 5-hydroxyindoleacetic acid, than that of Fischer rats [14]. Fischer rats are reported to have a hyperactive hypothalamic-pituitary-adrenal (HPA) axis relative to Sprague-Dawley rats and serotonin, acting via 5-HT_{1A} receptors, is considered to be an important stimulator of the HPA axis [14]. Burnet et al. [15] reported that Fischer rats have more raphe 5-HT transporter mRNA than Sprague-Dawley rats. Uphouse et al. [89] also observed a strain difference between Fischer and Sprague-Dawley females in 8-OH-DPAT's potency to inhibit lordosis behavior. Although the drug

inhibited lordosis behavior in both strains, the behavior was reduced by a lower dose in Sprague-Dawley females. Since Sprague-Dawley rats are reported to have more 5-HT in the hypothalamus than Fischer rats, it is possible that less 8-OH-DPAT was required to inhibit lordosis behavior. It was also noted that Sprague-Dawley rats showed more flat body posture than did Fischer females after treatment with 8-OH-DPAT. 8-OH-DPAT-mediated flat body posture and inhibition of lordosis behavior reflect an action of the drug on postsynaptic 5-HT1A receptors.

It is possible that Fischer and Sprague-Dawley rats differ in their sensitivity to female gonadal hormones [93]. Since lordosis is an estrogen dependent behavior, in the experiment by Uphouse et al. [89], it was not possible to determine if strain differences in lordosis behavior reflected differential responses to 8-OH-DPAT or to the hormonal priming. However, strain differences in flat body posture were independent of hormonal priming. Since both of these behaviors reflect effects of 8-OH-DPAT at postsynaptic receptors, it is not clear if behaviors mediated by 5-HT1A receptors in several locations show strain differences or if such differences occur only for behaviors resulting from activation of postsynaptic 5-HT1A receptors.

The following experiments were designed to determine if :1) comparable strain differences are present for a behavior resulting from the activation of somatodendritic 5-HT1A autoreceptors and : 2) such strain differences are dependent on female gonadal hormones. Ovariectomized Fischer and Sprague-Dawley rats, with and without hormonal priming, were used. The following questions were addressed:

1) Does there exist a strain difference in the ability of the 5-HT1A receptor

agonist, 8-OH-DPAT, to induce hyperphagia? Sprague-Dawley rats were more sensitive to the effects of 8-OH-DPAT on behaviors mediated by postsynaptic 5-HT1A receptors [89]. It was important to determine if the same sensitivity to 8-OH-DPAT by Sprague-Dawley rats exists for a behavior mediated by presynaptic 5-HT1A receptors. If strain differences exist in both pre and postsynaptic 5-HT1A receptors, Sprague-Dawley rats were expected to show more 8-OH-DPAT-induced hyperphagia and a lower dose responsivity to the drug.

2) Do female gonadal hormones decrease 8-OH-DPAT-induced hyperphagia? Since Fischer rats are reported to be more sensitive to estrogen-like compounds, estrogen's attenuation of 8-OH-DPAT-induced hyperphagia was expected to be greater in Fischer rats than in Sprague-Dawley rats. Of course, there may also be a strain difference in the ability of female gonadal hormones to decrease food intake. If so, Fischer rats were expected to show a greater decrease in food intake after estrogen priming.

CHAPTER II

MATERIALS AND METHODS

A. MATERIALS

Methoxyflurane (Metofane®) was obtained from Pitman Moore (Mundelein, IL). Suture material was obtained from Butler Co (Arlington, TX). EB, P and sesame seed oil were purchased from Sigma Chemical Co. (St. Louis, MO). 8-Hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) was purchased from Research Biochemicals International (Natick, MA). All other supplies were purchased from Fischer Scientific (Houston, TX).

B. GENERAL METHODS

Animals and Housing Conditions

Age-matched (60 days) female Fischer and Sprague-Dawley rats were purchased from Sasco Laboratories (Wilmington, MA). After their arrival, rats were housed 2 or 3 per cage in polycarbonate shoebox cages with free access to food and water. Rats were housed in a 12 hr/12 hr light/dark cycle with lights off at 12 noon.

Surgical Procedures

Two weeks after their arrival, rats were anaesthetized with Metofane®. The abdominal muscles were opened with a single incision across the abdomen. The ovarian tissue was found, ligated and removed. The muscle incision was sutured and the epidermal layer was stapled with a tissue stapler. Rats were given two weeks to recover before the start of the experiments.

Hormone and Drug Administration

Two weeks after ovariectomy, rats were injected with EB followed forty-eight hr later by P or oil. EB and P were dissolved in sesame seed oil and were injected s.c.. Between 1:30 and 3:00 PM (four hr after P or oil) rats were injected s.c. with 8-OH-DPAT. 8-OH-DPAT was dissolved in 0.9% saline and was injected in a volume of 0.1 ml/100 g rat at a dose of 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg or 0.3 mg/kg. An individual rat was used for only one treatment condition on a single test day. Within specific experiments, groups were counter-balanced to include each experimental treatment condition each day. Multiple test days were used to accumulate the desired sample size per experiment.

C. SPECIFIC EXPERIMENTS

EXPERIMENT 1

Dose dependent effects of 8-OH-DPAT on hyperphagia in Fischer and Sprague-Dawley rats.

In the first experiment, rats were ovariectomized but received no hormonal priming. Two weeks after ovariectomy, rats were housed individually in suspended metal cages for two days prior to the day on which food intake was to be measured. The rats had free access to food and water. On the day of the experiment, 1 1/2 hr after lights out (after 1:30 PM), food was removed from the cages. Rats were injected s.c. with either saline or 8-OH-DPAT (0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg or 0.3 mg/kg). Rats were returned to their home cages and a premeasured quantity of food was placed in each cage. Construction paper was placed under each cage to capture food waste. A five min interval was given between injections to keep a precise timing in measurement of food

intake. Two and four hr after injection, intake was recorded by removing the food remaining in the cage, weighing it and replacing it back in the cage. Food intake (in grams) was determined by subtracting the remaining food (corrected for waste) from the original food allotment. Data were recorded as grams of food intake during the first and second two-hr intervals after injection and over the entire four-hr (total) period. Food intake between two and four hr was determined by subtracting the two-hr food intake from the four-hr intake.

Total food intake (in grams) was compared across drug treatments and between strains with a two way ANOVA. Linearity in response to the dose of 8-OH-DPAT was evaluated by simple regression procedures. A second ANOVA was performed on food intake after 8-OH-DPAT relative to the average of the saline control for each strain and time interval to eliminate strain differences in food intake. Food intake during the first relative to the second two-hr interval after injection was compared with a two-way (dose x strain) repeated measures ANOVA with the time interval as the repeated factor. An alpha level of 0.05 was required for the rejection of the null hypothesis [96]. The Tukey test was used to compare differences between treatments (within strain) and strains (within treatment). Dunnett's test was used to evaluate food intake relative to the saline control.

EXPERIMENT 2

Dose-dependent effects of estrogen plus P on food intake and 8-OH-DPAT-induced hyperphagia in Fischer and Sprague-Dawley rats.

Two weeks after ovariectomy, Fischer and Sprague-Dawley rats were injected s.c. with sesame seed oil or with 0.17 μg/g or 0.033 μg/g of EB dissolved in sesame seed oil. After injection, rats were housed individually in

suspended metal cages with free access to food and water. Forty-eight hr after EB or oil, the rats were primed either with 500 µg P or oil. All EB treated rats received P and all oil treated rats received oil. This produced three groups of rats of each strain: control group (oil/ oil) and two hormone treated groups (0.17 µg/g EB /500 µg P or 0.033 µg/g EB /500 µg P). One and one-half hr after lights out (after 1:30 PM), food was removed from the cages. Four hr after the injection with P or oil, rats were injected s.c. with 0.15 mg/kg of 8-OH-DPAT and were replaced in their cages with a premeasured quantity of food. A five min interval was used between rats as described in experiment 1. Two and four hr after injection, food intake was recorded as described for experiment 1.

Total food intake (in grams) was compared across hormone doses and between strains. ANOVA was also performed of the two-hr food intake after 8-OH-DPAT relative to the average of the saline control as described earlier to eliminate strain differences in food intake. Food intake during the first relative to the second two-hr interval after 8-OH-DPAT injection was compared with a two-way (dose x strain) repeated measures ANOVA with the time interval as the repeated factor. An alpha level of 0.05 was required for rejection of the null hypothesis [96]. The Tukey test was used to compare differences between treatments (within strain) and strains (within treatment). Dunnett's test was used to evaluate hyperphagia relative to the saline control.

EXPERIMENT 3

Strain differences in attenuation of 8-OH-DPAT-induced hyperphagia in rats primed with EB only or P only.

The third experiment was designed to compare effects of EB or P, alone, on the response to 8-OH-DPAT. Two weeks after ovariectomy, rats were

injected s.c. with sesame seed oil or with 0.033 μg/g of EB. After injection, the rats were housed individually in suspended metal cages. Forty-eight hr later, rats were primed s.c. with P or oil to produce four groups per strain: oil /oil, 0.033 μg/g of EB /oil, oil /500 μg of P and 0.033 μg/g of EB /500 μg of P. One and one-half hr after lights out (after 1:30 PM), food was removed from the cages. Four hr after the injection with P or oil, rats were injected s.c. with 0.15 mg/kg of 8-OH-DPAT or with saline and were returned to their cages as described above. A five min interval was used between rats. Two and four hr after injection, food intake was determined as described earlier. Differences in food intake were evaluated by ANOVA as described earlier.

EXPERIMENT 4

Strain differences in the dose dependent effect of estrogen alone on 8-OH-DPAT-induced hyperphagia in Fischer and Sprague-Dawley rats.

The fourth experiment was designed to compare the dose dependent effects of estrogen alone on the response to 8-OH-DPAT in ovariectomized Fischer and Sprague-Dawley rats. Two weeks after ovariectomy, rats of the two strains were injected s.c. with sesame seed oil or with different doses of EB (0.0067, 0.0222 or 0.17 μg/g). After injection, the rats were housed individually in suspended metal cages with free access to food and water. Forty-eight hr later, rats were injected s.c. with sesame seed oil. This produced four groups of rats of each strain: oil /oil, 0.0067 μg/g EB /oil, 0.0222 μg/g EB /oil, 0.17 μg/g EB /oil. Four hr after the injection with sesame seed oil, food was removed from the cages and a premeasured quantity of food was placed in each cage. Rats of each strain were injected with 0.15 mg/kg of 8-OH-DPAT or with saline and were returned to their cages as described earlier. Food intake was measured at

two and four hr after the injection as described earlier. Differences in food intake between the two strains were compared with ANOVA as described earlier.

CHAPTER III

RESULTS

EXPERIMENT 1: Strain differences in the dose dependent effect of 8-OH-DPAT on food intake in ovariectomized Fischer and Sprague-Dawley rats.

A: Strain differences in the effects of 8-OH-DPAT on food intake during the total four-hr time period.

The first experiment was designed to see if there was a strain difference in the dose dependent effect of 8-OH-DPAT on food intake. Food intake during the four hr after injection with different doses of 8-OH-DPAT is shown in Figure 1. In agreement with prior studies [76], there was a dose dependent effect of 8-OH-DPAT on food intake ($F_{4, 60} = 3.34$, $p \leq .02$). However, relative to the saline control, a significant increase in food intake was present only in Sprague-Dawley rats and only at 0.15 mg/kg 8-OH-DPAT [Dunnett's ($q_{60, 5} = 3.46$, $p \leq .02$)]. Sprague-Dawley rats ate more than Fischer rats ($F_{1, 60} = 13.14$, $p \leq .0006$) and this was true for all experimental conditions ($q_{60, 5} = 1.91$, $p > .05$). Consequently, the interaction between strain and drug dose was not significant ($F_{4, 60} = 0.49$, $p > .05$).

Food intake after 8-OH-DPAT was evaluated as a ratio to the mean of food intake of the same-strain saline control in order to eliminate strain effects on food intake that were independent of the drug (Figure 2). Although drug effects continued to be significant ($F_{4, 60} = 3.55$, $p \leq .02$), the strain differences

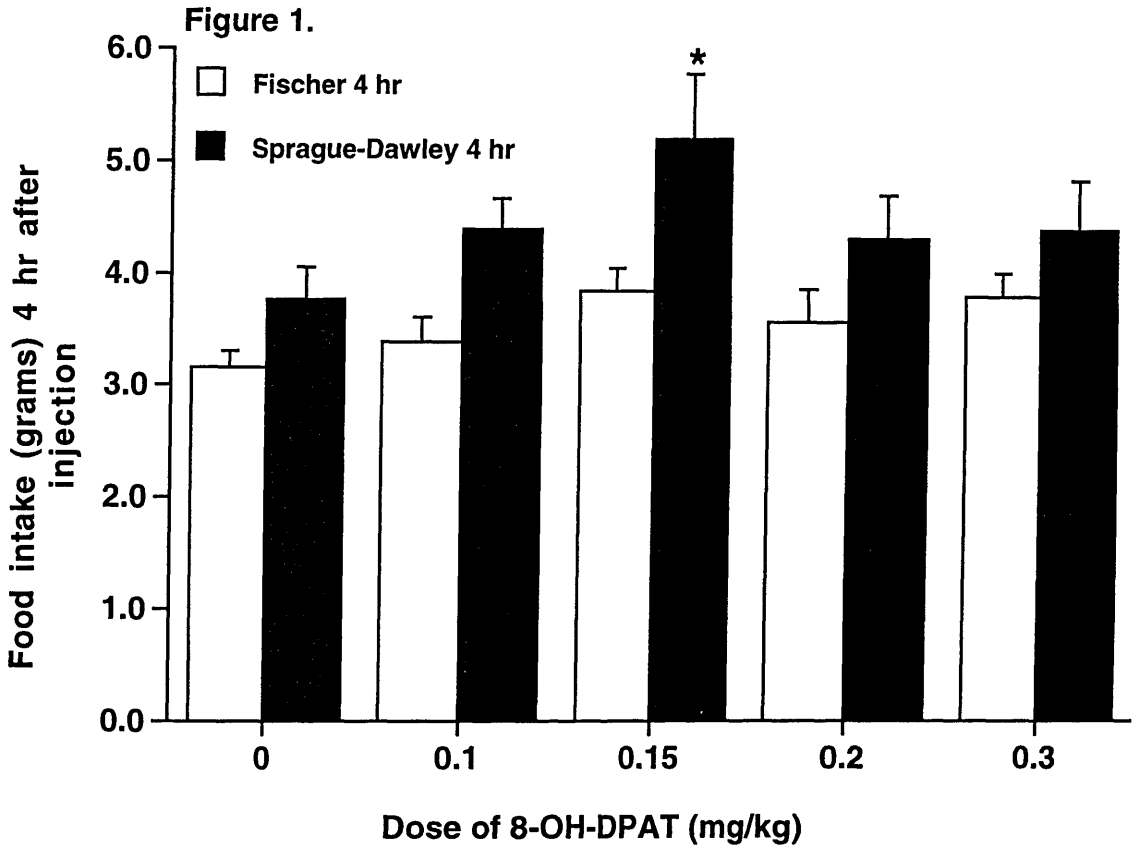


Figure 1: Four-hr food intake after treatment with saline or 8-OH-DPAT. The mean ± S.E. grams of food intake after treatment with saline (0) or 8-OH-DPAT (0.1, 0.15, 0.2, 0.3 mg/kg) for ovariectomized Fischer and Sprague-Dawley rats are shown. N's for 0, 0.1, 0.15, 0.2 and 0.3 mg/kg 8-OH-DPAT are 11, 6, 9, 3, 5 for Fischer rats and 11, 6, 9, 5, 5 for Sprague-Dawley rats. The asterisk indicates a significant difference from the saline (0) control of the same strain.

