Combination unilateral amygdaloid and ventromedial hypothalamic lesions: evidence for a feeding pathway

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Lesions of the most posterodorsal part of the amygdala result in hyperphagia and excessive weight gain in rats (42). Female rats with bilateral lesions typically gain 50–80 g in 20 days, and gains of as much as 100 g in 20 days have been observed (15, 16, 18, 20, 21). The obesity syndrome resembles that which follows lesions of the ventromedial hypothalamus (VMH) in many respects. For example, the weight gains are greater in female rats than in male rats (23), and the animals are hyperinsulinemic even when food restricted (16) and do not respond appropriately to caloric challenges (24).

Excessive weight gains have been produced with small lesions limited exclusively to the posterodorsal medial amygdaloid nucleus and the intra-amygdaloid bed nucleus of the stria terminalis (42). Examination of anterograde degeneration by the amino-cupric silver method in the brains of rats given unilateral posterodorsal amygdala (PDA) lesions revealed a dense pattern of degenerating terminals in areas that have previously been shown to be involved in various aspects of feeding behavior and/or body weight regulation (17). Particularly dense staining was observed in the VMH ipsilateral to the PDA lesion, but not in the contralateral VMH. There was little to no staining in the paraventricular hypothalamic nucleus.

The pattern of anterograde degeneration observed after unilateral PDA lesions suggests an ipsilateral pathway mediating feeding behavior and body weight regulation. The major pathway between the PDA and VMH is the stria terminalis (5, 6, 39), and after unilateral PDA lesions there is dense degeneration in the dorsal component and medial part of the ventral component, but not in the commissural component (17). Three studies reported no excess weight gain after transections of the stria terminalis and concluded that it was not involved in feeding behavior (2, 3, 36). However, all three studies used male rats. A more recent study that used female rats found both hyperphagia and excessive weight gain when the stria terminalis was transected just as it exits the amygdala (22). Hyperphagia and obesity have also been observed in rats given coronal knife cuts just anterior to the VMH (11, 37, 45). Although not verified histologically, these cuts presumably would have transected the fibers of the dorsal component of the stria terminalis terminating in the VMH.

The present study used unilateral lesions to ascertain whether the effects of PDA and VMH lesions on body weight are additive. If both are part of an ipsilateral pathway involved in feeding behavior and body weight regulation, unilateral PDA lesions ipsilateral to a unilateral VMH lesion should result in no greater weight gain than a unilateral VMH lesion alone. On the other hand, weight gain after a unilateral VMH lesion and a contralateral PDA lesion would be additive.

METHODS

Animals

A total of 70 adult female Long-Evans hooded rats were used (Harlan Sprague-Dawley, Indianapolis, IN). All animals were individually housed in standard wire-mesh rat cages (9.5 in. long × 7 in. wide × 7 in. high; no activity wheel) in a temperature-controlled colony (21–24°C) with a 12:12-h light-dark cycle (lights on at 7 AM) throughout the experiment.

Lesions and Histology

Unilateral PDA and VMH lesions were produced under 85 mg/kg ketamine HCl (plus 10 mg/kg xylazine) anesthesia by passing a 1.5-mA anodal current for 20 s between the uninsulated tip (0.2 mm for PDA; 0.5 mm for VMH) of an insulated stainless steel electrode (Plastics One, Roanoke, VA) and a rectal cathode. Electrodes were positioned with a Kopf small-animal stereotaxic instrument. With the upper incisor bar positioned horizontally with the interaural line, the electrodes for PDA lesions were positioned 1.7 mm posterior to bregma, 4.5 mm lateral to the midsagittal suture, and 8.4 mm below the surface of the skull. For VMH lesions, the electrodes were placed on the surface of the skull. For VMH lesions, the electrodes were...
positioned 1.2 mm posterior to bregma, 0.6 mm lateral to the mid-sagittal suture, and 9.9 mm below the surface of the skull. Animals with sham lesions had holes drilled in the skull at the same coordinates, and electrodes were lowered to a depth 1.0 mm above the target site.

Upon completion of the study, the rats with lesions were euthanized and perfused with physiological saline followed by a 10% Formalin solution. The brains were stored in 10% Formalin and later frozen and sliced into 40-μm coronal sections. The sections were stained with cresyl violet, and initial histological analysis was performed in a blinded fashion (i.e., without knowing changes in body weight) by light microscopic examination. The extent of the lesions was determined with the use of the stereotaxic atlas by Paxinos and Watson (38).

**Procedure**

Five groups of animals were given combination lesions 20 days apart: sham VMH lesions-sham PDA lesions \( (n = 9) \), sham VMH lesions-unilateral PDA lesions \( (n = 14) \), unilateral VMH lesions-sham PDA lesions \( (n = 11) \), unilateral VMH lesions-ipsilateral PDA lesions \( (n = 18) \), and unilateral VMH lesions-contralateral PDA lesions \( (n = 18) \). All animals were fed Harlan Teklad mouse/rat diet LM-485 for 20 days after each surgery. Body weight and food intake (corrected for spillage) were measured daily.

