Sex differences in body weight gains following amygdaloid lesions in rats

Bruce M. King, Bethany L. Rollins, Samuel G. Stines, Sofia A. Cassis, Holland B. McGuire, and Michelle L. Lagarde

Sex differences in body weight gains following amygdaloid lesions in rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R975–R980, 1999.—Lesions of the most posterodorsal aspects of the amygdala resulted in equal weight gains (mean = 58 g) in male and female rats during a 22-day observation period. However, the absolute weight gains in the first 5 days after lesions were greater in females (+41.4 g) than in males (+18.8 g), as were the longer-term gains relative to their respective control groups. In a second study with female rats, it was found that amygdaloid lesions had little effect on the estrous cycle and that ovariectomy resulted in additional excessive weight gains in both rats with sham lesions and those with amygdaloid lesions. The weight gains produced by amygdaloid lesions and ovariectomy were additive. It is concluded that there is a sex difference in weight gains after amygdaloid lesions, but that the lesion-induced obesity is independent of estrogen levels. Similarities to lesions of the ventromedial hypothalamus are noted, and an amygdaloid-ventromedial hypothalamic pathway for the regulation of feeding behavior is proposed.

Amygdala; feeding behavior; estrous cycle; ovariectomy

Lesions of the most posterodorsal aspects of the amygdala result in moderate obesity in female rats (24, 26, 27). The animals typically gain 50–80 g in a dynamic phase lasting from 15 to 20 days, but gains of as much as 100 g have occasionally been observed. The rats then enter a static phase in which the excessive weight gains are maintained indefinitely (24). The obesity-inducing lesions are within an area of the amygdala that projects heavily to the ventromedial hypothalamus (VMH) (1, 10, 31). Thus the lesion syndrome resembles in some respects that which follows lesions of the VMH (22, 24, 29).

Many researchers have noted a sex difference in the weight gain induced by VMH lesions; i.e., females gain more weight than males (3, 9, 17, 25, 41, 43, 46, 47). As a result, it has long been suspected that gonadal hormones play some role in the obesity syndrome. The VMH has a high density of estrogen-binding neurons (33, 40), and estrogen acts on the ventromedial hypothalamic nuclei to reduce food intake and body weight (19, 53). Ovariectomy results in hyperphagia and obesity in rodents (6, 12, 52). However, two studies have reported that ovariectomized rats with VMH lesions gain more weight than nonovariectomized rats with lesions (30, 51), suggesting that estrogen also influences food intake at sites outside the VMH.

The amygdala and bed nucleus of the stria terminalis also have a high density of estrogen receptors (39, 49), and hyperphagic rats with amygdaloid lesions are similar in some respects (e.g., lack of finickiness) to ovariectomized rats (28; see Ref. 13). Moreover, previous pharmacological studies have shown that the medial amygdala is sexually dimorphic (34). The present study examined whether rats with posterodorsal amygdaloid lesions display a sex difference in weight gain and also whether ovariectomy induces further weight gain in female rats with lesions.

METHODS

Subjects

Sixty-one adult female and twenty-four adult male Long-Evans hooded rats were used (Harlan Sprague Dawley, Indianapolis, IN). All animals were individually caged in a temperature-controlled colony (21–24°C) with a 12:12-h light-dark cycle throughout the experiment.

Surgeries and Histology

Bilateral electrolytic lesions were produced under pentobarbital sodium anesthesia (50 mg/kg) by passing a 1.5-mA anodal current between the 0.25-mm uninsulated tip of an insulated stainless steel electrode (no. 0 insect pin) and a rectal cathode for 20 s. Electrodes were positioned with use of a Kopf small animal stereotaxic instrument. A series of pilot lesions were performed to equate lesion placements in male and female rats. With the upper incisor bar positioned horizontally with the interaural line, the electrodes were positioned 2.1 (females) or 2.0 mm (males) posterior to bregma, 4.5 mm lateral to the midsagittal suture, and 8.4 (females) or 8.5 mm (males) below the surface of the skull. For control animals, holes were drilled in the skull, and the electrode was lowered 7.4 mm below the surface of the skull.

Ovariectomies were performed under pentobarbital sodium anesthesia through a dorsolateral incision. In animals with sham ovariectomies, a dorsolateral incision was made without disturbing the ovaries.