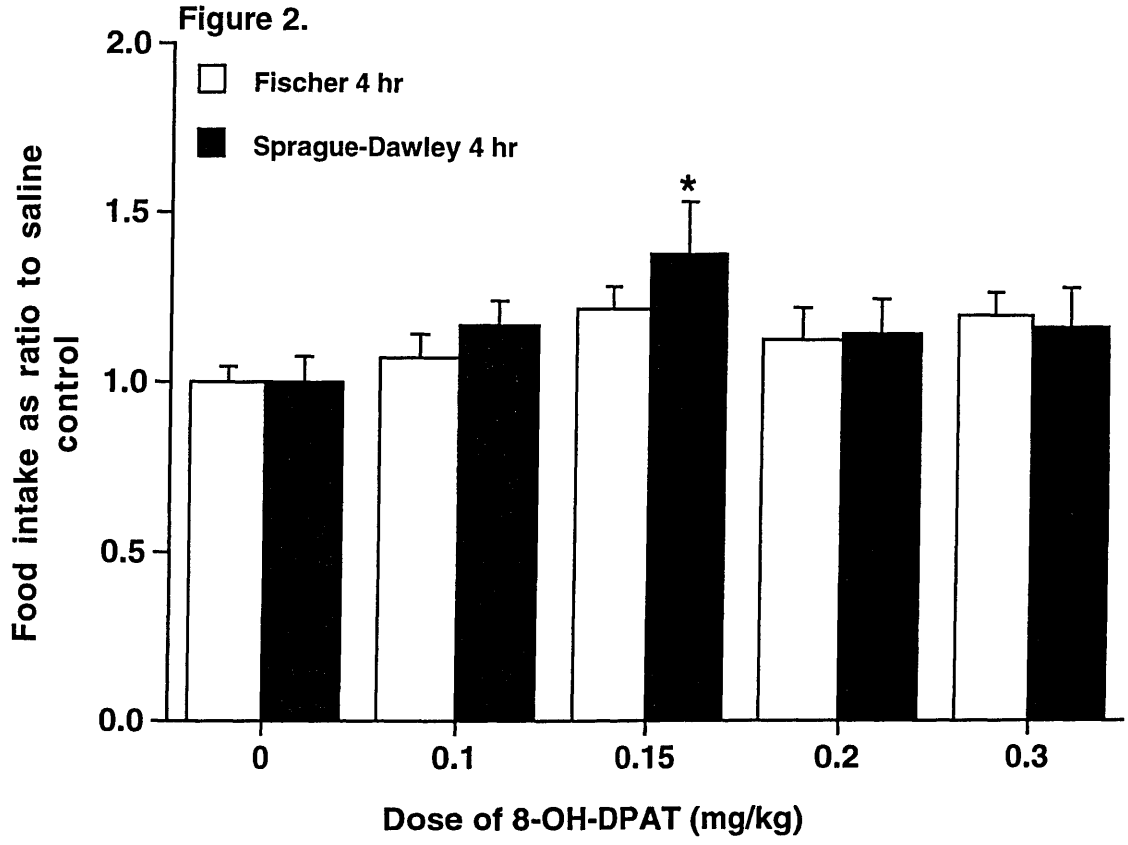


Figure 2: Four-hr food intake as a ratio to the saline control.
The mean ± S.E. ratio to the average food intake of the same-strain saline control are shown. Ovariectomized Fischer and Sprague-Dawley rats were injected with saline (0) or 8-OH-DPAT (0.1, 0.15, 0.2, 0.3 mg/kg). N's for 0, 0.1, 0.15, 0.2 and 0.3 mg/kg 8-OH-DPAT are 11, 6, 9, 3, 5 for Fischer rats and 11, 6, 9, 5, 5 for Sprague-Dawley rats. The asterisk indicates a significant difference from the same-strain saline control.

disappeared ($F_{1, 60} = 0.54$, $p > .05$) and there was no interaction between strain and dose of 8-OH-DPAT ($F_{4, 60} = 0.38$, $p > .05$). In Sprague-Dawley rats, the 0.1 and 0.15 mg/kg dose of 8-OH-DPAT increased food intake, but only at 0.15 mg/kg of 8-OH-DPAT was this difference significant (Dunnett's $q_{60, 5} = 4.71$, $p \leq .05$). After the higher doses, food intake tended to decline. This non-linear response to the increasing dose of 8-OH-DPAT was especially evident in Sprague-Dawley rats. To further evaluate this apparent strain difference in the effect of dose, food intake was examined with simple regression procedures. For Fischer females, there was a slight, but significant, correlation between dose of 8-OH-DPAT and food intake ($F_{1, 32} = 7.11$, $p \leq .01$, $r = .426$). For Sprague-Dawley females, what appeared to be a linear dose responsivity up to 0.15 mg/kg 8-OH-DPAT ($F_{1, 24} = 6.41$, $p \leq 0.02$, $r = .459$) disappeared when the two higher doses were included in the analysis ($F_{1, 34} = 1.95$, $p > .05$).

B: Effects of 8-OH-DPAT on food intake during the first and second two-hr interval after injection.

Since the effects of 8-OH-DPAT may have dissipated in less than four hr, food intake during the first and second two-hr intervals were examined (Figure 3). Breakdown of food intake into these two periods allowed examination of the early and late response to 8-OH-DPAT in the two strains. Data were compared with a two way repeated measure ANOVA with the time interval as the repeated factor. Over all groups, food intake was higher in the first relative to the second two-hr eating interval ($F_{1, 60} = 17.35$, $p \leq .0001$). Since this pattern was evident in both

Figure 3.

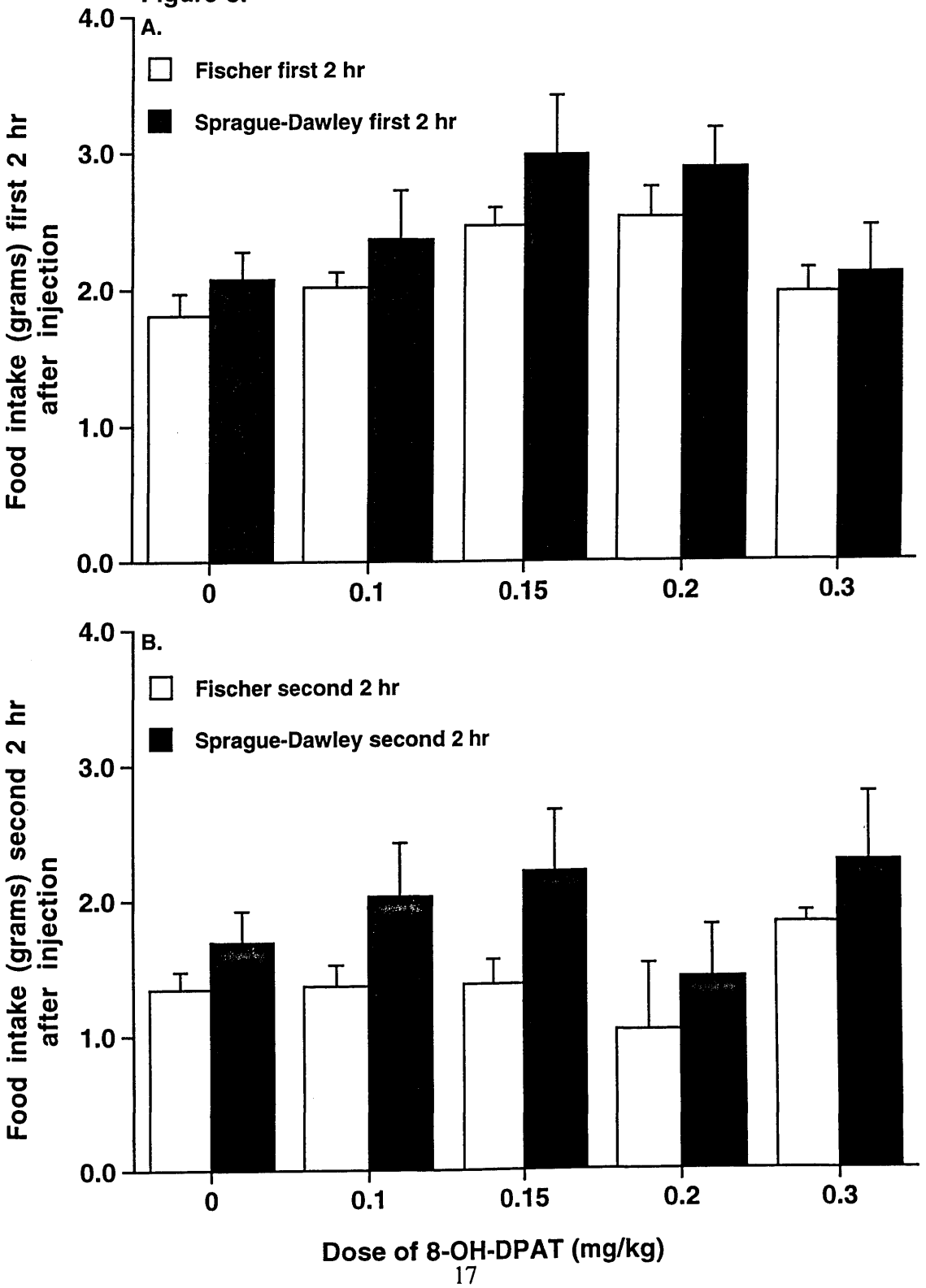

A.
Fischer first 2 hr
Sprague-Dawley first 2 hr
Food intake (grams) first 2 hr after injection
4.0
3.0
2.0
1.0
0.0
0
0.1
0.15
0.2
0.3
B.
Fischer second 2 hr
Sprague-Dawley second 2 hr
Food intake (grams) second 2 hr after injection
4.0
3.0
2.0
1.0
0.0
0
0.1
0.15
0.2
0.3
Dose of 8-OH-DPAT (mg/kg)

Figure 3: Effects of 8-OH-DPAT or saline on food intake in the first and second two-hr interval after treatment.

The mean ± S.E. grams of food intake after treatment with saline (0) or with 8-OH-DPAT in the first and second two hr after injection are shown. Data are from the same rats shown in Figure 1. N's for 0, 0.1, 0.15, 0.2 and 0.3 mg/kg 8-OH-DPAT are 11, 6, 9, 3, 5 for Fischer rats and 11, 6, 9, 5, 5 for Sprague-Dawley rats. Data for the first two hr are shown in Figure A; data for the second two hr interval are shown in Figure B.

the saline-treated rats and rats injected with 8-OH-DPAT, this probably reflects a normal eating pattern during the early portion of the light/dark cycle. There was a significant effect of strain ($F_{1, 60}$ =13.14, $p \leq .0006$) and the dose of 8-OH-DPAT ($F_{4, 60}$ = 3.35, $p \leq .02$), but none of the interaction terms with the time interval were significant (all $p > .05$). None of the posthoc comparisons were significant. When food intake was examined as a ratio to the same-strain and same-time saline control for the first two-hr relative to the second two-hr food intake (Figure 4), there was no significant main effect of strain ($F_{1,60}$ = 0.69, $p > .05$). The drug effect continued to be significant ($F_{4, 60}$ = 3.06, $p \leq .03$). Also there was no significant interaction between drug and strain ($F_{4, 60}$ = 0.42, $p > .05$). There was no significant difference in the effects of 8-OH-DPAT on food intake in the first relative to the second two hr after injection ($F_{1, 60}$ = 1.54, $p > .05$, all $q_{60, 5}$ = 2.22, $p > .05$).

EXPERIMENT 2: Strain differences in the effects of female gonadal hormones on food intake in saline treated and 8-OH-DPAT treated ovariectomized Fischer and Sprague-Dawley rats.

A: Strain differences in eating in oil-treated rats.

In the second experiment ovariectomized rats were hormonally primed with EB and P to determine 1) if hormonal priming altered the response to 8-OH-DPAT and 2) if Fischer and Sprague-Dawley females differed in the hormonal modulation of 8-OH-DPAT induced hyperphagia. Rats were hormonally primed with 0.033 or 0.17 µg/g of EB per gram body weight followed

Figure 4

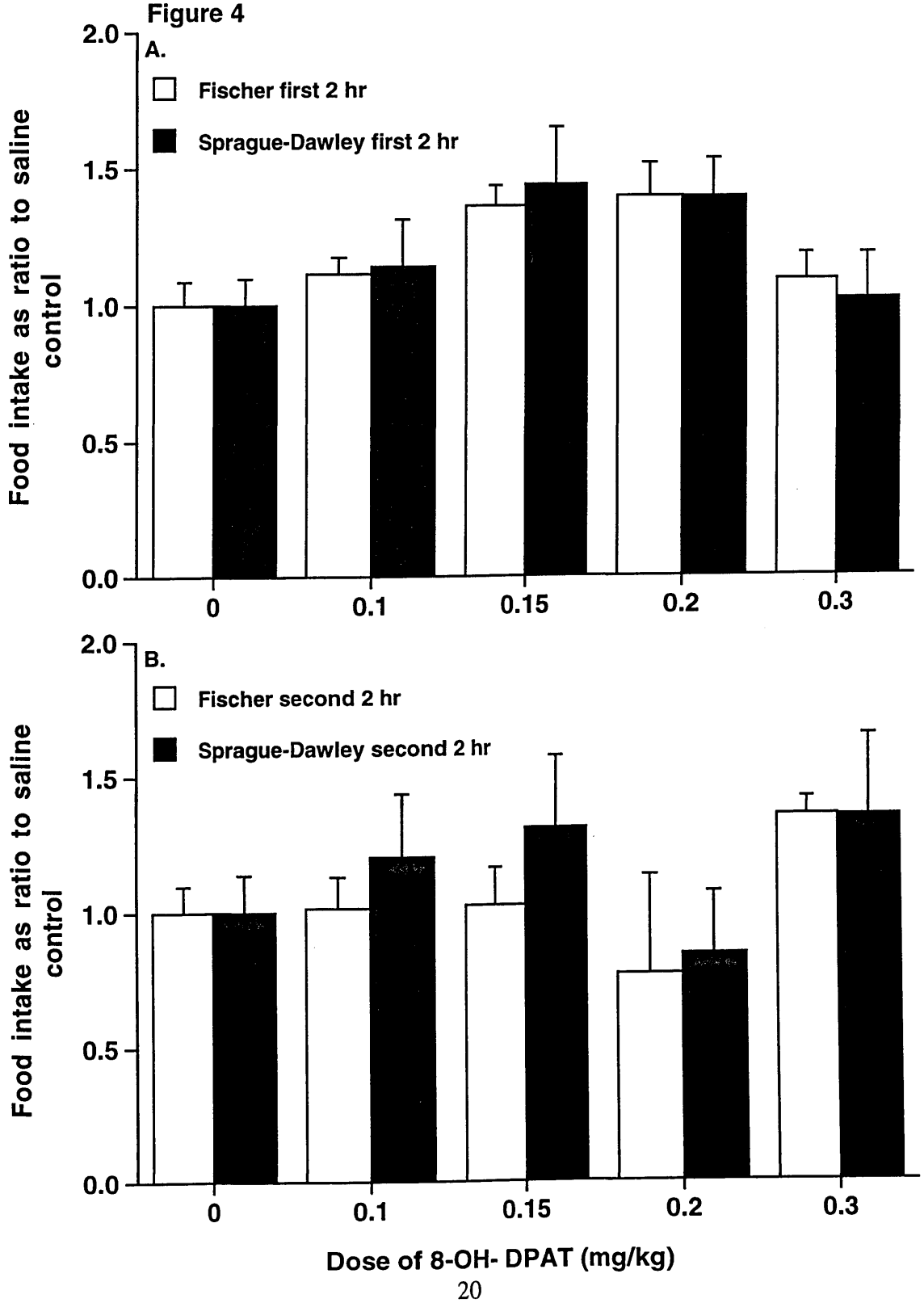

A.
Fischer first 2 hr
Sprague-Dawley first 2 hr
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
0
0.1
0.15
0.2
0.3
B.
Fischer second 2 hr
Sprague-Dawley second 2 hr
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
0
0.1
0.15
0.2
0.3
Dose of 8-OH- DPAT (mg/kg)

Figure 4: First and second two-hr food intake as a ratio to the saline control.

The mean ± S.E. grams of food intake as a ratio to the same-strain saline control are shown. Data are for the first and second two hr after injection and are for the same rats in Figure 3. N's for 0, 0.1, 0.15, 0.2 and 0.3 mg/kg 8-OH-DPAT are 11, 6, 9, 3, 5 for Fischer rats and 11, 6, 9, 5, 5 for Sprague-Dawley rats. Data for the first two hr are shown in Figure A; data for the second two-hr interval are shown in Figure B.

forty-eight hr later with 500 µg P. The oil-treated rats are shown in Figure 5 and hormonally primed rats are shown in Figure 6. A single dose of 8-OH-DPAT (0.15 mg/kg) was used. There was a significant effect of hormone treatment ($F_{1, 105} = 81.02$, $p \leq .0001$) as well as a significant interaction between hormone treatment and strain ($F_{2, 105} = 18.38$, $p \leq .0001$) but the main effect of strain was not significant ($F_{1, 105} = 0.51$, $p > .05$). Overall, there was a significant effect of eating interval on food intake ($F_{1, 105} = 69.71$, $p \leq .0001$) with both strains eating more during the first than the second two hr of the study. There was also a significant interaction between time and strain ($F_{1, 105} = 3.80$, $p \leq .05$), time and drug ($F_{1, 105} = 12.33$, $p \leq .0007$), time and hormonal priming ($F_{2, 105} = 4.63$, $p \leq .000l$) as well as a significant 3 way-interaction among time, strain and hormonal priming ($F_{2, 105} = 4.37$, $p \leq .02$).

Food intake for oil-treated rats injected either with saline or with 8-OH-DPAT is shown in Figure 5. Consistent with the findings of the first experiment, 8-OH-DPAT increased food intake ($F_{1,105} = 10.59$, $p \leq .002$). Also Sprague-Dawley rats ate more than did Fischer rats, but this was true only for the first two-hr food intake interval ($q_{105, 4} = 6.88$, $p \leq .05$). Although rats ate more during the first than the second two-hr interval, this difference was significant only after 8-OH-DPAT treatment (for Fischer and Sprague-Dawley, respectively $q_{105, 4} = 4.17$ and 8.24, $p \leq .05$).

B: Effects of hormones in saline-treated rats.

