Amygdaloid-lesion hyperphagia: impaired response to caloric challenges and altered macronutrient selection

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King, Bruce M., Kirk N. Rossiter, Samuel G. Stines, Gelana M. Zaharan, Jack T. Cook, Misty D. Humphries, and David A. York. Amygdaloid-lesion hyperphagia: impaired response to caloric challenges and altered macronutrient selection. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R485–R493, 1998.—Lesions of the most posterodorsal aspects of the amygdala in female rats result in hyperphagia and moderate obesity. In the present study, rats with amygdaloid lesions did not increase their daily food intake when their powdered diet was diluted with 25 or 50% nonnutritive bulk. Control animals adjusted their food intake appropriately. In a second study, rats with lesions ate less food (lab chow pellets) than controls when allowed to eat for only 1 h/day for 10 days. In experiment 3, rats were offered a three-choice macronutrient diet. Whereas four of six control animals preferred the high-fat diet, all eight of the rats with amygdaloid lesions displayed a distinct preference for the high-carbohydrate diet, including those that had preferred the high-fat diet before surgery. These results, along with the previous finding that identical lesions result in hyperinsulinemia, indicate that the amygdala is involved in both the homeostatic regulation of food (caloric) intake and the selection of macronutrients.

amygdala; caloric regulation; macronutrient selection; rats

Lesions of the most posterodorsal aspects of the amygdala result in hyperphagia and moderate obesity in female rats fed a standard lab chow diet (19, 20, 22, 24, 25, 27). Food intake begins to return to normal levels within a few days after surgery, but the excessive weight gains (typically 50–80 g) are maintained indefinitely (22, 24). Unlike hyperphagic animals with ventromedial hypothalamic (VMH) lesions, rats with lesions of the posterodorsal amygdala are not finicky, i.e., they do not overrespond, regardless of the taste stimuli added to their diet or water supply (26). These results are similar to those reported in much older studies of cats, dogs, and primates given amygdaloid lesions or temporal lobectomies (e.g., 1, 4, 5, 12, 15, 36, 43, 58).

The nature of the feeding disorder that results from amygdaloid lesions has yet to be determined. Some early investigators suggested that hyperphagic animals with amygdaloid lesions were best described as polyphagic, as they would readily consume usually nonpreferred foods, or even feces (1, 4, 5, 43). Others have hypothesized that the amygdala is important in matching current stimuli with past experiences and activating appropriate motivated responses (e.g., feeding) for which the effector mechanisms are located in the hypothalamus (14). Lesions centered in the posterodorsal aspects of the amygdala do, in fact, result in considerable degeneration of the VMH nuclei (21).

Preliminary studies from this lab suggest that the amygdala may additionally play a role in the homeostatic regulation of feeding behavior. Similar to rats with VMH lesions, rats with lesions of the posterodorsal aspects of the amygdala are hyperinsulinemic even when food restricted to the intake of control animals (20). The present series of studies directly tests whether this area of the amygdala is involved in homeostatic functions related to feeding. The first two studies examine the ability of animals with amygdaloid lesions to regulate their intake of calories, i.e., their ability to adjust their intake when the diet is diluted with nonnutritive bulk or when food is presented for only a short period each day.

When rodents are given a dietary choice, the selection of macronutrients varies both within and across strains (38, 49). The mechanisms underlying these macronutrient preferences are unclear, but it has been established that they may be influenced by various neuropeptides and metabolic inhibitors. Thus neuropeptide Y promotes carbohydrate ingestion (31), κ-opioids may promote fat intake (39, 47), and enterostatin inhibits fat intake (11, 34, 38, 59), while both 2-deoxy-D-glucose and β-mercaptoacetate promote carbohydrate feeding (3, 17). Although there have been no systematic studies of the sites at which these agents influence macronutrient choice, several studies have found that rats with VMH lesions prefer a high-fat diet (2, 6, 8, 16, 28). Recent results suggest, on the other hand, that rats with posterodorsal amygdaloid lesions do not overrespond when switched from lab chow to a high-fat diet (24, 26). Therefore, a third study examines the effects of amygdaloid lesions on macronutrient selection.

METHOD

Subjects

Forty-four adult female 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.

Lesions 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. With the upper incisor bar positioned horizontally with the interaural line, the electrodes were positioned 2.1 mm posterior to bregma, 4.5 mm lateral to the midsagittal suture, and 8.4 mm below the surface of the skull. For control animals, holes were drilled in the skull, but the electrode was not lowered into the brain because previous studies demonstrated that damage dorsal to the effective site results in weight loss (10, 24). After completion of the experiment, the animals 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 (41).