After completion of the study, the rats with lesions were killed and perfused, and their brains were removed and placed in a 10% Formalin solution. Frozen coronal 40-µm sections were taken through the region of brain tissue containing the lesion. The sections were stained with cresyl violet, and histological analysis was performed by light microscopic examination. The extent of the lesions was determined with use of the stereotaxic atlas by Paxinos and Watson (38). The lesions were serially reconstructed, and the sections were overlaid so that areas of overlap among the animals could be determined.

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Procedure

Part 1. Four groups were tested: female rats with amygdaloid lesions (n = 15), female rats with sham lesions (n = 9), male rats with amygdaloid lesions (n = 16), and male rats with sham lesions (n = 8). All animals were fed Harlan Teklad rat diet LM-485 for 22 days after surgery. Body weight and food intake were measured daily.

Part 2. All animals were given two surgeries 15 days apart. Four groups of female rats were tested: those that received amygdaloid lesions followed by ovariectomy (AMYG-OVX, n = 14), amygdaloid lesions followed by sham ovariectomy (AMYG-SOVX, n = 13), sham lesions followed by ovariectomy (SAMYG-OVX, n = 5), or sham lesions followed by sham ovariectomy (SAMYG-SOVX, n = 5). Because the purpose of the study was to see if the excess weight gains after amygdaloid lesions and ovariectomy are additive, for inclusion in the study the animals with amygdaloid lesions had to achieve a minimum criterion of 35 g weight gain in 15 days. The animals were monitored for an additional 30 days after the second surgery. Vaginal smears were taken daily starting 10 days before the first surgery to determine the effect of lesions on the estrous cycle. Male rats were housed in the animal colony during the period when estrous cycles were recorded. Body weight and food intake were also measured daily.

Statistical Analyses

The data were analyzed with use of planned comparisons (t), ANOVA, and, where post hoc tests were appropriate, Tukey’s honestly significant difference test. In addition to P, effect size (η²) is reported where appropriate.

RESULTS

Fifteen of thirty-one animals (7 of 15 females and 8 of 16 males) given lesions in part 1 and 13 of 27 animals in part 2 had damage centered bilaterally in the most posterodorsal aspects of the amygdala ("posterodorsal" refers to an area, not a specific nucleus). The "hit rate" is low because the critical site is very small. Weight gain was greatest in animals with damage to the posterodorsal medial amygdala and the intra-amygdaloid division of the bed nucleus of the stria terminalis. Weight gain was attenuated when lesions extended into the globus pallidus immediately dorsal to the amygdala. Representative lesions of a male and female rat are shown in Fig. 1. There was considerable variability among the lesions that missed, and the data for these animals were not included in the analyses. More
detailed histological analysis is provided elsewhere (21, 27). One male rat died shortly after surgery.

Part 1

Mean daily body weights are displayed in Fig. 2. Sham lesions resulted in a small weight loss for both males and females in the first 4–6 days after surgery. Amygdaloid lesions, on the other hand, resulted in immediate weight gains, but this was more dramatic in females than males. By day 5, male rats with amygdaloid lesions had gained a mean of 18.8 ± 3.7 g compared with 41.4 ± 4.2 g for females (t = 3.97, df = 13, P < 0.001; η² = 0.55). Thereafter, all four groups displayed sex-typical weight gains, so that total mean weight gains on day 22 were 10.8 ± 2.3 and 58.0 ± 4.3 g for sham and lesioned females, respectively, compared with 34.3 ± 4.3 and 58.5 ± 3.9 g for sham and lesioned males, respectively. A two-factor (2 × 2) ANOVA of total weight gains revealed a significant effect for lesion (F = 102.22; df = 1, 27; P < 0.001; η² = 0.79), sex (F = 11.55; df = 1, 27; P < 0.002; η² = 0.30), and interaction (F = 10.60; df = 1, 27; P < 0.003; η² = 0.28). Data for food intake reflected those for weight gain (see Fig. 3). Food consumption increased substantially in the first few days after amygdaloid lesions (mean daily intakes on days 3–6 of 37.5 ± 2.0 g for females and 39.9 ± 1.4 g for males compared with 21.7 ± 0.7 and 30.1 ± 0.7 g for male and female controls, respectively), but had returned to normal levels by day 15.