The effects of hormone treatment in saline-treated rats are shown in

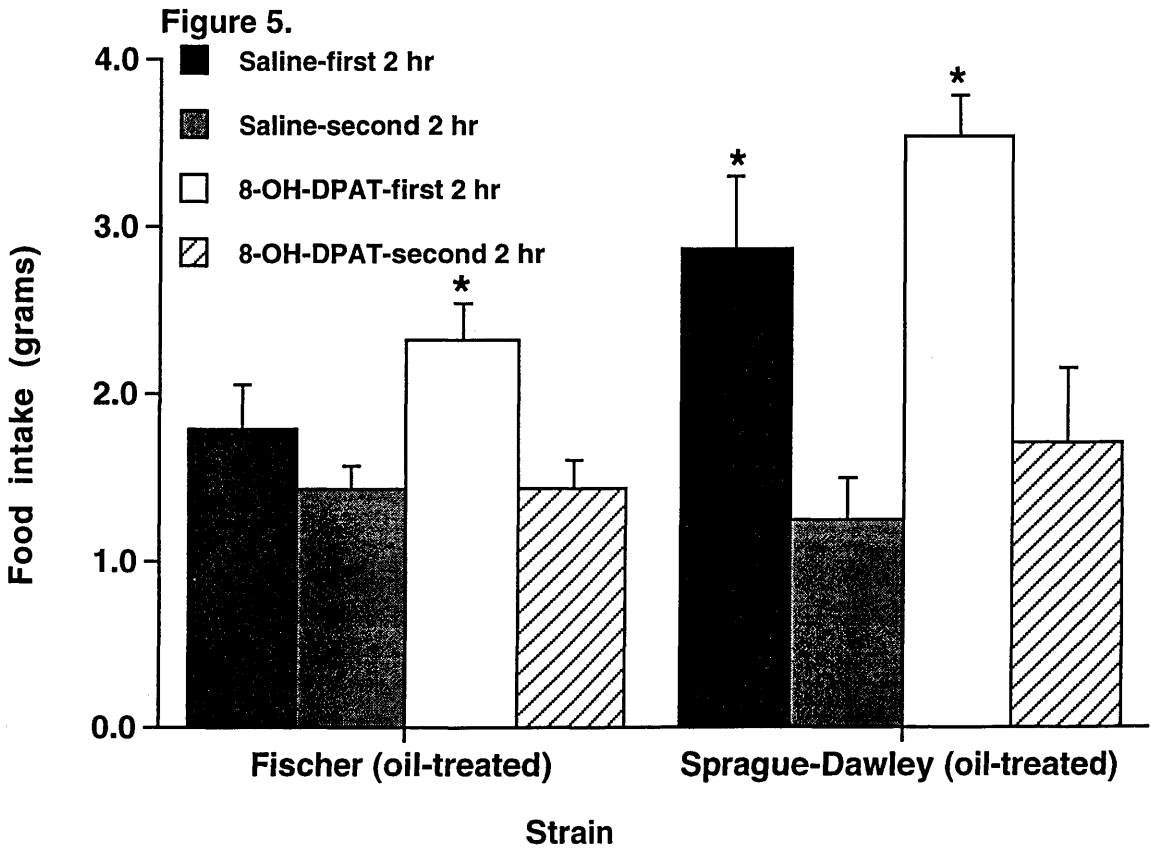


Figure 5: Food intake in oil-treated ovariectomized rats.
The mean ± S.E. grams of food intake in response to 0.15 mg/kg of 8-OH-DPAT (s.c.) or saline are shown for ovariectomized Fischer and Sprague-Dawley rats injected with oil as described in methods. Data are for the first and second two hr after injection. N's for saline or 8-OH-DPAT are 9 and 11 for Fischer and 8 and 10 for Sprague-Dawley rats. A single asterisk indicates significant differences, within groups, between the first and second two-hr eating interval.

Figure 6.

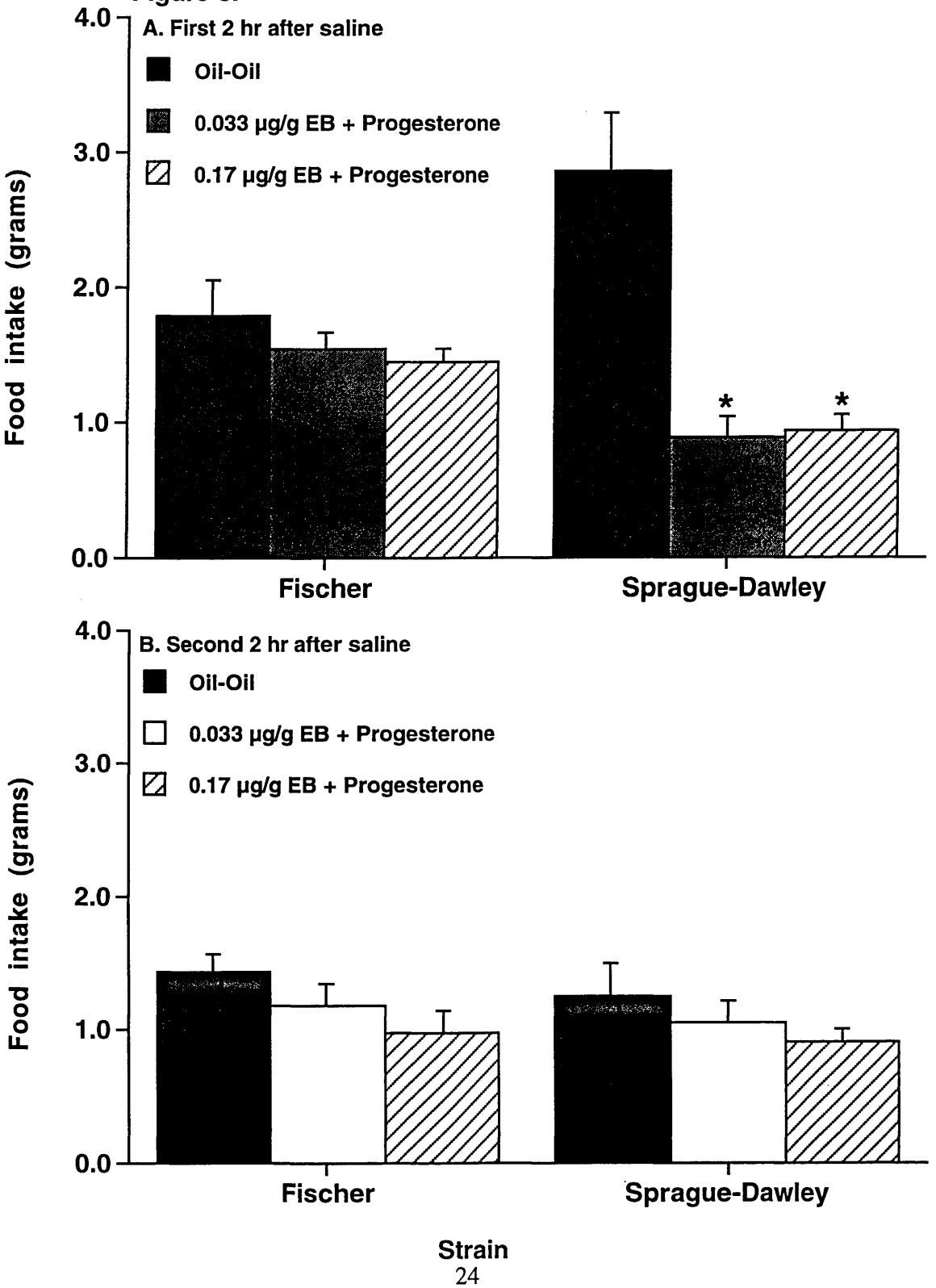

A. First 2 hr after saline
Oil-Oil
0.033 µg/g EB + Progesterone
0.17 µg/g EB + Progesterone
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
*
*
Fischer
Sprague-Dawley
B. Second 2 hr after saline
Oil-Oil
0.033 µg/g EB + Progesterone
0.17 µg/g EB + Progesterone
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
Fischer
Sprague-Dawley
Strain

Figure 6.

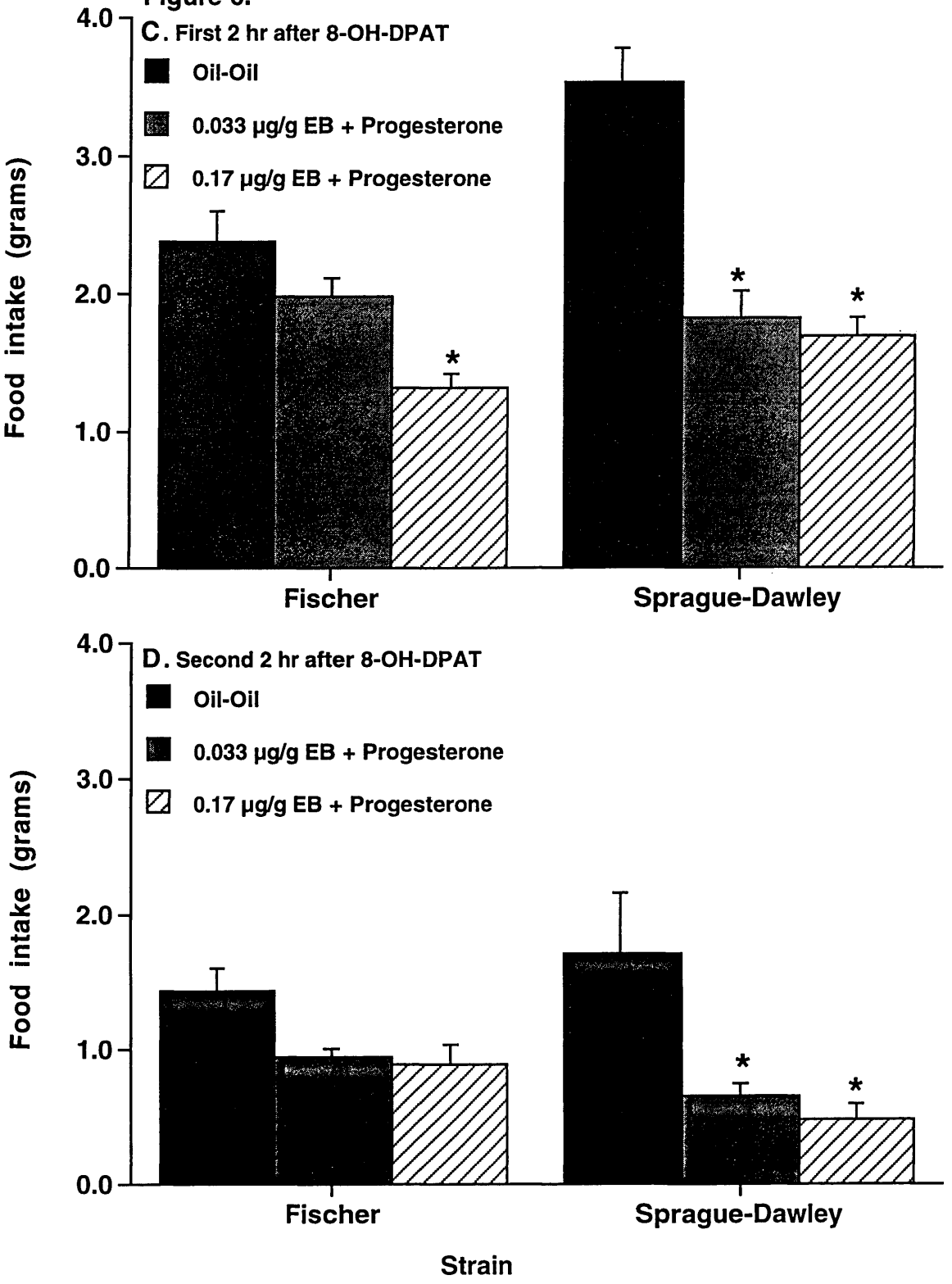

C. First 2 hr after 8-OH-DPAT
Oil-Oil
0.033 µg/g EB + Progesterone
0.17 µg/g EB + Progesterone
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
*
*
*
Fischer
Sprague-Dawley
D. Second 2 hr after 8-OH-DPAT
Oil-Oil
0.033 µg/g EB + Progesterone
0.17 µg/g EB + Progesterone
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
*
*
Fischer
Sprague-Dawley
Strain

Figure 6: Effects of hormonal priming on food intake.
Data are the mean ± S.E. grams of food intake for Fischer and Sprague-Dawley rats primed with oil or with 0.033 or 0.17 μg/g EB followed forty-eight hr later with 500 μg P. Data are for the first and second two-hr intervals after injection in rats treated with saline (6 A, B) or with 8-OH-DPAT (6 C, D). N's for saline treated rats for oil, 0.033 and 0.17 μg/g EB plus 500 μg P are 9, 9, 8 for Fischer and 8, 10, 10 for Sprague-Dawley rats, respectively. For 8-OH-DPAT treated rats, N's for oil, 0.033 and 0.17 μg/g EB plus 500 μg P are 11, 11, 10 for Fischer and 10, 11, 10 for Sprague-Dawley rats, respectively. Oil-saline control groups are the same data shown in figure 5. Asterisks indicate significant differences from the oil-oil control of the same strain and same treatment within the same time interval.

Figure 6 A and B. Hormonal priming with either dose of EB plus P significantly reduced food intake of Sprague-Dawley females during the first two hr of the study (for 0.033 and 0.17 µg EB, respectively, $q_{105, 3} = 8.37$ and 8.16, $p \leq .05$) (Figure 6 A). There was no effect of hormone priming during the second two hr of the experiment (Figure 6 B) and never did Fischer females show a significant decrease after hormone priming (all $q > .05$). This strain difference was reflected in the significant interactions between strain and hormone priming and between time interval, strain, and hormone priming.

C: Effects of hormones in 8-OH-DPAT-treated rats.

The effects of hormone treatment in rats injected with 8-OH-DPAT are shown in Figure 6 C and D. Hormone-induced reductions in food intake were evident in both strains following treatment with 8-OH-DPAT. However, this difference was accentuated in Sprague-Dawley relative to Fischer females. For Sprague-Dawley females, a reduction in food intake of 8-OH-DPAT-treated rats was evident for both eating intervals and following both doses of EB (for the first two-hr and second two-hr interval, respectively, for 0.033 and 0.17 µg EB, respectively, $q_{105, 3} = 7.90$ and 8.29 and 4.86 and 5.90, all $p \leq .05$). In contrast, a significant hormonal suppression of eating after 8-OH-DPAT treatment was evident in Fischer females only at the highest dose of EB priming and only during the first two-hr eating interval ($q_{105, 3} = 4.60$, $p \leq .05$).

D: Strain differences.

In order to more fully evaluate the strain differences in both the response

to 8-OH-DPAT and the hormonal priming, data for the first two-hr interval after eating were converted to a ratio of food intake for the same-strain, oil-oil, saline-treated condition. By normalizing food intake within the two strains, it was possible to compare treatment effects between strains without the confounding variable of strain differences in food intake. These ratios are shown in Figure 7. Main effects of strain, drug, and hormonal priming were significant (respectively, $F_{1,105}$, $F_{1,105}$, $F_{2,105}$ = 31.59, 18.28, and 37.44, all $p \leq .0001$). The only significant interaction was that between strain and dose of estrogen ($F_{2,105} = 7.68$, $p \leq .0008$). In saline-treated rats (Figure 7 A), both doses of EB priming had a significantly greater suppressing effect on food intake in Sprague-Dawley than in Fischer females (for 0.033 and 0.17 µg EB, respectively, $q_{105,6} = 6.13$ and 5.36, $p \leq .05$). However, in 8-OH-DPAT-treated rats (Figure 7 B), the lower dose of EB hormonal priming significantly differentiated the strains ($q_{105,6} = 5.22$, $p \leq .05$).

EXPERIMENT 3: Strain differences in the effect of EB or 500 µg of P on food intake in 8-OH-DPAT treated or saline treated ovariectomized Fischer and Sprague-Dawley rats.

A: Strain differences in eating in oil-treated rats.

The third experiment was designed to evaluate the potential contribution of P to the hormonal effects identified in the prior study. Ovariectomized rats were primed with EB + P, EB + Oil, or Oil + P and treated with saline or 8-OH-DPAT as in previous studies. A single dose of EB (0.033 µg/g) and a

Figure 7.

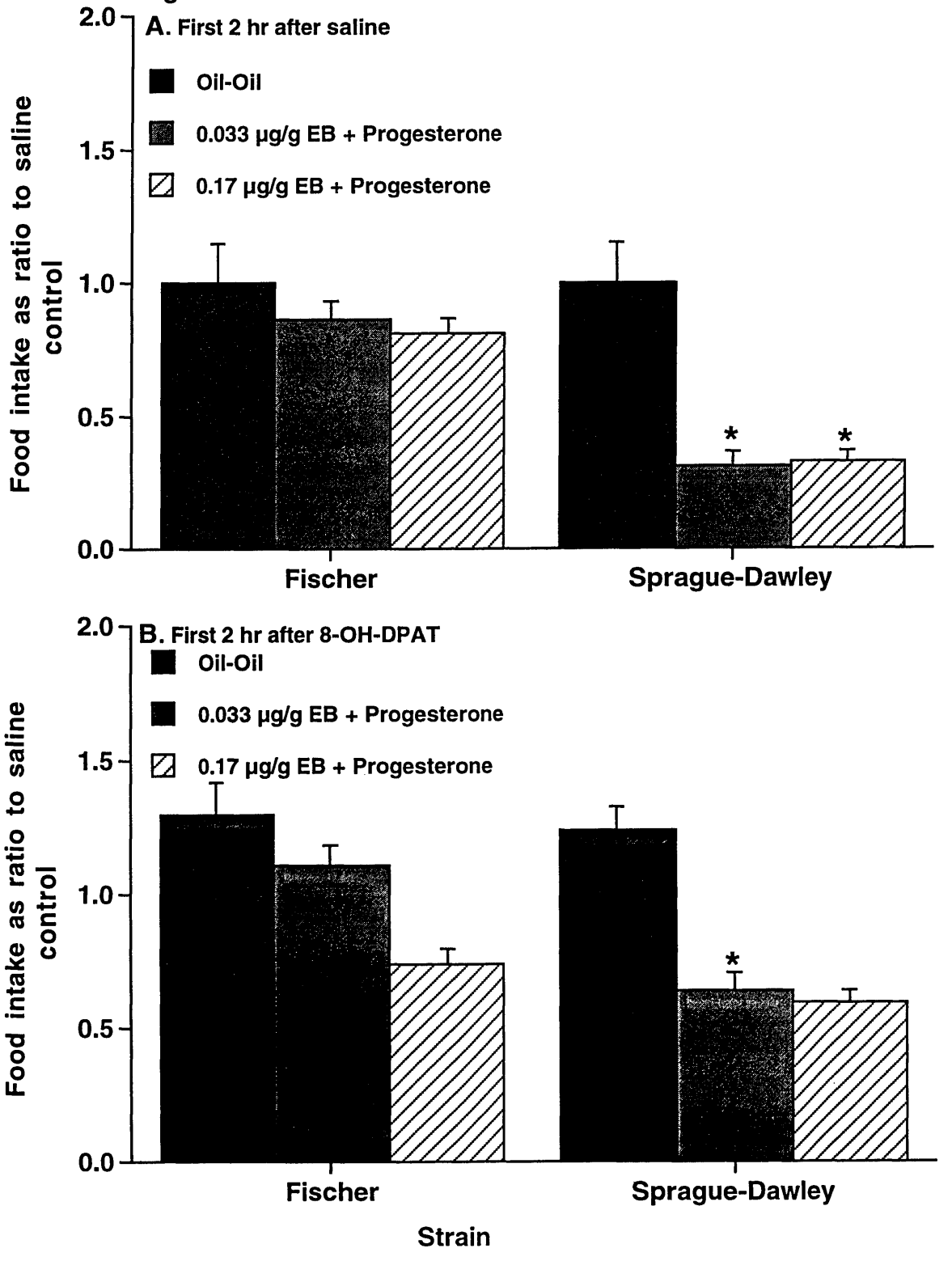

A. First 2 hr after saline
Oil-Oil
0.033 µg/g EB + Progesterone
0.17 µg/g EB + Progesterone
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
*
*
Fischer
Sprague-Dawley
B. First 2 hr after 8-OH-DPAT
Oil-Oil
0.033 µg/g EB + Progesterone
0.17 µg/g EB + Progesterone
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
*
Fischer
Sprague-Dawley
Strain

Figure 7: Food intake as a ratio to that of oil-treated, saline injected control.