Procedure

Part 1. The first study examined the response of rats with amygdaloid lesions (n = 16) and control animals (n = 6) to dilution of their diets with nonnutritive bulk (Alphacel; ICN Biomedicals, Aurora, OH). All animals had been maintained postoperatively for 9 days on a powdered high-carbohydrate diet for another study (26) (1 animal from each group in the previous study was eliminated at the start of this study because their incisions had opened). They were then switched to powdered lab chow (Harlan Teklad rat diet LM-485) for the next 4 days. On postoperative days 14–17, the animals were fed a 25% Alphacel-diluted diet, followed by a 50% Alphacel-diluted diet on days 18–21. Spillage was collected on paper towels. Food intake and body weight were measured daily.

Part 2. On the completion of part 1, the animals were returned to a pellet lab diet (LM-485) for 5 days. On postoperative day 26 they were deprived of food for 23 h and then allowed to eat for 1 h. A feeding schedule of 23-h deprivation and 1-h food presentation was then maintained for 9 more days. Spillage was collected on paper towels. Food intake and body weight were measured daily.

Part 3. This study compared the intake of rats with amygdaloid lesions (n = 16) and control animals (n = 6) on a three-choice macronutrient diet used previously by Smith et al. (50) (see Table 1). The animals were allowed to choose from three food cups (secured to the inside of the cages with springs) that contained either a carbohydrate, fat, or protein diet, each supplemented with vitamins and minerals. Spillage was collected on paper towels. Food intake and body weight were measured daily. All the animals were presented with the diets for 7 days before surgery. After surgery, the animals were maintained on the diets for 10 additional days.

Statistical Analyses

The data were analyzed with use of the t-test, two- or three-way ANOVA, and, when appropriate, Tukey's honestly significant difference test.

RESULTS

Seven of sixteen animals given electrolytic lesions in parts 1 and 2 and 8 of 16 animals in part 3 had damage centered bilaterally in the most posterdorsal aspects of the amygdala ("posterodorsal" refers to an area, not a specific nucleus). Two representative lesions are shown in Fig. 1. Damage included the posterodorsal medial amygdala and the intra-amygdaloid division of the bed nucleus of the stria terminalis. Previous work has shown that weight gain is best related to damage of the latter and that ancillary damage to the caudal portions of the central nucleus (which often occurs) is negatively correlated with weight gain (21). Lesions that missed varied considerably (some missed unilaterally, some bilaterally, some damaged motor areas, whereas others did not, etc.), and the data for these animals were not included in the analysis. The "hit rate" is low because the critical site has proven to be quite small. Damage just medial to the site destroys the optic tract(s), whereas damage just posterior to the site results in damage to the hippocampal formation rather than to the amygdala. Damage just dorsal to the effective site results in damage to areas of the brain involved in motor control. More detailed histological analysis is provided elsewhere (19, 21, 24).

Part 1

In the first week after surgery, the rats with lesions were hyperphagic compared with control animals [mean daily food intakes for days 2–8 of 31.1 ± 1.8 and 18.5 ± 3.0 g, respectively; t = 3.72, degrees of freedom (df) = 11, P < 0.005]. However, food consumption of the rats with lesions decreased thereafter, and the daily predilution food intake (averaged over 2 days) was not significantly different between groups (19.2 ± 1.5 g for controls and 22.4 ± 1.5 g for rats with lesions). Mean weight gains before dilution of the diet were 11.7 ± 4.0 g for controls and 50.0 ± 4.6 g for rats with lesions (t = 6.17, df = 11, P < 0.001). Mean daily food intakes, expressed as a percentage of predilution intake, are displayed in Fig. 2. A two-factor (2 × 8) ANOVA with repeated measures on one factor revealed a significant main effect for groups (F = 8.37; df = 1,11; P < 0.05) and for the interaction (F = 8.71; df = 7,77; P < 0.001). Control animals adjusted their daily intake in accord with the caloric density of the diet, displaying a significant increase on the 25%-diluted diet and a further increase on the 50%-diluted diet (mean of third and fourth days, P < 0.01). The rats with amygdaloid lesions, on the other hand, failed to increase their food intake significantly when the powdered diet was diluted with Alphacel. As a result, the mean postoperative weight gain for the rats with lesions was only 31.8