Part 2

Neither the sham lesions nor the amygdaloid lesions disrupted the estrous cycles for more than a few days. The range for which rats first displayed estrus after surgery was 3–9 days for animals with sham lesions and 2–6 days for rats with lesions. Mean daily body weights and food intake are displayed in Figs. 4 and 5, respectively. The mean changes in body weight that followed the various surgical procedures are displayed in Table 1. A two-factor (4 × 2) ANOVA with repeated measures on one factor revealed a significant main effect for groups (F = 44.33; df = 3, 19; P < 0.001; η² = 0.88), surgeries (F = 8.36, df = 1,19; P < 0.01; η² = 0.31), and interaction (F = 35.04, df = 3,19; P < 0.001; η² = 0.85). Post hoc analysis indicated that both groups with amygdaloid lesions gained significantly more than either group with sham lesions before the second surgery (P < 0.001). After the second surgery, both groups with ovariectomies gained more than either group with sham ovariectomies (P < 0.005), and the SAMYG-OVX group gained significantly more weight than the AMYG-OVX group (P < 0.05). Among the AMYG-OVX animals, there was a negative correlation of −0.60 between weight gain after lesions and weight gain after ovariectomies, but this was not statistically significant. Data for food intake paralleled that for weight changes in the first 15 days after lesions. On days 16–45, OVX rats gained excess weight while displaying only a very small increase in food intake (see Fig. 5).

DISCUSSION

In the first study, male rats with posterodorsal amygdaloid lesions gained as much weight as female
Table 1. Weight changes 15 days after amygdaloid or sham lesions and 30 days after ovariectomy or sham ovariectomy

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Initial Weight</th>
<th>Day 15</th>
<th>Day 45</th>
<th>Total Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMYG-SOVX</td>
<td>5</td>
<td>308.2 ± 2.4</td>
<td>1.6 ± 1.0</td>
<td>-0.2 ± 2.3</td>
<td>1.4 ± 2.0</td>
</tr>
<tr>
<td>SAMYG-OVX</td>
<td>5</td>
<td>311.8 ± 8.7</td>
<td>2.4 ± 3.0</td>
<td>42.6 ± 4.7</td>
<td>45.0 ± 6.4</td>
</tr>
<tr>
<td>AMYG-SOVX</td>
<td>7</td>
<td>314.7 ± 8.7</td>
<td>45.3 ± 5.3</td>
<td>-15.4 ± 3.5</td>
<td>30.0 ± 2.8</td>
</tr>
<tr>
<td>AMYG-OVX</td>
<td>6</td>
<td>309.2 ± 6.5</td>
<td>43.2 ± 3.7</td>
<td>23.8 ± 5.2</td>
<td>67.0 ± 4.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals. Weight changes were measured 15 days after amygdaloid (AMYG) or sham lesions (SAMYG; day 15) and 30 days after ovariectomy (OVX) or sham ovariectomy (SOVX; day 45).

The results of Part 2 suggest that the sex difference is also not due to a disruption in the estrous cycle. VMH lesions are often followed by ovarian atrophy and a prolonged period of diestrous (20, 32, 45), but the disruption in estrous cycles is sometimes as short as 2 wk and it is not always correlated with obesity (18, 50). The amygdaloid lesions in female rats in the present study had little effect on the estrous cycle.

The role of estrogen in the weight gains of female rats is of particular interest. If estrogen played no role in lesion-induced obesity, then the weight gains that follow lesions and ovariectomies would be additive. Although two studies reported that rats with VMH lesions gained additional weight when given ovariectomies, in one of them the extra weight gain induced by ovariectomy was far less than that observed in ovarioctomized rats with sham lesions (30), and in the other the authors provided no data (51). A third study reported that ovariectomy did not result in additional weight gain in goldthioglucose-treated obese mice (5), whereas a fourth reported that obese VMH rats given ovariectomies gained less than control rats given ovariectomies (2). Thus in the case of VMH lesions, the authors provided no data (51). A third study reported that ovariectomy did not result in additional weight gain in goldthioglucose-treated obese mice (5), whereas a fourth reported that obese VMH rats given ovariectomies gained less than control rats given ovariectomies (2). Thus in the case of VMH lesions, the cumulative evidence suggests that a lesion-induced decrease in estrogen levels does play a role in the weight gain of female animals. It should be noted that the weight gains after ovariectomy and parasagittal hypothalamic knife cuts do appear to be additive (13), but it is unclear whether knife cuts have the same effect on the ovaries and estrogen levels as do VMH lesions.