Data are the mean ± S.E. ratio of food intake of each treatment group relative to the same strain, oil-treated, saline injected control. Data are for the first two hr after injection with saline (7 A) or with 8-OH-DPAT (7 B) and are from the same rats shown in Figure 6. Asterisks indicate significant strain differences within the same treatment condition.

single dose of 8-OH-DPAT (0.15 mg/kg) and of P (500 µg) were examined.

Consistent with the previous study, there were significant effects of strain ($F_{1, 106} = 45.29$, $p \leq .0001$), drug ($F_{1, 106} = 8.80$, $p \leq .004$) and hormonal priming ($F_{3, 106} = 38.57$, $p \leq .0001$) (Figures 8 and 9). The interaction between hormone and strain ($F_{3, 106} = 12.89$, $p \leq .0001$) and hormone and drug ($F_{3, 106} = 3.01$, $p \leq .0335$) were also significant. Similarly, there was a significant effect of eating interval on food intake ($F_{1, 106} = 59.82$, $p \leq .0001$) with both strains eating more during the first than the second two-hr interval.

Data for oil-treated females treated with saline or 8-OH-DPAT are shown in Figure 8. Consistent with the previous experiments, 8-OH-DPAT increased food intake ($F_{1, 106} = 8.80$, $p \leq .004$) but this increase was significant only in Sprague-Dawley females. After treatment with saline, there was no significant difference between the first and second two-hr intervals, but after treatment with 8-OH-DPAT, eating was greater during the first relative to the second two-hr interval and was significant for both Sprague-Dawley rats and Fischer rats (respectively for Fischer and Sprague-Dawley rats, $q_{106, 4} = 4.78$ and 3.80, $p \leq .05$).

<u>B: Effects of hormones on saline treated and 8-OH-DPAT treated rats.</u>

The effect of hormone priming with EB, P or both on the response to 8-OH-DPAT are shown in Figure 9. The overall significant effect of hormone was primarily due to the suppressive effect of 0.033 µg/g EB + P and of 0.033 µg/g EB on food intake in Sprague-Dawley females. In saline treated Sprague-

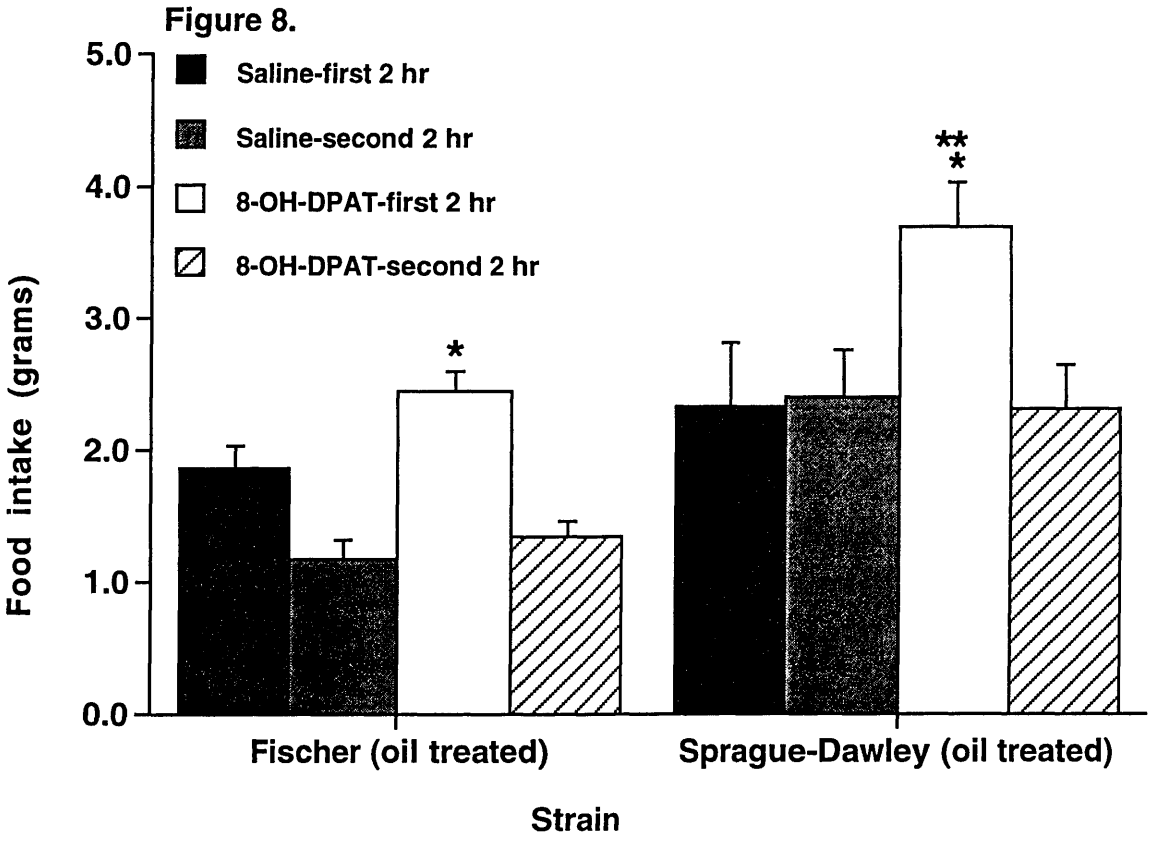


Figure 8: Food intake in oil-treated ovariectomized rats.
The mean ± S.E. grams of food intake in response to 8-OH-DPAT or saline are shown for ovariectomized Fischer and Sprague-Dawley rats injected with oil as described in the methods. Data are for the first and second two hr after injection. N's for saline or 8-OH-DPAT for Fischer and Sprague-Dawley rats are 8, 7 and 7, 7 respectively. Single asterisks indicate a significant difference, within strain and treatment, between the first and second eating interval. A double asterisk indicates a significant increase in food intake after 8-OH-DPAT treatment when compared to the saline control of the same strain and eating interval.

Figure 9.

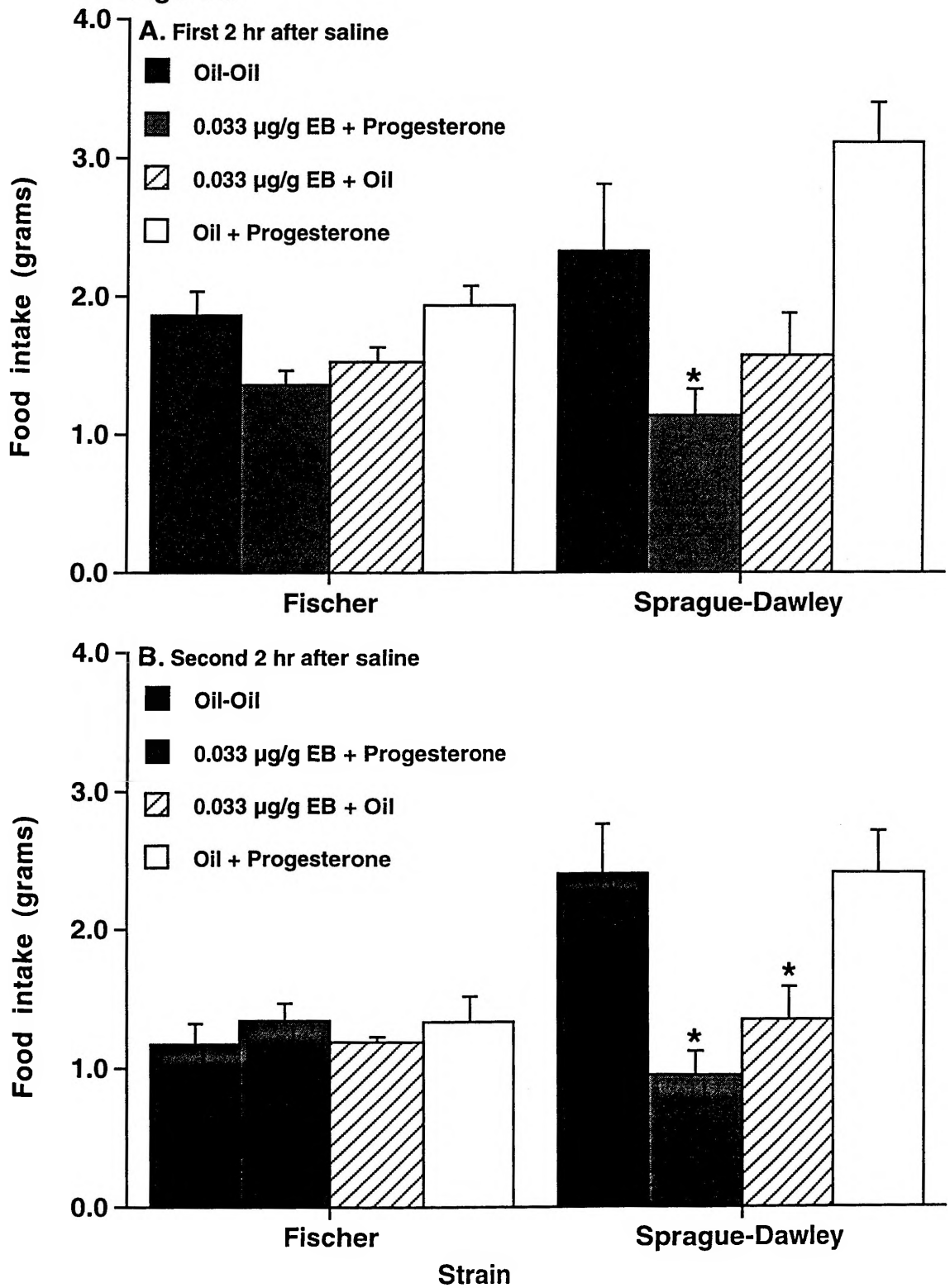
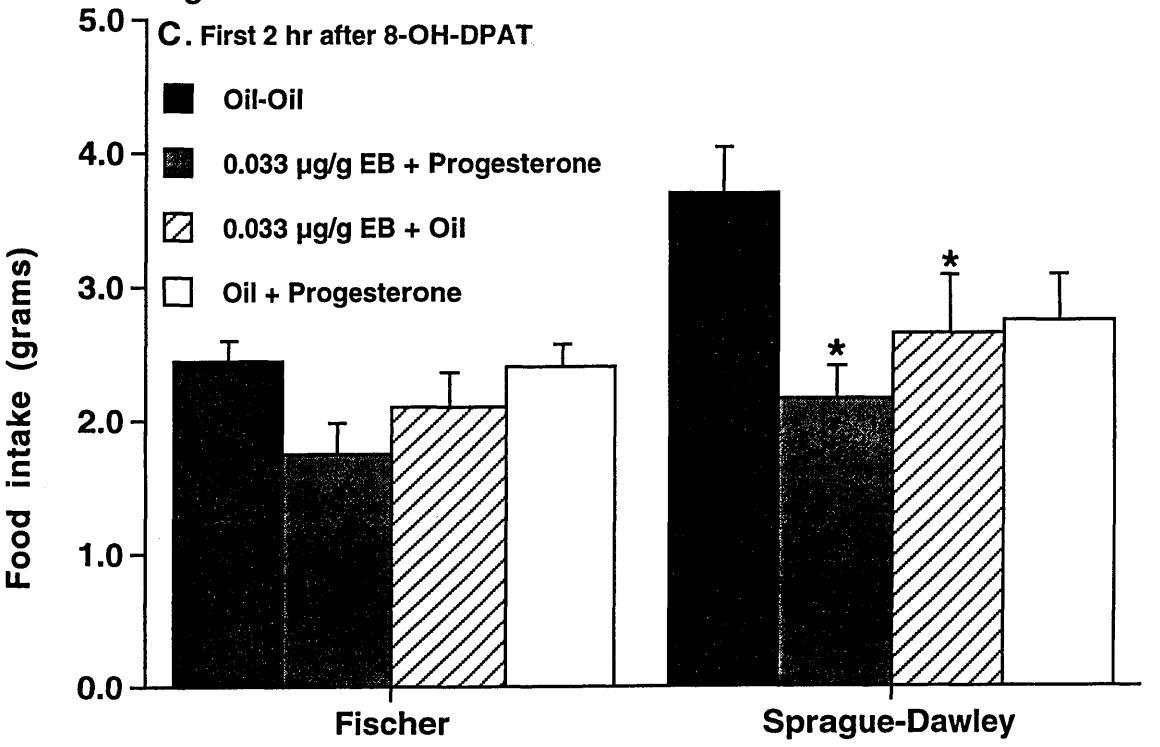

A. First 2 hr after saline
Oil-Oil
0.033 µg/g EB + Progesterone
0.033 µg/g EB + Oil
Oil + Progesterone
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
*
Fischer
Sprague-Dawley
B. Second 2 hr after saline
Oil-Oil
0.033 µg/g EB + Progesterone
0.033 µg/g EB + Oil
Oil + Progesterone
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
*
*
Fischer
Sprague-Dawley
Strain

Figure 9.


C. First 2 hr after 8-OH-DPAT
Oil-Oil
0.033 µg/g EB + Progesterone
0.033 µg/g EB + Oil
Oil + Progesterone
Food intake (grams)
5.0
4.0
3.0
2.0
1.0
0.0
*
*
Fischer
Sprague-Dawley

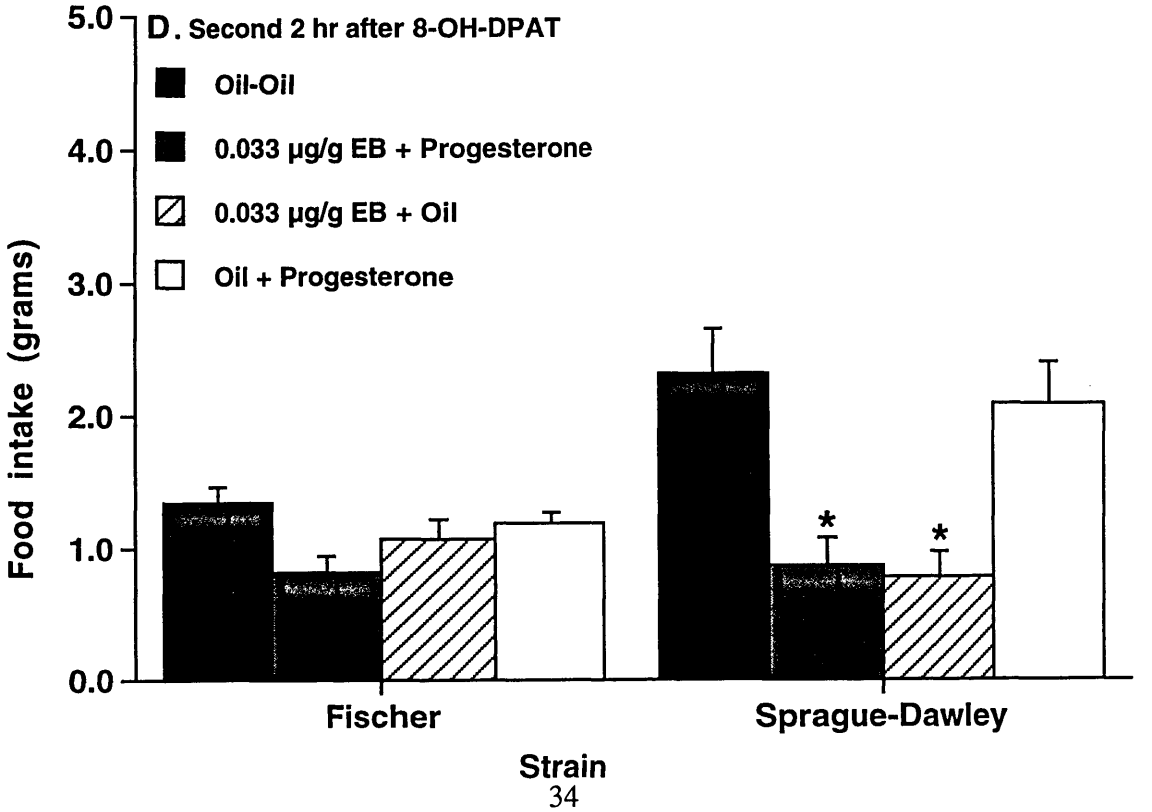

D. Second 2 hr after 8-OH-DPAT
Oil-Oil
0.033 µg/g EB + Progesterone
0.033 µg/g EB + Oil
Oil + Progesterone
Food intake (grams)
5.0
4.0
3.0
2.0
1.0
0.0
*
*
Fischer
Sprague-Dawley
Strain

Figure 9: Effect of EB /P, EB or P on food intake.