Table 1. Composition of three-choice macronutrient diet

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>58.11</td>
<td>0.00</td>
</tr>
<tr>
<td>Powdered sugar</td>
<td>29.06</td>
<td>0.00</td>
</tr>
<tr>
<td>Casein</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.11</td>
<td>0.20</td>
</tr>
<tr>
<td>Vegetable shortening</td>
<td>0.00</td>
<td>75.12</td>
</tr>
<tr>
<td>AIN-76A vitamin mix</td>
<td>0.77</td>
<td>1.49</td>
</tr>
<tr>
<td>AIN-76A mineral mix</td>
<td>3.07</td>
<td>5.95</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.18</td>
<td>0.34</td>
</tr>
<tr>
<td>Cellulose (Alphacel)</td>
<td>8.72</td>
<td>16.91</td>
</tr>
<tr>
<td>Energy density (kcal/g)</td>
<td>3.53</td>
<td>6.85</td>
</tr>
</tbody>
</table>

Values are % by weight.
Fig. 1. Coronal sections of bilateral lesions of the posterodorsal aspects of the amygdala in 2 rats (A and B) at the level of maximum damage. Effective lesions always included the intra-amygdaloid division of the bed nucleus of the stria terminalis.
planned comparison of food intakes after the first 23 h of deprivation (day 1, Fig. 3) revealed that the control animals consumed significantly more than the animals with lesions (t = 3.61; df = 11; P < 0.005). In the 10 days of testing, mean weight loss for control animals was 38.7 ± 4.3 g compared with 41.1 ± 4.0 g for the animals with lesions. By the tenth day the rats with amygdaloid lesions weighed 3.7 ± 4.8 g less than they had preoperatively, while the control animals weighed 20.2 ± 7.2 g less than preoperatively (t = 1.95, df = 11, P > 0.05).

Part 3

Mean daily intakes of the three macronutrients for the 3 days immediately before surgery and for two 3-day postoperative periods that show the acute (days 1–3) and longer-term (days 8–10) effects of the surgeries are displayed in Figs. 4 (control animals) and 5 (animals with lesions). A three-factor (2 × 3 × 3) ANOVA with repeated measures revealed a significant main effect for macronutrients (F = 11.79; df = 2, 24; P < 0.0001), the groups × days interaction (F = 4.39; df = 2, 24; P < 0.05), the days × macronutrient interaction (F = 2.66; df = 4, 48; P < 0.05), and the groups × days × macronutrient interaction (F = 3.14; df = 4, 48; P < 0.05). A univariate F test revealed a significant group × day interaction for the prelesion period vs. the first 3 days after lesions (F = 5.15, P < 0.05). The major contributing factor was a difference between groups in the intake of the high-carbohydrate diet after surgery (P < 0.05).

At the time of surgery, the animals could clearly be divided into three groups: those that preferred the high-fat diet (Figs. 4 and 5, A), those that preferred the high-carbohydrate diet (Figs. 4 and 5, B), and those that did not show a preference between the high-fat and high-carbohydrate diets (Figs. 4 and 5, C). Among the control animals, those that preferred the high-fat or high-carbohydrate diets continued to do so throughout the 10 days after surgery. The two control rats that showed no preference at the time of surgery gradually developed a preference for the high-fat diet by the end of the study. Thus four of six control animals eventually came to prefer the high-fat diet.

All eight of the rats with amygdaloid lesions preferred the high-carbohydrate diet. The effect of the lesions was immediate and often dramatic. As can be seen in Fig. 5, A and C, rats that had displayed a preference for the high-fat diet before surgery displayed a strong preference for the high-carbohydrate diet afterward, and the same was true of rats that had displayed no clear preference before surgery.

During the 10 postoperative days on the macronutrient diets, the rats with amygdaloid lesions gained 41.6 ± 3.7 g compared with 19.8 ± 9.3 g for the control animals (t = 2.41, df = 12, P < 0.05). Two of the control animals that greatly preferred the high-fat diet gained as much as most of the rats with lesions.

Fig. 2. Mean daily intake (±SE) of control rats and rats with amygdaloid lesions on a diet of ground lab chow diluted with 25% nonnutritive bulk (days 1–4) or 50% nonnutritive bulk (days 5–8). Intakes are expressed as a percentage of predilution food intake (dotted line).