In the present study, ovariectomy resulted in excessive weight gains in animals with both sham and amygdaloid lesions. The mean weight gain was greater in rats with sham lesions, but one must consider that sham ovariectomies caused a mean weight loss of 15.4 g in animals with lesions. The total mean differential gain of the two groups with lesions was about the same compared with their respective control groups (i.e., +42.6 g vs. -0.2 g for rats with sham lesions compared with +23.8 g vs. -15.4 g for rats with lesions). Individual comparisons are consistent with this. For example, the AMYG-OVX rat that gained the most weight after lesions (+60 g) gained the least amount of weight after...
ovariectomy (+8 g), which explains the negative correlation reported in part 2. However, among the AMYGSOX rats, sham ovariectomy caused the greatest weight loss (~35 g) in the rat that had gained the most weight. The difference in this comparison (43 g) is about the same as that for the group means.

The weight loss displayed by rats with amygdaloid lesions after sham ovariectomy was unexpected. When undisturbed and fed ad libitum, rats with posterdorsal amygdaloid lesions maintain their excess body weight indefinitely (24). Although the present results suggest that rats with lesions may not defend their excess weight, it should also be noted that rats with amygdaloid lesions have been found to gain excess weight even when fed an unpalatable powdered diet that caused weight loss in control animals (28).

The etiology of the excessive weight gains after amygdaloid lesions and ovariectomy appear to be quite different. Amygdaloid obesity was associated with an initial dynamic phase of hyperphagia, whereas ovariectomy resulted in excess weight gains in absence of a substantial increase in food intake, thus reflecting an enhanced metabolic efficiency.

In conclusion, the obesity-inducing effects of ovariectomy appear to be independent of, and additive to, the excessive weight gains produced by posterdorsal amygdaloid lesions. This suggests that the amygdaloid lesions had little effect on estrogen levels, and explains in large part why the sex difference is less pronounced in rats with amygdaloid lesions than in rats with VMH lesions.

Perspectives

Studies conducted over 25 years ago reported hyperphagia and obesity in cats, dogs, and primates with temporal lobectomies or amygdaloid lesions (e.g., 7, 8, 11, 15, 35, 55). However, the role of the amygdala in feeding behavior went largely ignored when later studies did not replicate these results in rats (see Ref. 26 for a review). Recent studies have demonstrated that specific amygdaloid lesions in rats do result in hyperphagia and obesity (21–24, 26–29). The present results add to the growing evidence that the lesion-induced syndrome resembles in many (22, 24, 29), but not all (28, 29), respects that which follows lesions of the VMH. This includes a dynamic and static phase (24), hyperinsulinaemia when food-restricted (22), and impaired responses to caloric challenges (29). Anatomical pathways between medial aspects of the amygdala and ventromedial hypothalamus are well established (1, 10, 31), and recent anterograde degeneration studies from our own lab confirm that obesity-inducing amygdaloid lesions result in heavy degeneration in the ventromedial hypothalamus (23). There is no degeneration in the paraventricular nuclei after the amygdaloid lesions. These results suggest an amygdaloid-ventromedial hypothalamic pathway that is critically involved in the regulation of feeding behavior.

One likely pathway is the stria terminalis. Electrical stimulation of parts of the medial amygdala suppresses food intake in food-deprived animals, and this is prevented by severing the stria terminalis (54). Two studies reported no excessive weight gain in rats given transections of the stria terminalis (4, 36), but, pertinent to the results of the present study, both studies used males. In studies that placed coronal knife cuts anterior to the VMH (which presumably would sever some of the fibers of the stria terminalis that terminate in the ventromedial hypothalamus), two of three studies that used female rats reported some excessive weight gain (16, 45, 48), and a fourth reported hyperphagia in five of nine male rats (37). These results indicate that future studies should reexamine the role of the stria terminalis in feeding behavior.

Address for reprint requests and other correspondence: B. M. King, Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148.

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