The mean ± S.E. grams of food intake in saline and 8-OH-DPAT treated ovariectomized Fischer and Sprague-Dawley rats are shown. Data are for the first and second two hr after injection with saline (9 A, B) or with 0.15 mg/kg of 8-OH-DPAT (9 C, D) (s.c.). Rats were hormonally primed with 0.033 µg/g EB and 500 µg P, with 0.033 µg/g EB and oil, or with oil and 500 µg P. N's for oil/oil, 0.033 µg/g EB and 500 µg P, 0.033 µg/g EB and oil, oil and 500 µg P are 8, 7, 8 and 9 for Fischer rats and 7, 8, 8 and 8 for Sprague-Dawley rats for saline-treated conditions. N's are 7, 6, 7 and 8 for Fischer rats and 7, 8, 8 and 8 for Sprague-Dawley rats, respectively, for 8-OH-DPAT treatment. Asterisks indicate significant differences, within strains and injection condition, from the oil-oil control within the same eating interval.

Dawley females (Figure 9 A and B), 0.033 μg/g EB + P significantly decreased food intake during the first and second two-hr eating intervals ($q_{106, 4}$ = 4.25 and 5.17 respectively, $p \leq .05$). EB, alone, significantly decreased food intake of Sprague-Dawley rats during the second (but not the first) two-hr eating interval ($q_{106, 4}$ = 4.88, $p \leq .05$). In 8-OH-DPAT treated Sprague-Dawley rats (Figure 9 C and D), both EB + P and EB + Oil decreased food intake during the first and second two-hr eating intervals [($q_{106, 4}$ = 5.486 and 5.17 for first and second two hr, respectively, $p \leq .05$ for EB + P) and ($q_{106, 4}$ = 3.74 and 5.49 for first and second two hr respectively, $p \leq .05$ for EB alone)]. Although EB + P decreased the effect of 8-OH-DPAT in Fischer females, none of the posthoc comparisons to the oil control were significant.

C: Strain differences.

Data for the first and second two-hr interval after eating were converted to a ratio of food intake for the same strain, oil-oil, saline-treated condition as described earlier. These ratios are shown in Figure 10. There were significant effects of strain ($F_{1, 106}$ = 19.28, $p \leq .0001$), drug ($F_{1, 106}$ = 6.41, $p \leq .02$) and hormone ($F_{3, 106}$ = 34.75, $p \leq .0001$). The interaction between strain and hormone ($F_{3, 106}$ = 6.93, $p \leq .0003$) and between hormone and drug ($F_{3, 106}$ = 3.26, $p \leq .03$) were also significant. None of the pair-wise posthoc comparisons between strains were significant during the first two hr of treatment. On the other hand, during the second two hr of food intake, EB + P and EB + Oil suppressed food intake of saline treated and 8-OH-DPAT treated Sprague-Dawley rats

Figure 10.

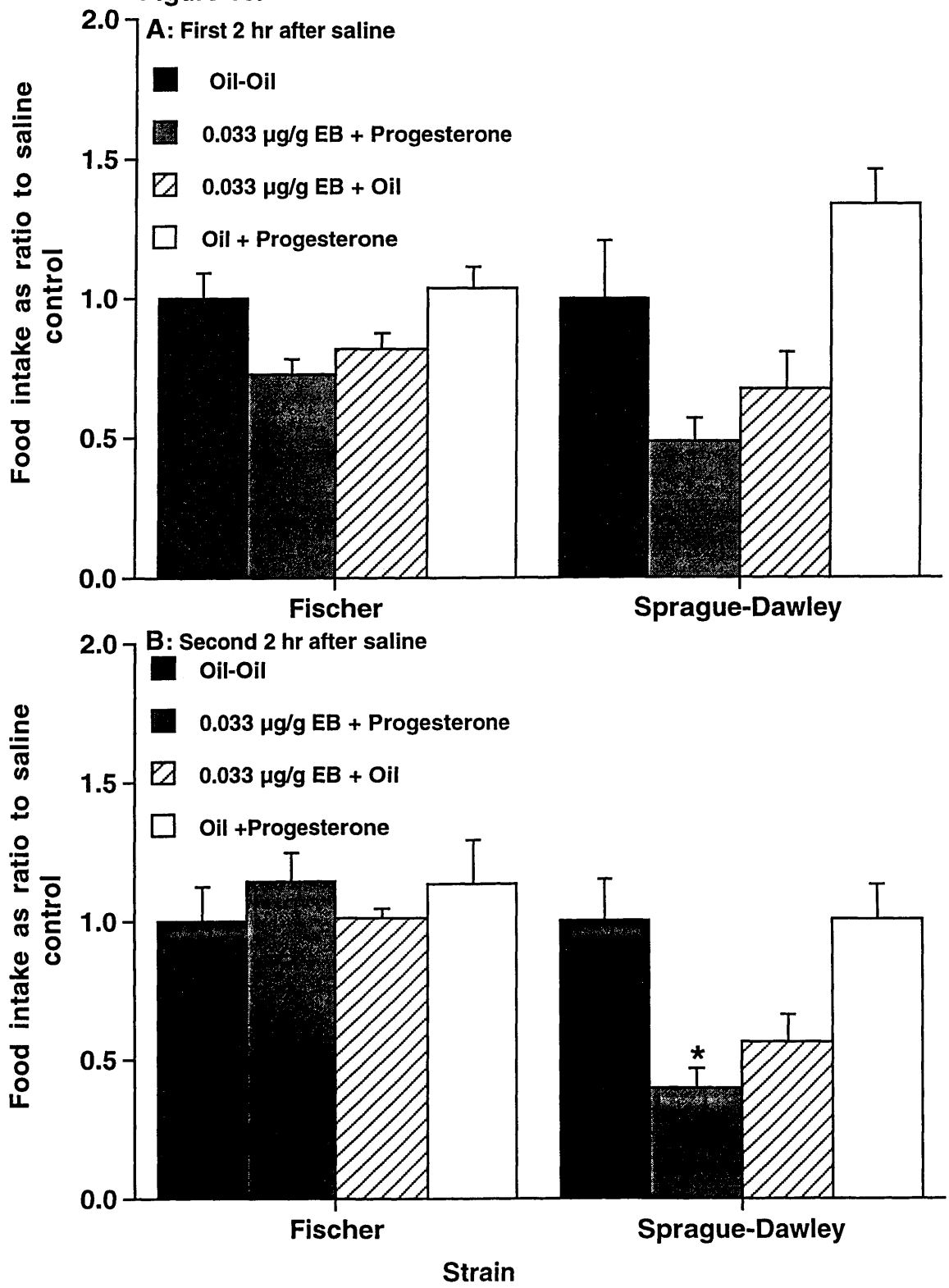

A: First 2 hr after saline
Oil-Oil
0.033 µg/g EB + Progesterone
0.033 µg/g EB + Oil
Oil + Progesterone
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
Fischer
Sprague-Dawley
B: Second 2 hr after saline
Oil-Oil
0.033 µg/g EB + Progesterone
0.033 µg/g EB + Oil
Oil +Progesterone
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
*
Fischer
Sprague-Dawley
Strain

Figure 10.

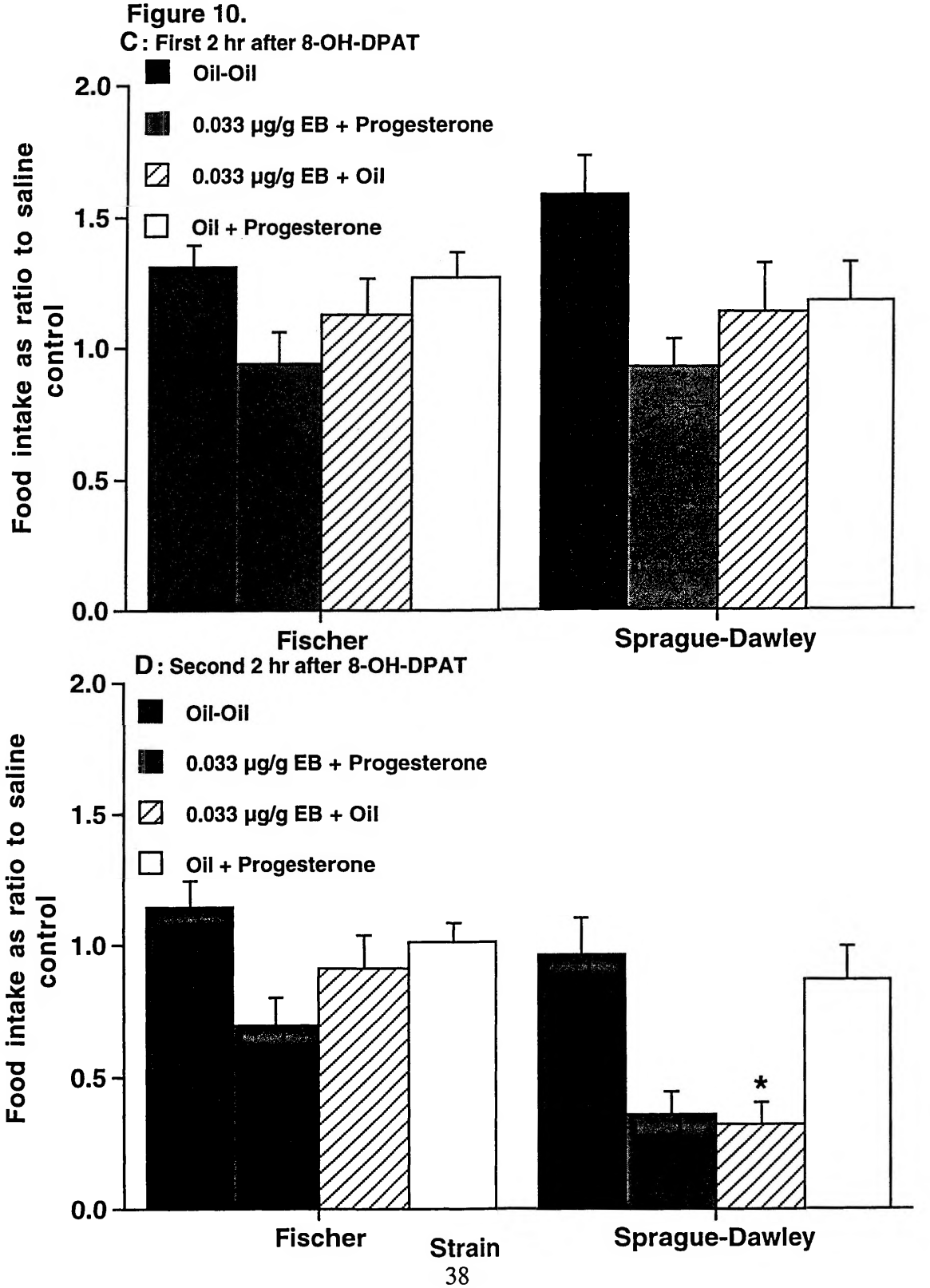

C: First 2 hr after 8-OH-DPAT
Oil-Oil
0.033 µg/g EB + Progesterone
0.033 µg/g EB + Oil
Oil + Progesterone
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
Fischer
Sprague-Dawley
D: Second 2 hr after 8-OH-DPAT
Oil-Oil
0.033 µg/g EB + Progesterone
0.033 µg/g EB + Oil
Oil + Progesterone
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
*
Fischer
Strain
Sprague-Dawley

Figure 10: Strain differences in the food intake of saline and 8-OH-DPAT treated Fischer and Sprague-Dawley rats.

The mean ± S.E. ratio of food intake of each treatment group relative to the same strain, oil-treated, saline injected control are shown. Data are for the first and second two hr after injection with the saline (10 A, B) and with 8-OH-DPAT (10 C, D) and are from the same rats shown in Figure 9. Asterisks indicate significant strain differences within the same treatment condition.

[(respectively, $q_{106, 8} = 5.55$, $p \leq .05$) and ($q_{106, 8} = 4.38$, $p \leq .05$)] but not Fischer rats.

EXPERIMENT 4: Strain differences in the dose dependent effect of EB on food intake in 8-OH-DPAT treated and saline treated ovariectomized Fischer and Sprague-Dawley rats.

A: Strain differences in food intake of oil-treated rats.

The final experiment was designed to extend the doses of EB examined. Rats were injected with oil or with 0.0067, 0.0222 or 0.17 µg/g of EB prior to treatment with saline or 8-OH-DPAT. Overall, there were significant main effects of dose of EB ($F_{3, 124} = 24.39$, $p \leq .0001$) and drug ($F_{1, 124} = 25.39$, $p \leq .0001$). Consistent with earlier studies, food intake was higher in the first than in the second two-hr interval ($F_{1, 124} = 39.41$, $p \leq .0001$) and the time by drug interaction was significant ($F_{1, 124} = 23.54$, $p \leq .0001$). Food intake in oil-treated rats is shown in Figure 11. In agreement with prior studies, 8-OH-DPAT increased food intake and the drug induced hyperphagia was greater in the first than in the second two hr of the study. Time dependent effects were significant only in Sprague-Dawley rats and only after 8-OH-DPAT treatment ($q_{124, 4} = 5.34$, $p \leq .05$). Similarly, posthoc comparisons between saline and 8-OH-DPAT were only significant in Sprague-Dawley females in the first two-hr eating interval ($q_{124, 4} = 4.49$, $p \leq .05$). These accounted for the significant interactions between drug and strain ($F_{1, 124} = 6.15$, $p \leq .02$) and between drug and eating interval ($F_{1, 124} = 23.54$, $p \leq .0001$).

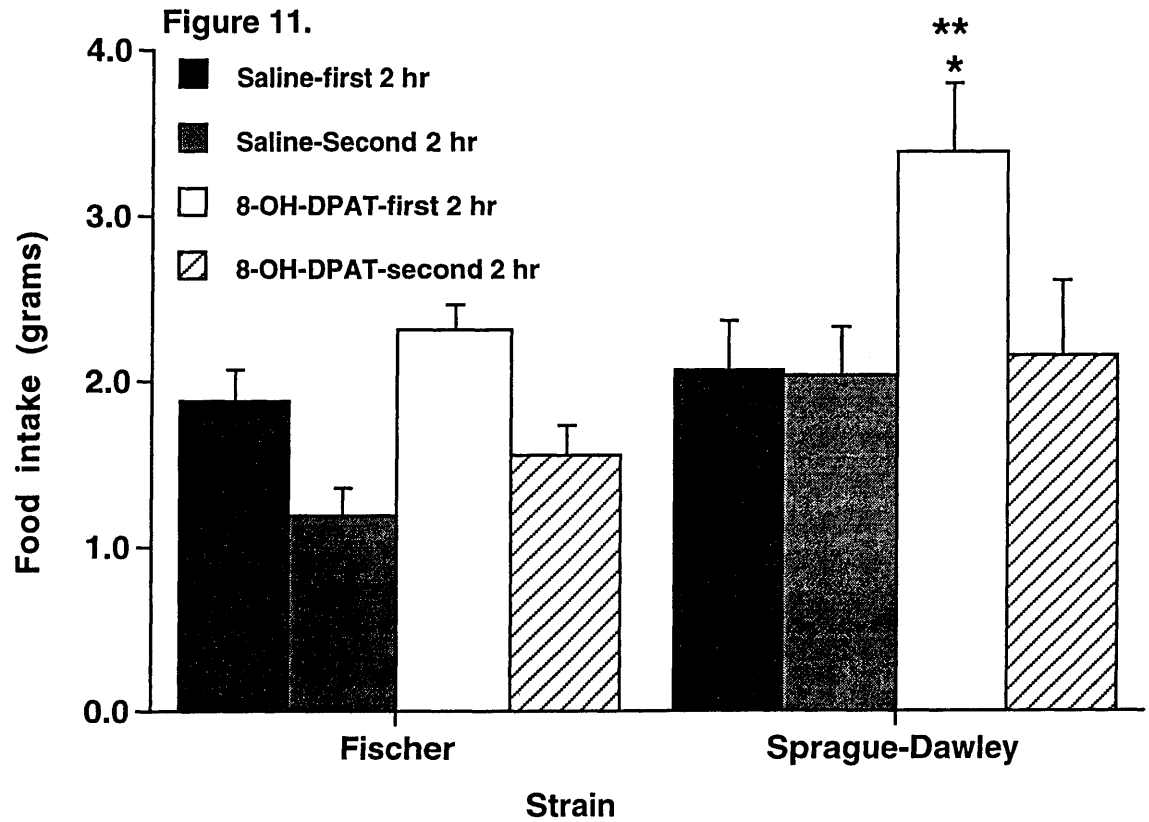


Figure 11: Food intake in oil-treated ovariectomized rats.
The mean ± S.E. grams of food intake in response to 0.15 mg/kg of 8-OH-DPAT or saline are shown for ovariectomized Fischer and Sprague-Dawley rats injected with oil as described in the methods. Data are for the first and second two hr after injection. Nine animals are represented in each group. The single asterisk indicates a significant difference, within strain, between the first and second two-hr eating interval. The double asterisk indicates a significant increase in food intake after 8-OH-DPAT treatment.