Fig. 3. Mean food intakes (±SE) of control rats and rats with amygdaloid lesions allowed to eat (lab chow pellets) for only 1 h/day for 10 days.
DISCUSSION

The results of the first study indicate that the amygdala plays a role in monitoring caloric intake. Control animals adjusted their food intake quickly and accurately both times their diet of ground lab chow was diluted with nonnutritive bulk. The rats with amygdaloid lesions, on the other hand, were unresponsive and continued to consume the same amount of food (by weight) as they had before dilution. Thus they displayed a decline in daily caloric intake during the period of dilution. The diluted diets were first presented 14 days after surgery, and, although it is unknown whether the results would have been the same had the study been conducted in the immediate postsurgical period, the difference between groups cannot easily be attributed to obesity. At the time the animals were fed the 50%-diluted diet, the groups differed in weight gain by only $\pm 30$ g.

Lesions of the posterodorsal aspects of the amygdala result in degeneration within the shell and core of the VMH nuclei (21), so comparisons of the two lesion-induced obesity syndromes are inevitable. Similar to the present results, several studies have found that VMH-lesioned rats also do not increase food intake appropriately when their powdered diet is diluted with nonnutritive bulk (33, 53, 54). Others have reported normal caloric adjustments with dilution of liquid or solid diets (52, 53, 57), and numerous studies have found that already hyperphagic VMH-lesioned rats greatly increase their daily caloric intake when switched to a high-fat (high-caloric density) diet (e.g., Refs. 6, 8, 37, 53) or when their diet is sweetened with sugar (33, 54). In summary, studies of the ability of VMH-lesioned rats to regulate caloric intake suggest a dysfunction, but interpretation is limited by the confounding factor that the animals are also hyperreactive to the palatability of their diet.

The results of the present study cannot easily be attributed to hyperreactivity to the taste and/or texture of food by rats with lesions. In anticipation of conduct-
ing this study, rats with posterodorsal amygdaloid lesions were previously tested for their reactivity to a powdered diet, a sucrose solution, and quinine in the food or water supply (26). The animals were not finicky. In fact, when placed on a very fine powdery, high-carbohydrate diet, control animals lost weight while animals with lesions gained weight. Rats with amygdaloid lesions also did not overrespond when switched from lab pellets or powder to a high-fat diet and back again (24, 26).

The results of the second study further support the conclusion that the amygdaloid lesions disrupted homeostatic functions regulating food intake. Here animals were allowed to eat standard lab pellets for only 1 h/day. Although rats with posterodorsal amygdaloid lesions are hyperphagic when allowed lab pellets ad libitum, they consumed less than control animals and thus appeared to be less hungry throughout the 10 days of testing. In fact, after the first 23-h period of deprivation, control animals consumed over twice as much food as the rats with lesions. One must be cautious when interpreting results of different behavioral tests conducted on the same animals over time, not only because of the possibility of different functional states in the brain after lesions, but also because experience in one paradigm may influence behavior in a subsequent one (e.g., Ref. 9). However, the first day of part 2 followed the last day of part 1 by only 6 days, and, although the two caloric challenges were quite different, on neither day did the rats with lesions respond as well as control animals.

Three studies have reported that rats with VMH lesions eat less than control animals after food deprivation or when on a restricted feeding schedule (35, 40, 51), but another study, which observed just the opposite for VMH-lesioned rats early in the dynamic phase of hyperphagia, attributed some of the earlier results to a lack of hyperphagia (i.e., poor lesions). The rats in one of the studies had already gained 23.6% of body weight, and obesity has proven to be an important confounding
factor in many studies of the motivation for food of VMH-lesioned rats (see Ref. 18). In the present study (part 2), because of prior testing procedures, the rats with amygdaloid lesions had gained only slightly more weight than controls at the start of the first day of deprivation. There was no significant difference between groups by day 10, when both groups were below their preoperative body weights. Thus, as in part 1, obesity should not have been a confounding factor.

The third experiment of the present study demonstrates a major difference between rats with VMH lesions and those with lesions of the posterodorsal amygdala. Several studies have found that rats with VMH lesions greatly prefer a high-fat diet over any other diet (e.g., Refs. 2, 6, 8, 16, 28). Even VMH-lesioned animals that do not overeat (high carbohydrate) lab chow become hyperphagic and obese when switched to a high-fat diet (35, 37). Two studies found that hyperphagic VMH-lesioned rats initially preferred a high-carbohydrate diet when allowed to self-select macronutrients, but the animals quickly switched their preference to the high-fat diet (45, 46). The preference for high-fat diets cannot be explained solely on the basis of palatability preferences (finickiness) (6). In contrast, the rats with amygdaloid lesions clearly showed an initial preference for the high-carbohydrate diet and maintained that preference over the 10 days of testing. Even those animals that preferred the high-fat diet before surgery very quickly switched to the high-carbohydrate diet afterward. This marked and sustained preference for carbohydrates helps explain why, when maintained on just a high-carbohydrate diet, rats with amygdaloid lesions gained weight while control animals lost weight (26).