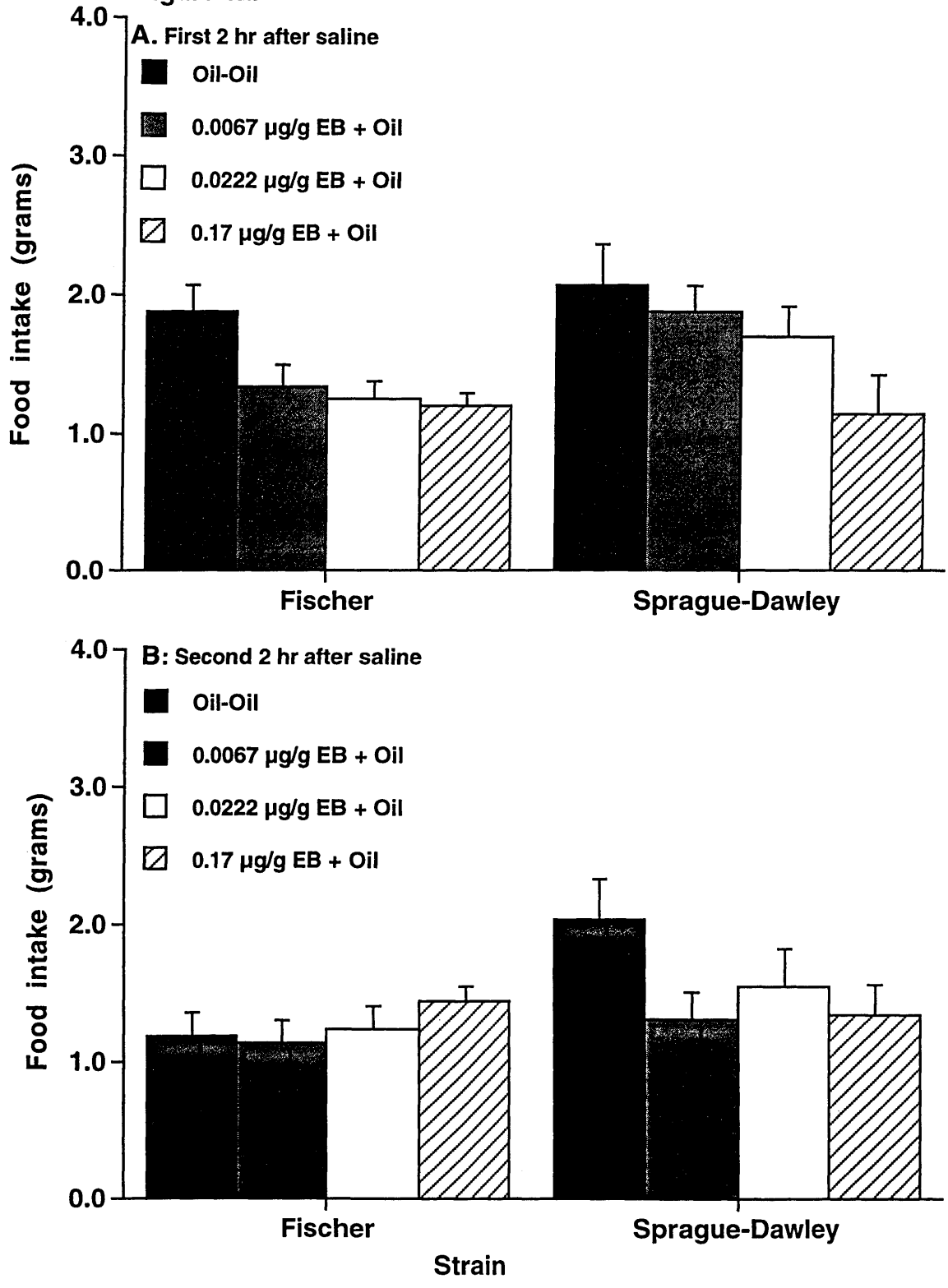
B: Effects of hormones on saline treated and 8-OH-DPAT treated rats.

The effect of EB on food intake is shown in Figure 12. Although EB decreased food intake in saline treated rats (Figure12 A and B), none of the post-hoc comparisons to the oil-oil control were significant (all Tukey, $p > .05$). Similar to the hormone effects in saline treated rats, EB reduced food intake in response to 8-OH-DPAT (Figure12 C and D). However, this reduction was significant only in Sprague-Dawley rats for 0.17 µg/g of EB and only during the second two-hr eating interval ($q_{124, 4} = 4.85$, $p \leq .05$).

C: Strain differences.

Ratios of food intake to that of the same strain, oil-oil, saline treated controls are shown in Figure 13. Overall, there was a significant effect of drug ($F_{1, 124} = 15.26$, $p \leq .0002$) and hormone ($F_{3, 124} = 22.63$, $p \leq .0001$), but the main effect of strain was not significant ($F_{1, 124} = 0.37$, $p > .05$). There was, however, a significant drug by strain interaction ($F_{3, 124} = 6.61$, $p \leq .02$) as well as a significant interaction between drug and dose of estrogen ($F_{3, 124} = 3.09$, $p \leq .02$). The time interval ($F_{1, 124} = 7.64$, $p \leq .007$) as well as the interaction between time and drug ($F_{1, 124} = 24.51$, $p \leq .0001$) were significant. No other interactions with strain were significant. No posthoc comparisons were significant.

Figure 12.


A. First 2 hr after saline
Oil-Oil
0.0067 µg/g EB + Oil
0.0222 µg/g EB + Oil
0.17 µg/g EB + Oil
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
Fischer
Sprague-Dawley
B: Second 2 hr after saline
Oil-Oil
0.0067 µg/g EB + Oil
0.0222 µg/g EB + Oil
0.17 µg/g EB + Oil
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
Fischer
Sprague-Dawley
Strain

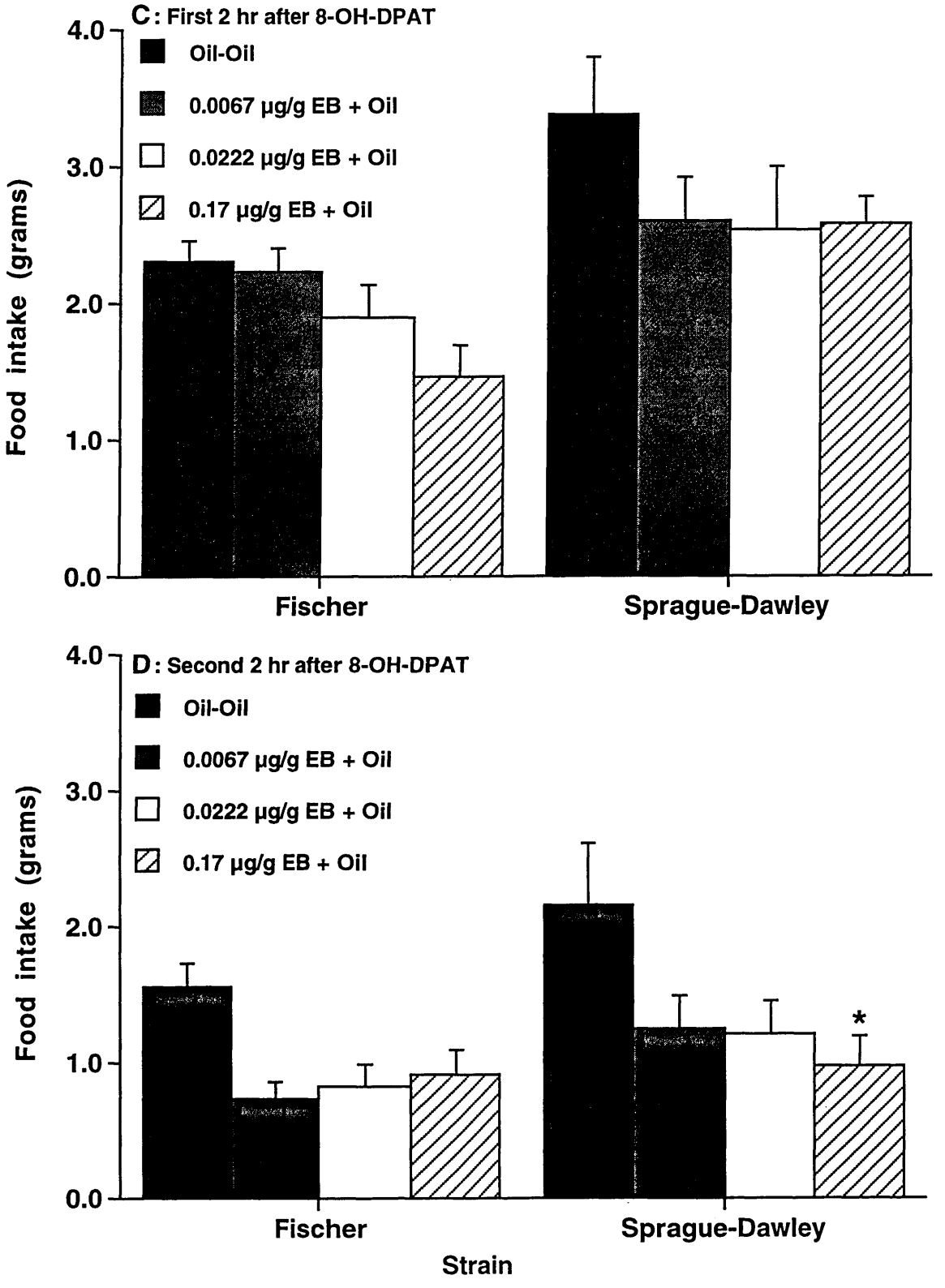

C: First 2 hr after 8-OH-DPAT
Oil-Oil
0.0067 µg/g EB + Oil
0.0222 µg/g EB + Oil
0.17 µg/g EB + Oil
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
Fischer
Sprague-Dawley
D: Second 2 hr after 8-OH-DPAT
Oil-Oil
0.0067 µg/g EB + Oil
0.0222 µg/g EB + Oil
0.17 µg/g EB + Oil
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
*
Fischer
Sprague-Dawley
Strain

Figure 12: Dose responsivity of EB on food intake.
Data are the mean ± S.E. grams of food intake during the first and second two hr after injection with saline or with 8-OH-DPAT. Ovariectomized Fischer and Sprague-Dawley rats were hormonally primed with oil or with 0.17, 0.0222, 0.0067 μg/g of EB and oil as described in the methods. Data for saline-treated rats are shown in Figure12 A and B. N's for oil and for 0.17, 0.0222 and for 0.0067 μg/g of EB are 9, 9, 8 and 8 for Fischer rats and 9, 9, 8 and 9 for Sprague-Dawley rats, respectively. Data for 8-OH-DPAT treated rats are shown in Figure 12 C and D. N's for oil and for 0.17, 0.0222 and for 0.0067 of EB are 9, 8, 8 and 9 for Fischer rats and 9, 9, 9 and 10 for Sprague-Dawley rats respectively. Oil treated females are the same rats shown in Figure 11. The single asterisk indicates a significant difference from the control group within the same strain and eating interval.

Figure 13.

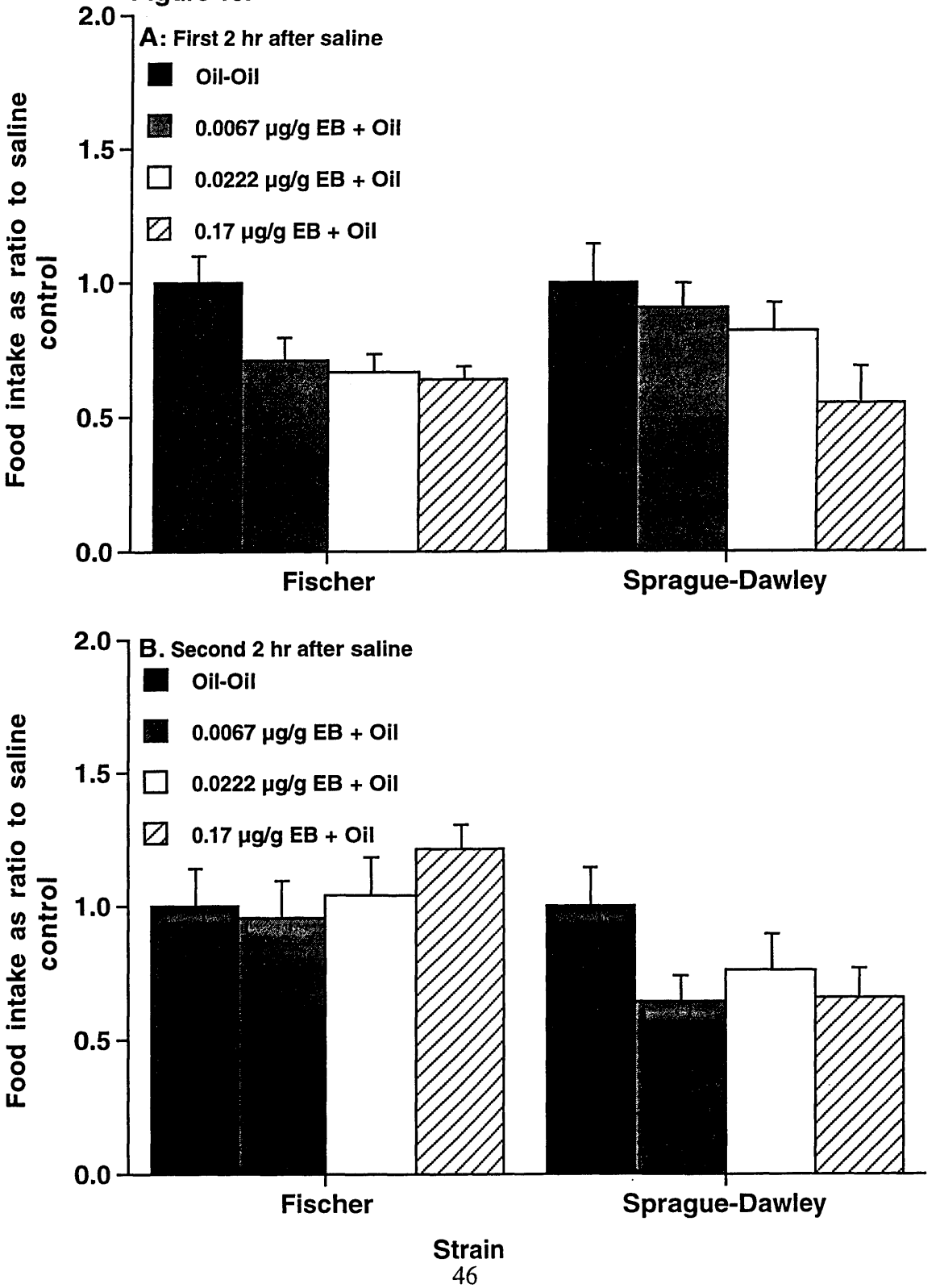

A: First 2 hr after saline
Oil-Oil
0.0067 µg/g EB + Oil
0.0222 µg/g EB + Oil
0.17 µg/g EB + Oil
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
Fischer
Sprague-Dawley
B. Second 2 hr after saline
Oil-Oil
0.0067 µg/g EB + Oil
0.0222 µg/g EB + Oil
0.17 µg/g EB + Oil
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
Fischer
Sprague-Dawley
Strain

Figure 13.

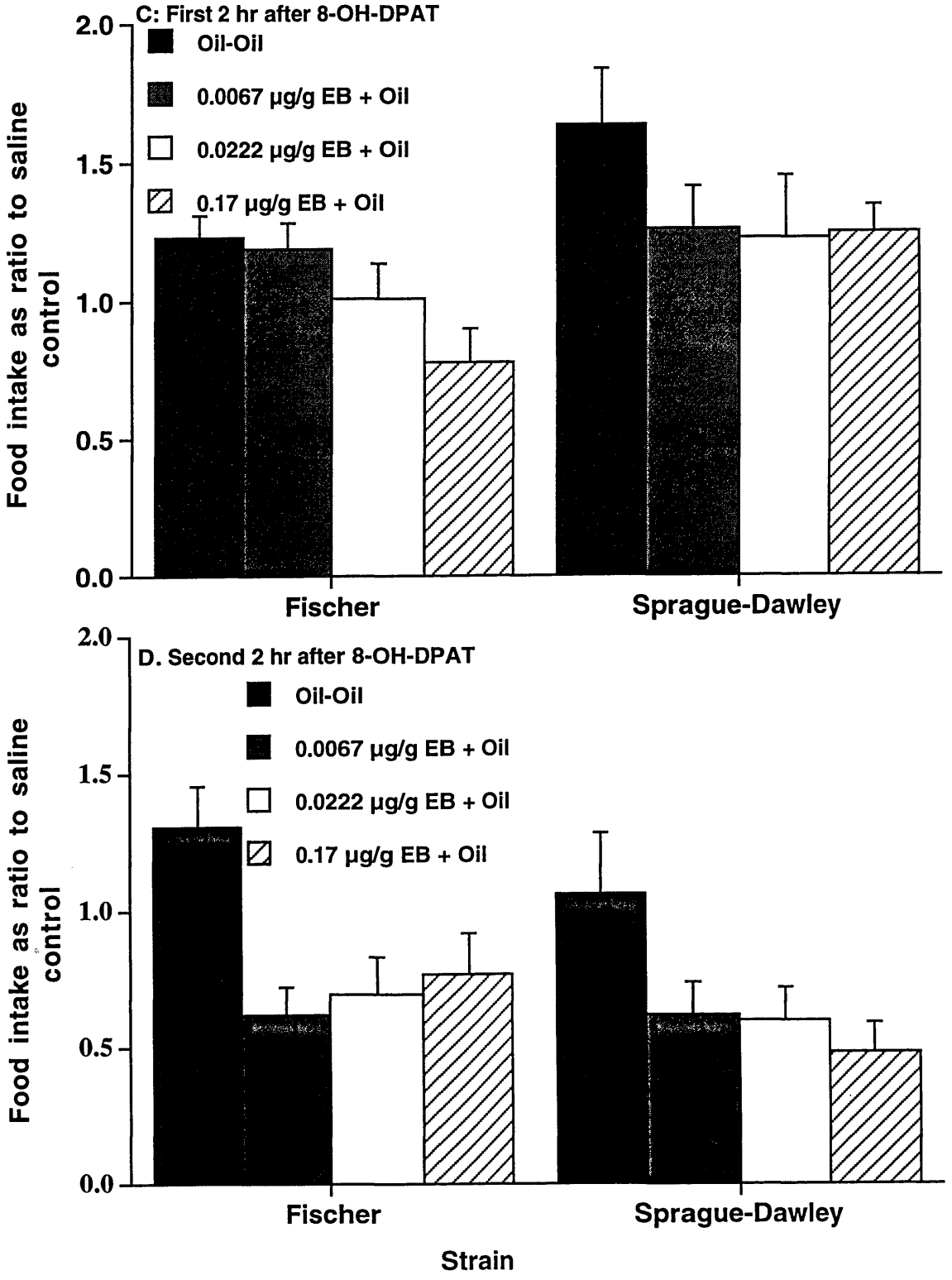

C: First 2 hr after 8-OH-DPAT
Oil-Oil
0.0067 µg/g EB + Oil
0.0222 µg/g EB + Oil
0.17 µg/g EB + Oil
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
Fischer
Sprague-Dawley
D. Second 2 hr after 8-OH-DPAT
Oil-Oil
0.0067 µg/g EB + Oil
0.0222 µg/g EB + Oil
0.17 µg/g EB + Oil
Food intake as ratio to saline control
2.0
1.5
1.0
0.5
0.0
Fischer
Sprague-Dawley
Strain

Figure 13: Strain differences in dose responsivity of EB on food intake.

Data are the mean ± S.E. ratio of food intake of each treatment group relative to the same strain, oil-treated, saline injected control. Data are for the first and second two hr after injection with saline (13 A, B) and with 8-OH-DPAT (13 C, D) and are from the same rats shown in Figure 12.

SUMMARY OF ALL EXPERIMENTS

Data for the oil-treated, saline-treated groups from all the individual experiments were combined and analyzed with experiments as a main factor. This was done in order to determine if group differences were consistent across experiments. Since there were no differences attributable to experiment number ($F_{2, 91} = 0.58$, $p > .05$), it was deemed appropriate to pool the relevant data to generate a dose response curve for EB. In a separate ANOVA, relevant data were pooled to compare EB, alone, versus EB plus P.

Data for food intake of saline and 8-OH-DPAT treated rats across all the doses of EB examined are shown in Figure 14. In EB treated rats, there was a dose-dependent decrease in food intake ($F_{4, 218} = 11.89$, $p \leq .001$) that was most clearly evident in Sprague-Dawley rats. In saline-treated Sprague-Dawley rats, every dose of EB significantly decreased food intake relative to oil-treated control (Dunnetts, $q_{218, 5} \geq 2.51$, $p \leq .001$). In saline-treated Fischer rats, the food intake of EB treated rats was never significantly different from the oil-oil control and there was little dose-dependency (Dunnetts, $q_{218, 5} = 2.27$, $p \geq .05$). However, there was no interaction between strain and dose of EB ($F_{4, 218} = 1.08$, $p > .05$).

8-OH-DPAT continued to increase food intake in EB treated rats (ANOVA for main effect of drug, $F_{1, 218} = 49.38$, $p \leq .0001$) but food intake was decreased by EB as in the saline-treated rats. This EB-induced decrease in 8-OH-DPAT treated rats also resembled that in saline treated animals with a greater effect in

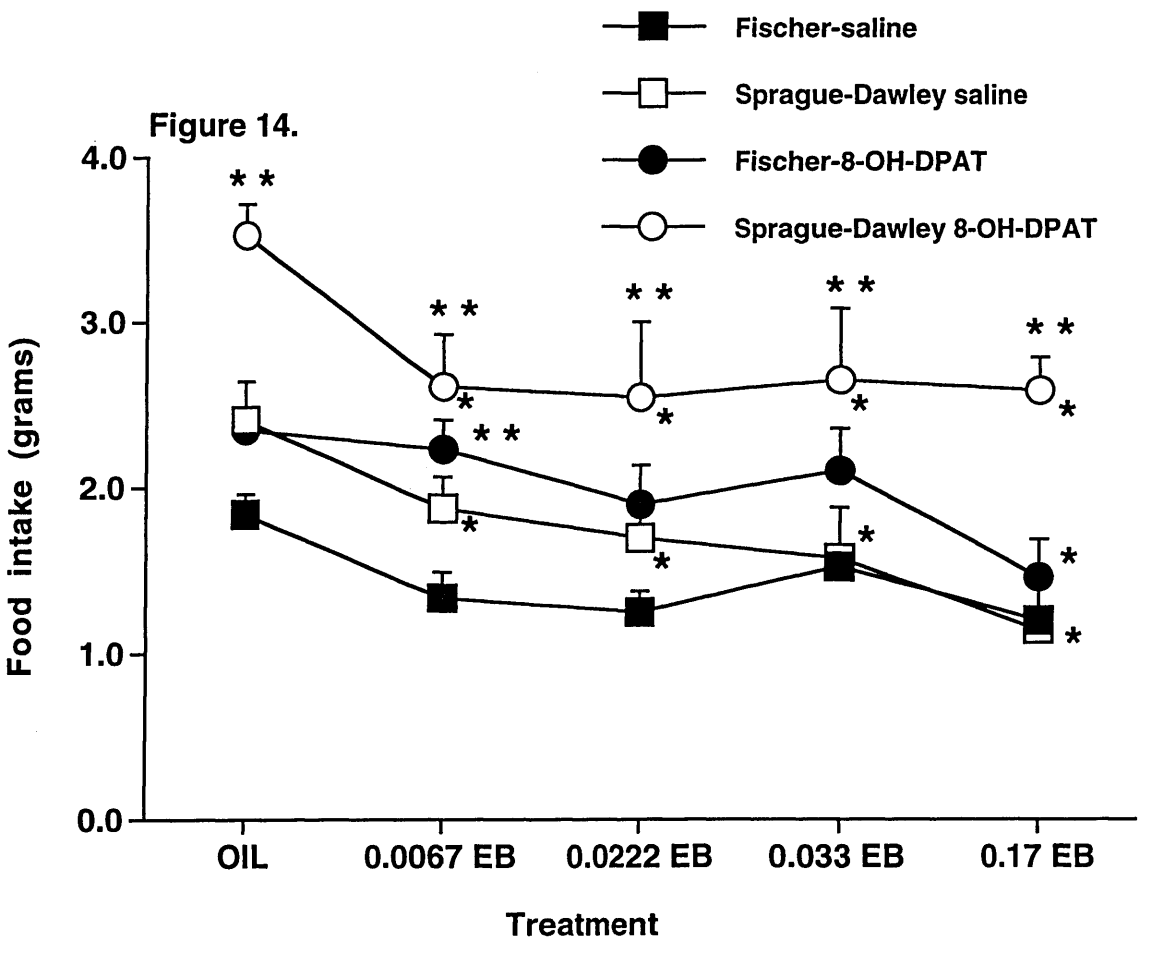


FIGURE 14: Food intake in hormone treated rats.
The mean+ S.E. grams of food intake in response to 0.15 mg/kg of 8-OH-DPAT or saline in hormone treated ovariectomized Fischer and Sprague-Dawley rats are shown. Data are for the first two hr after injection with saline or 8-OH-DPAT. N's for saline and 8-OH-DPAT for oil, 0.0067, 0.0222 and 0.033, 0.17 µg/g of EB respectively for Fischer rats are 26, 27, 8, 9, 8, 8, 8, 7, 9, 8 and for Sprague-Dawley rats are 24, 26, 9, 10, 8, 9, 8, 8, 9 and 9. The single asterisk indicates a significant difference relative to same strain oil control. The double asterisk indicates a significant increase in food intake relative to the saline control of the same hormonal treatment within strain.

Sprague-Dawley rats than in Fischer rats. Every dose of EB decreased food intake in Sprague-Dawley rats relative to the oil-oil-8-OH-DPAT treated control (Dunnetts, $q_{218, 5} \geq 3.12$, $p \leq .05$) while a significant decrease in 8-OH-DPAT treated Fischer rats was evident only at the highest EB dose as compared to saline-oil treated controls (Dunnett's $q_{218, 5} = 3.13$, $p \leq .05$).

In spite of the EB-induced decrease in food intake, 8-OH-DPAT continued to produced hyperphagia relative to the saline treated rats ($F_{1, 218} = 49.38$, $p \leq .0001$). This hyperphagia was more evident in Sprague-Dawley rats as compared to Fischer rats and led to a significant strain by drug interaction ($F_{4, 218} = 11.897$. $p \leq .05$). 8-OH-DPAT significantly increased food intake in Sprague-Dawley rats in every treatment condition except at .0067 µg/g EB ($q_{218, 2} \geq 2.99$, $p \leq .05$). In contrast a significant hyperphagia was evident in Fischer only at 0.0067 µg/g EB ($q_{218, 2} = 3.51$, $p \leq .05$).

A comparison between the effects of EB and EB plus P is shown in Figure 15. Only two doses of EB (0.033 and 0.17 µg/g) plus and minus 500 µg/g of P could be compared. Only EB and EB and P rats were included in the ANOVA. There was a significant effect of EB versus EB plus P ($F_{1, 158} = 11.29$, $p \leq .001$) but there was also a significant interaction between EB versus EB plus P and strain ($F_{1, 158} = 8.67$, $p \leq .004$). Moreover, the interaction between EB versus EB plus P and drug only marginally escaped statistical significance ($F_{1, 158} = 3.14$, $p \leq .07$). In Sprague-Dawley rats (Figure 15 B) P accentuated the suppressing effect of EB on food intake. At the lowest (0.033 µg/g) EB dose, the EB plus P

Figure 15.

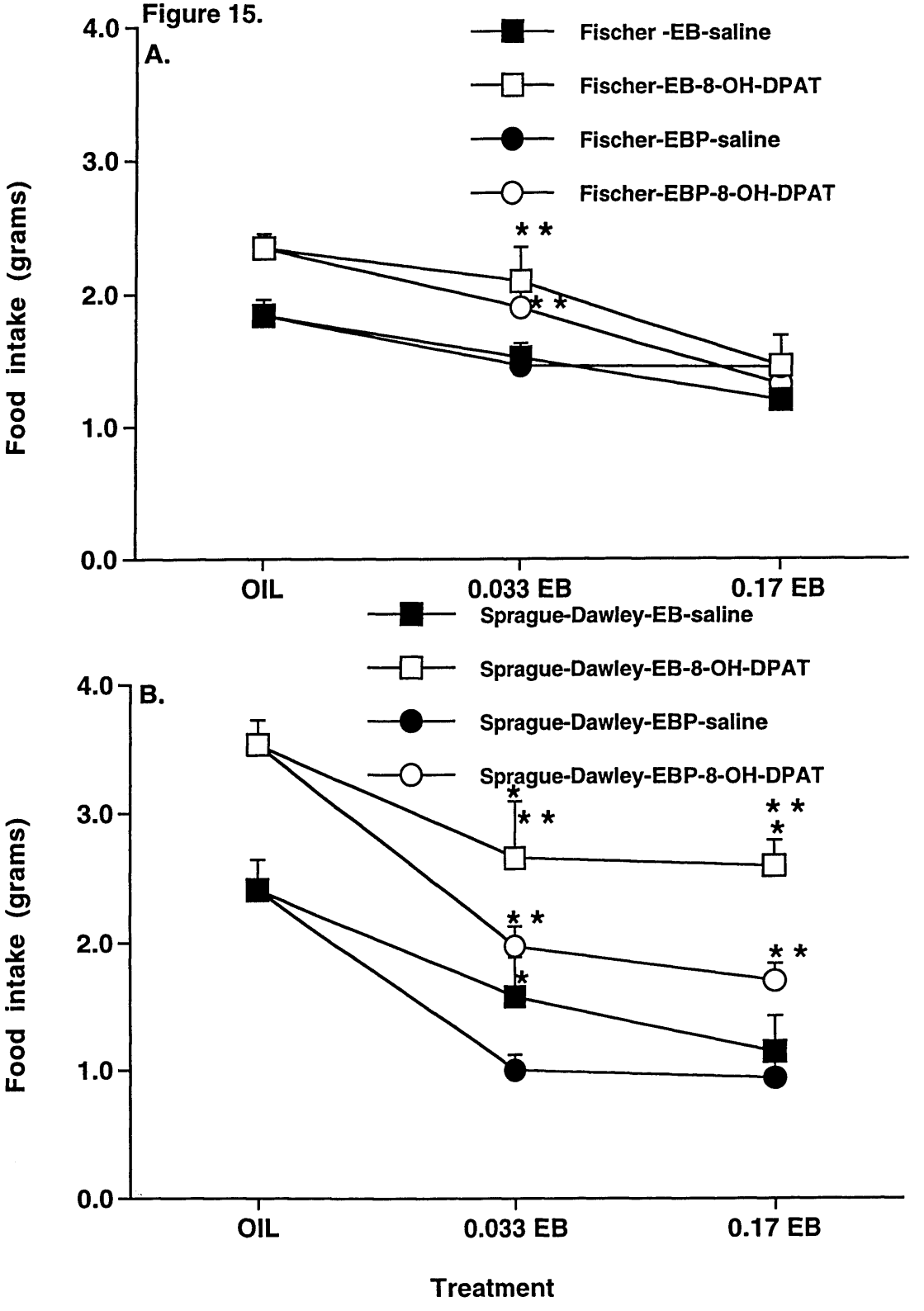

A.
Fischer -EB-saline
Fischer-EB-8-OH-DPAT
Fischer-EBP-saline
Fischer-EBP-8-OH-DPAT
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
OIL
0.033 EB
0.17 EB
B.
Sprague-Dawley-EB-saline
Sprague-Dawley-EB-8-OH-DPAT
Sprague-Dawley-EBP-saline
Sprague-Dawley-EBP-8-OH-DPAT
Food intake (grams)
4.0
3.0
2.0
1.0
0.0
OIL
0.033 EB
0.17 EB
Treatment

Figure 15: Food intake in EB versus EB plus P treated ovariectomized Fischer and Sprague-Dawley rats.

The mean ± S.E. grams of food intake for Fischer and Sprague-Dawley rats injected with oil, EB alone or EB plus P are shown. Data are for the first two hr after injection with saline or 8-OH-DPAT for Fischer rats (15 A) and for Sprague-Dawley rats (15 B) and are pooled across all the experiments. N's for oil, 0.033 and 0.17 μg/g of EB for saline or 8-OH-DPAT treated Fischer rats are, respectively, 26, 27, 8, 7, 9, 8 and for EB plus P are 26, 27, 16, 17, 18 and 10. N's for oil, 0.033 and 0.17 μg/g of EB for saline or 8-OH-DPAT treated Sprague-Dawley rats are, respectively, 24, 26, 8, 8, 9, 9 and for EB plus P are 24, 26, 18, 19, 10 and 10. A single asterisk indicates a significant difference between EB and EB plus P of the same dose. The double asterisk indicates a significant difference between saline and 8-OH-DPAT of the same treatment.

Sprague-Dawley treated rats ate significantly less food than EB treated rats after both 8-OH-DPAT and saline treatment (Tukeys, $q_{158, 2} \leq 3.09$, $p \leq .05$). There was no significant difference between the effects of EB and EB plus P in Fischer rats (all $q_{158, 2} < 1.34$, $p > .05$). Neverthless, in Sprague-Dawley rats, 8-OH-DPAT-induced hyperphagia was present under both EB and EB plus P conditions ($q_{158, 2} \geq 4.08$, $p \leq .05$) while hyperphagia was present in Fischer rats only at the lower EB dose (Tukey, $q_{158, 2} = 3.09$, $p \leq .05$).

CHAPTER IV

Discussion

The objective of these studies was to examine the strain difference in the ability of the 5-HT1A receptor agonist, 8-OH-DPAT, to induce hyperphagia and to determine if this behavior was modulated differently by female gonadal hormones in Fischer and Sprague-Dawley rats. Evidence for a strain difference in the effect of 8-OH-DPAT was initially provided by Uphouse et al. [89] who reported that the ability of the drug to inhibit lordosis behavior and to produce flat body posture was greater in Sprague-Dawley than in Fischer rats. Since both of these behaviors are thought to result from the activation of postsynaptic 5-HT1A receptors [59,84,88], Uphouse et al. [89] concluded that these two strains of rats may differ in their postsynaptic responsivity to 5-HT1A receptor agonists. Whether the same conclusion extends to behaviors mediated by the activation of somatodendritic 5-HT1A receptors was the focus of the current studies.