Recent studies of the effects of various manipulations on macronutrient intake indicate that the source of carbohydrate influences the extent to which animals select a high-carbohydrate diet. A high-carbohydrate diet is much more likely to be preferred over other diets when the main source is sucrose rather than corn starch (13, 32). In the present study, the high-carbohydrate diet consisted of 58.1% corn starch, 29.1% sugar, and 8.7% cellulose. We cannot be certain that the results would have been the same with an even greater proportion of corn starch, but the rapidity with which the change in preference often occurred and the lack of evidence for any lesion-induced change in the affective response to the taste or texture of their diet (26) suggest that the lesions had an effect on central mechanisms involved in macronutrient selection.

The background preference of individual animals also strongly influences their macronutrient intake observed after experimental manipulations (37, 56). In the present study, some of the animals had just established a preference for fat or carbohydrate at the time of surgery and a few had not yet established a preference. We, again, cannot be certain that all rats initially preferring a high-fat diet would switch to a high-carbohydrate diet after lesions if allowed a longer presurgical period of adaptation.

To date, the brain structure that has been most implicated in macronutrient selection, particularly carbohydrate selection, has been the hypothalamic paraventricular nucleus (PVN) (e.g., Refs. 29, 30, 50, 55). However, anterograde tracing reveals no degeneration in the PVN after obesity-producing lesions of the posterior amygdala (21), so it is doubtful that the effects found in the present study are directly mediated by this structure. Recent evidence suggests that insulin may play a role. Chavez et al. (7) reported that insulin injected into the third ventricle of rats offered a choice of three macronutrients resulted in a selective reduction of dietary fat. Rats with posterodorsal amygdaloid lesions are hyperinsulinemic even during an initial period of food restriction to levels of control animals (20). Rats with VMH lesions are also hyperinsulinemic (see Ref. 23), but it is likely that such lesions would also destroy many of the insulin receptors.

Enterostatin, a peptide released by both the exocrine pancreas and the gastric mucosa, acts both peripherally and centrally to selectively reduce fat intake (11, 59). Recently, one of us (34) mapped the central sites of action of enterostatin and showed the central bed nucleus of the amygdala to be most sensitive, with dosages as low as 10 pmol inhibiting fat intake at that site. The amygdala is also responsive to galanin, a peptide that was initially thought to preferentially enhance fat intake but is now thought to affect intake of the preferred macronutrient (48). Thus it is possible that the lesions used in these current studies, although in a different subarea of the amygdala, disrupted an enterostatin-sensitive pathway that normally promotes fat intake and that in this situation rats eat carbohydrate to maintain their caloric intakes. Alternatively, it is possible that there is also an inhibitory system for carbohydrate feeding located in the amygdala, although no such system has yet been identified. The β-mercaptoacetate stimulation of feeding, which is carbohydrate selective when macronutrient choices are available (3), is also blocked by lesions of the central nucleus of the amygdala (44).

Perspectives

Investigations of the role of the brain in feeding behavior have focused almost exclusively on hypothalamic regulation. Results of early studies demonstrating hyperphagia and obesity in cats, dogs, and primates with temporal lobe damage (e.g., Refs. 1, 4, 5, 12, 15, 36, 43, 58) were largely ignored when numerous studies did not replicate the findings in rats (see Ref. 24 for a review). However, recent studies demonstrate that lesions in the most posterodorsal aspects of the amygdala (including the intra-amygdaloid division of the bed nucleus) result in hyperphagia and moderate obesity in rats and that the effective site projects heavily to the VMH (19–22, 24, 25, 27).

Although the function of the amygdala in feeding behavior has usually been viewed as modulating the hypothalamus with respect to appetite or affect, the present results indicate that at least part of the amygdala is involved in homeostasis. A previous study found
that rats with posterodorsal amygdaloid lesions were hyperinsulinemic even when food restricted (20). The present results indicate that the lesions also impair the ability to adjust food intake in response to caloric challenges and that this area of the brain is directly involved in macronutrient selection. Similar results are challenges and that this area of the brain is directly involved in modulating the role of the VMH in hunger.

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