The results of this study demonstrated the following: 1) there was no strain difference in 8-OH-DPAT-induced hyperphagia in ovariectomized non-hormonally treated Fischer and Sprague-Dawley rats; 2) although estrogen reduced the food intake of saline treated rats of both strains, Fischer rats seemed to be less sensitive to estrogen's effect on food intake; 3) in estrogen treated rats, 8-OH-DPAT produced almost no hyperphagia in Fischer rats, but Sprague-Dawley rats continued to show 8-OH-DPAT-induced hyperphagia; 4) in

EB /P treated rats, P accentuated the depressing effect of estrogen on food intake in saline-treated Sprague-Dawley rats but seemed to attenuate the effect of estrogen on food intake in Fischer rats; 5) P alone increased food intake in saline treated Sprague-Dawley rats but had no effect in saline-treated Fischer rats; 6) in EB /P and 8-OH-DPAT treated rats of both strains, P had no effect on 8-OH-DPAT-induced hyperphagia; 7) in oil /P treated Sprague-Dawley rats, P alone decreased the 8-OH-DPAT-induced hyperphagia but had no effect in Fischer rats.

We examined the dose dependent effect of 8-OH-DPAT-induced hyperphagia in ovariectomized, non-hormonally treated Fischer and Sprague-Dawley rats. In agreement with prior studies [29,50], the 5-HT1A receptor agonist increased eating in both strains. However, there was no strain difference in the agonist-induced hyperphagia. The ability of 8-OH-DPAT to elicit hyperphagia is thought to result from the agonist's activation of somatodendritic 5-HT1A autoreceptors in the raphe nuclei. The infusion of the drug into dorsal or median raphe elicits a hyperphagic response [49]. Activation of these somatodendritic 5-HT1A autoreceptors reduces firing of 5-HT neurons, decreases release of 5-HT and is thereby thought to reduce the inhibitory effect of 5-HT on food intake [80]. The absence of a strain difference in 8-OH-DPAT-induced food intake in the present studies contrasts with the report by Uphouse et al. [89] of a strain difference in 8-OH-DPAT induced inhibition of lordosis behavior and the development of flat body posture. Since 8-OH-DPAT's effects on these latter two

behaviors is believed to result from activation of postsynaptic 5-HT1A receptors, there may be a strain difference in the responsivity of postsynaptic, but not presynaptic, receptors to the 5-HT1A receptor agonist.

There is some evidence that 5-HT1A receptors located on 5-HT cell bodies differ from those located postsynaptic to 5-HT nerve terminals. For example, 5-HT1A receptors located at 5-HT cell bodies may have a greater receptor reserve as compared to postsynaptic regions [59]. With a high receptor reserve, a lower dose of the drug is required to give a maximum effect because there are more coupled receptors available than are required to elicit the response. Since Fischer and Sprague-Dawley rats did not differ in agonist-induced hyperphagia, it is possible that high receptor reserve at somatodendritic locations masked the existence of any strain difference in receptor function. Such strain differences might be more readily observed for postsynaptic receptors where receptor reserve is low. If Sprague-Dawley rats have greater receptor reserve at postsynaptic sites compared to Fischer rats, this might account for the lower dose of 8-OH-DPAT required in Sprague-Dawley rats to inhibit lordosis behavior and to produce flat body posture.

Another difference in the responsivity between somatodendritic 5-HT1A receptors and those located postsynaptic to 5-HT terminals may be in the effector mechanisms used by the receptors. 5-HT1A receptors belong to the superfamily of G protein coupled receptors and utilize a variety of different G proteins to mediate their action [86]. The most prominent effects recognized are

the reduction in activity of adenylyl cyclase and the opening of a K^+ channel. Different G proteins are thought to mediate these effects [86]. In the DRN, the somatodendritic 5-HT1A receptors are coupled predominantly to K^+ channels but may also be negatively coupled to phosphoinositide turnover [18,53,55]. Postsynaptic 5-HT1A receptors couple to both adenylyl cyclase and K^+ channels [22,70]. It is, therefore, possible that Fischer and Sprague-Dawley 5-HT1A receptors differ in their degree of coupling to adenylyl cyclase, but not to K^+ channels. Although it is not known which precise coupling events are responsible for the specific 8-OH-DPAT-induced behavior examined in this study and by Uphouse et al. [89], the 8-OH-DPAT-induced decrease of lordosis can be attenuated by an increase in cAMP. However, this has been investigated only in Fischer rats. It is, of course, also possible that the postsynaptic 5-HT1A receptors that are responsible for the decrease in lordosis behavior and flat body posture are coupled to different G proteins in the two strains. However, this seems less likely than does the possibility that the strains might differ in the relative concentrations of the various G proteins.

The third possible explanation for the absence of a strain difference in hyperphagia but the presence of a strain difference in the inhibition of lordosis behavior and development of flat body posture after 8-OH-DPAT is a strain difference in the synaptic availability of 5-HT. With a lower extracellular concentration of endogenous 5-HT, a higher concentration of 8-OH-DPAT would be required to elicit behaviors mediated by postsynaptic 5-HT1A receptors.

Fischer rats have been reported to have less available 5-HT than Sprague-Dawley within the mediobasal hypothalamus [14] and 5-HT1A receptors in this region are thought to be responsible for 8-OH-DPAT's decrease of lordosis behavior [88]. If Fischer rats have a lower concentration of extracellular 5-HT than do Sprague-Dawley rats, this could explain their lower sensitivity to the lordosis-inhibiting effects of the 5-HT1A receptor agonist. However, if Fischer rats have a lower availability of extracellular 5-HT at the raphe nuclei, Fischer rats would be expected to show less inhibition of cell firing and less inhibition of 5-HT release from nerve terminals. This would not be expected to produce the observed strain difference in the effects of 8-OH-DPAT on lordosis behavior or flat body posture but would be expected to produce a strain difference in 8-OH-DPAT-induced hyperphagia. Therefore, the possibility that Fischer rats have less extracellular 5-HT may not be a sufficient explanation to account for both the present and prior findings. However, it is important to note that differences in tissue concentration of 5-HT between Fischer and Sprague-Dawley rats vary in different brain areas [14] and comparative data for the raphe nuclei are not available.

A strain difference in extracellular 5-HT could arise independently of differences in either cell firing or neurotransmitter release. Since 5-HT receptor transporters are responsible for the removal of extracellular 5-HT [4], strain differences in the amount or activity of 5-HT transporters could lead to strain differences in synaptic availability or duration of action of 5-HT. Burnet et al. [15]

found a greater abundance of 5-HT transporter mRNA in the raphe of Fischer than Sprague-Dawley rats. If, relative to Sprague-Dawley rats, Fischer rats have a more efficient reuptake of released 5-HT, this would lead to a lower extracellular concentration of 5-HT. A higher concentration of the 5-HT1A receptor agonist might be required to activate 5-HT1A receptors and a higher concentration of the agonist would be required to elicit 8-OH-DPAT-induced hyperphagia. The absence of a strain difference in 8-OH-DPAT-induced hyperphagia argues against this possibility.

Of course, it is also possible that Fischer and Sprague-Dawley rats do not differ directly in any aspect of 5-HT or 5-HT1A receptor function. Instead, the strains may differ in a number of other physiological traits that could alter some, but not all, responses to the 5-HT1A receptor agonist. For example, Fischer rats were reported to display both higher basal levels of serum corticosterone and a greater serum corticosterone response to foot shock compared to that of Sprague-Dawley rats [74]. Fischer rats also have been reported to have a hyperfunctional hypothalamic-adrenal-pituitary (HPA) axis [14]. Mendelson and McEwen [68] reported a decreased binding at 5-HT1A receptors in the hippocampus of rats after chronic exposure to high levels of corticosterone. Moreover, chronic corticosterone treatment significantly decreased both 8-OH-DPAT induced flat body posture and hypothermia in rats [82]. Therefore, strain differences in circulating corticosterone may lead to apparent difference in postsynaptic 5-HT1A receptor function. Whether or not comparable changes

occur at the DRN are not known. However, the 5-HT1A receptor agonist, 8-OH-DPAT, increases circulating levels of corticosterone as well as ACTH, by activating 5-HT1A receptors presumably located in the PVN [7,40]. Therefore, a complex interaction between levels of corticosterone and HPA response to the drug could produce an apparent strain difference in the response to 5-HT1A receptor agonists and this might be expected to vary across different receptor-mediated behaviors.

Another possible explanation for the findings by Uphouse et al. [89] and those in the current study is a strain difference in the response to gonadal hormones. Since the rats used by Uphouse et al. [89] to study the strain difference in the inhibition of lordosis behavior were hormonally primed, it is possible that the strain difference in 8-OH-DPAT's decrease of lordosis behavior resulted from a strain difference in the modulation of 5-HT1A receptors by female gonadal hormones. Evidence for modulation of 5-HT1A receptors by estrogen was first provided by Lakoski [58] who reported that estrogen reduced the ability of 8-OH-DPAT to reduce the firing of dorsal raphe neurons. Since activation of somatodendritic 5-HT1A neurons leads to a decrease in neuronal firing, decreasing the activation of these receptors should lead to an increase in 5-HT neuronal firing, and as a result, an increase in 5-HT release and a decrease in food intake. Salamanca et al. [76] showed that, in response to 8-OH-DPAT, EB treated rats showed less eating than oil-treated controls. Maswood et al. [66] reported that treatment with EB reduced the effects of 0.25 mg/kg 8-OH-DPAT

on eating behavior, while having little effect on 8-OH-DPAT-induced flat body posture or hypothermia. However, other investigators reported that female gonadal hormones may also modulate postsynaptic 5-HT1A receptors. Uphouse et al. [87] reported that two estrogen treatments, separated by seven days reduced the ability of 8-OH-DPAT to inhibit lordosis behavior in ovariectomized rats. Jackson and Uphouse [51] reported a dose-dependent effect of the hormone that occurred within 56 hours. Although female gonadal hormones modulate both presynaptic and postsynaptic 5-HT1A receptors, it is possible that the presynaptic and postsynaptic 5-HT1A receptors are modulated differently by female gonadal hormones.

In the present experiment, food intake of estrogen-treated rats was reduced whether the rats were injected with saline or 8-OH-DPAT and this effect of estrogen was most apparent in Sprague-Dawley females. For Sprague-Dawley females, a dose-dependent effect of estrogen was clearly evident, while there was little dose-dependency in the hormone's effect in Fischer rats. Although the food intake after 8-OH-DPAT was reduced in estrogen-treated rats, this reduction resembled that seen in saline-treated rats, so that 8-OH-DPAT-induced hyperphagia continued to be present when food intake was compared to the same hormonal condition after saline treatment. However, such 8-OH-DPAT-induced hyperphagia was more evident in Sprague-Dawley than in Fischer females. These findings seem to be indicative of a strain difference in both the response to estrogen's suppressive effect on food intake and in

estrogen's effect on 8-OH-DPAT-induced hyperphagia. However, in those studies in which estrogen's effect on 8-OH-DPAT-induced hyperphagia was specifically examined, Fischer rats showed a relatively small hyperphagic response to the drug. It is, therefore, possible that the apparent strain difference in estrogen's suppression of 8-OH-DPAT-induced hyperphagia was confounded by the small effect of the drug in Fischer females.

In a prior experiment, Salamanca et al. [76] concluded that the ability of female gonadal hormones to reduce the effect of 8-OH-DPAT on food intake resulted from the effects of estrogen and not P. In those experiments with Fischer females, there was no difference between estrogen alone and estrogen plus P on 8-OH-DPAT-induced food intake. However, in the current experiments, there appeared to be clear differences between the effects of P in estrogen-treated Fischer and Sprague-Dawley females. In Sprague-Dawley rats, estrogen and P accentuated the suppressing effect of estrogen on food intake. In agreement with Salamanca et al. [76], there was little difference between estrogen and estrogen plus P in Fischer rats. Nevertheless, 8-OH-DPAT-induced hyperphagia was present in Sprague-Dawley rats under both conditions, while hyperphagia was less evident in Fischer females.

P's accentuation of the effects of estrogen on food intake in Sprague-Dawley females was surprising since P is generally thought to increase food intake [73,75,91]. In agreement with this expectation, P alone did slightly increase food intake in saline-treated Sprague-Dawley rats. However, in

Sprague-Dawley 8-OH-DPAT-treated rats, P was as effective as estrogen in reducing food intake. Thus, in Sprague-Dawley rats, P alone may have reduced the effects of 8-OH-DPAT. It is, however, important to note that this was an unexpected and unplanned observation, so that additional experiments will be required to determine if such a strain difference in the effects of P truly exist.

How estrogen affects the functioning of the 5-HT system is not fully understood but it may include serotonin synthesis, content, release, reuptake, degradation, binding sites and intracellular coupling mechanisms [8]. In the rat, serotonin neurons in the DRN do not contain estrogen receptors but non-5-HT neurons in the DRN do contain estrogen receptors [3]. Thus the potential exists for estrogen to indirectly influence the activity of serotonin neurons. Such neurons may be GABAergic, enkephalinergic or neurotensin containing interneurons [8]. Any or all of these interneuronal system may differ between Fischer and Sprague-Dawley rats.

The other mechanism by which estrogen may decrease food intake is by increasing the density of 5-HT2 receptors. Many investigators have reported that the activation of 5-HT2 receptors causes hypophagia [48,56,77,78]. Estrogen is known to increase 5-HT2 receptor density in many areas of brain including the DRN [9] and is also known to upregulate the expression of 5-HT2A mRNA [81]. Proestrous rats have more 5-HT2A receptors than diestrous rats [81]. It is possible that Fischer and Sprague-Dawley rats might differ in the density or function of 5-HT2A receptors which could explain their different response to

estrogen.

P may also influence the firing of 5-HT neurons. One mechanism by which P can reduce the firing of serotonin neurons is thought to involve the binding of P's metabolite, allopregnanolone, to GABAA receptor [6,44]. Drugs which activate GABAA receptors have been reported to decrease 5-HT neuronal firing [37,85]. P may also reduce the release of 5-HT from nerve terminals by increasing the density of 5-HT1B terminal autoreceptors [36] which reduce depolarization dependent release of 5-HT [34,45,47].

There is also the possibility that Fischer and Sprague-Dawley rats differ in their sensitivity to the complex interaction between estrogen and P. P can either facilitate or inhibit the effect of estrogen [10 ,63,71,72,91] depending on the time interval between exposure to estrogen and P and the specific parameter investigated. In many cases, effects of P require prior hormone priming with estrogen [10,92], and this is generally attributed to estrogen's effect on P receptors. One of the best recognized genomic responses to estrogen is the induction of P receptors (PR). However, P receptors are estrogen-inducible in some, but not all, brain areas [43,62,65]. In the hypothalamus and preoptic areas (as in the pituitary and uterus), the receptors are present in low concentration in the absence of estrogen and increase in number after estrogen treatment [65]. In contrast, in midbrain and cerebral cortex, P receptors are present at low levels and are not affected by short-term estrogen treatment [65]. Thus, some effects of P are independent of prior hormone priming. It is possible

that an interplay between the estrogen-inducible and estrogen-independent P receptor systems may underlie some of the differences between Fischer and Sprague-Dawley rats in their response to the hormonal treatment.

In the present study, the most unexpected observation was the greater effect of female gonadal hormones on food intake of Sprague-Dawley compared to Fischer rats. It has been reported that Fischer rats show a higher sensitivity to estrogen like xenobiotics [64] and Wiklund et al. [93], and Ying et al. [95] reported that Fischer rats were more sensitive to the development of pituitary tumors after treatment with diethylstilbestrol. Thus, if any strain differences occurred, a greater effect of female gonadal hormones was expected in Fischer rats, not Sprague-Dawley rats. However, other investigators have reported a minimal decline in food intake after estrogen in Fischer rats as compared to Sprague-Dawley rats [60].

One possible explanation for the greater food suppressing effect of the hormone in Sprague-Dawley rats is a lower endogenous hormone level in these rats. Although females were ovariectomized to remove the primary source of estrogen and P, other sources continued to be present. For example, the reported HPA hyperactivity of Fischer, relative to Sprague-Dawley rats, may result in enhanced adrenal steroid synthesis in Fischer rats. This may lead to hormone-induced suppression of food intake in the absence of exogenous hormone treatment.

Alternatively, there are a number of neurotransmitters and peptides that

influence food intake and are, in turn, modulated by female gonadal hormones. Cholecystokinin (CCK) is a gastrointestinal peptide which reduces food intake and it is modulated by female gonadal hormone [32,39]. Devazepide (CCK_A receptor antagonist) is reported to increase food intake during estrus but not during diestrus [32]. Estradiol treatment in ovariectomized rats also increases CCK-induced c-fos expression in brain regions implicated in satiety [33,39]. CCK is enhanced during estrous in intact rats and by estradiol treatment in ovariectomized rats [39]. There is also a strong interaction between serotonin and CCK_A [19,20,21]. It is possible that there is difference in the abundance of any or all of these systems in Fischer and Sprague-Dawley rats.

In summary, we have shown that there is a strain difference in female gonadal hormonal modulation of food intake. Fischer and Sprague-Dawley rats are used in many research laboratories but are seldom investigated within a single laboratory. Caution should be taken in generalizing the interpretation of data from one strain to the other strain because significant response differences may exist between these two strains.

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