**RESULTS**

Histological analysis revealed that 36 of 47 rats given VMH lesions had damage centered in the ventromedial hypothalamic area. Data for 11 animals were eliminated because the lesions extended beyond the midline (into the other hemisphere), beyond the fornix, or were too dorsal. Of the 36 rats with good VMH lesions, 29 received unilateral PDA lesions, as did 14 rats that initially received sham VMH lesions. Twenty-three of these animals had damage centered in the most posterodorsal part of the amygdala. The “hit rate” is low because the critical site is very small. The damage must be in the most posterodorsal part of the amygdala just adjacent to the tip of the optic tract, yet not extend dorsally into the immediately adjacent globus pallidus, damage to which attenuates weight gain. Lesions that are just lateral to this site result in very little weight gain. Detailed histological analysis of the effective lesion site has previously been described (17, 42). Coronal sections of the brains for six rats given both unilateral VMH and unilateral PDA lesions (3 ipsilateral and 3 contralateral) are provided in Fig. 1. The final sample sizes are provided in Table 1.

![Coronal sections of three rats with combination unilateral ventromedial hypothalamus (VMH)-ipsilateral posterodorsal amygdala (PDA) lesions (A–C) and three rats with combination unilateral VMH-contralateral PDA lesions (D–F). The first weight change is that observed after a unilateral VMH lesion (days 0–20) and the second weight change is that observed after a subsequent unilateral PDA lesion (days 20–40).](http://ajpregu.physiology.org/)
Mean daily body weights are displayed in Fig. 2, and the final 20-day mean (± SE) weight gains are displayed in Table 1. A planned comparison revealed that rats with unilateral VMH lesions gained significantly more weight than rats with sham lesions ($t = 46.54$, $df = 34$, $P < 0.001$, measure of effect size $r = 0.99$). The weight gain in rats with VMH lesions ranged from 29 to 77 g, compared with $-12$ g to $+15$ g for rats with sham lesions. The mean 20-day weight gain in all three groups with VMH lesions exceeded 50 g. The body weights of all 22 rats with unilateral VMH lesions reached a plateau (i.e., weight gain that did not exceed that of rats with sham VMH lesions) between postoperative days 10 and 16.

Weight gain after the second lesion depended on whether the unilateral amygdaloid lesion was ipsilateral or contralateral to the initial VMH lesion. A second planned comparison revealed that rats with contralateral PDA lesions gained significantly more weight than rats with ipsilateral lesions (32.0 g vs. 0.6 g, $t = 19.77$, $df = 34$, $P < 0.001$, $r = 0.96$). The mean 20-day weight gain in rats with contralateral PDA lesions was identical to that in rats given unilateral PDA lesions 20 days after receiving sham VMH lesions. The weight gain in rats with contralateral PDA lesions ranged from $+19$ g to $+43$ g (5 of the 8 rats gained $>35$ g) compared with $-14$ g to $+12$ g for rats that received sham lesions after initial unilateral VMH lesions. The body weights of rats with contralateral PDA lesions reached a plateau (i.e., weight gain that did not exceed that of rats with sham PDA lesions) between postoperative days 29 and 37 (9 to 17 days after the PDA lesions).

There was one outlier in the VMH-ipsilateral PDA lesion group: a rat that gained 51 g after the initial hypothalamic lesion (reaching a plateau on postoperative day 16) and then another 39 g after the PDA lesion (3.29 SD above the mean). However, during the first surgery, there was an error in reading the Vernier scale, and the lesion was dorsal to the VMH, sparing the VMH completely and instead damaging the dorsomedial hypothalamus and posterior half of the paraventricular nucleus (see Fig. 3). Data for this animal were eliminated from the analysis.

Food intake paralleled body weight gain. The animals with unilateral VMH lesions were initially hyperphagic, as were the animals with unilateral PDA lesions in the sham VMH-ipsilateral PDA and unilateral VMH-contralateral PDA group. The animals in the unilateral VMH-ipsilateral PDA group displayed no increase in food intake after the unilateral amygdaloid lesion. For animals that were hyperphagic, daily food intake was greatest 2 to 7 days after surgery (see Table 1) but had returned to control levels by day 20.

**DISCUSSION**

The results of the present study replicate previous studies that observed moderate hyperphagia and obesity after unilateral lesions of the VMH (31, 32) or PDA (17). It is clear from Fig. 2 that the rats with unilateral VMH lesions maintained their excess body weight (compared with the sham VMH-sham PDA lesion animals) for at least 40 days. A previous study found that female rats with bilateral PDA lesions defend their higher body weight for at least 60 days (18) (although at a lower level of weight gain than rats with bilateral VMH lesions), and the present results show that female rats with unilateral PDA lesions do so for at least 20 days. Thus it can be concluded that female rats with either VMH or PDA lesions, whether bilateral or unilateral, are similar in that both display a dynamic and static phase of weight gain (4). Previous studies, as well as the present one, have demonstrated that the rate of weight gain in rats with PDA lesions is directly reflective of the hyperphagia (e.g., 15, 20, 23), and the hyperinsulinemia (16) probably helps to maintain the excess body weight. Activity level in rats with PDA lesions has not yet been monitored.

A previous study of the anterograde degeneration after unilateral PDA lesions found a dense pattern of degenerating terminals in the dorsal component of the stria terminalis and ipsilateral VMH, but not in the contralateral VMH (17). Here, a combination of unilateral sham, VMH, and PDA lesions was used to ascertain the functional nature of this anatomic pathway between the PDA and VMH. Unilateral PDA lesions given 20 days after initial unilateral VMH lesions resulted in

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**Table 1. Changes in body weight and food intake after VMH and PDA lesions**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Initial Weight, g</th>
<th>Weight Change, g</th>
<th>Food Intake, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Days 0–20</td>
<td>Days 20–40</td>
</tr>
<tr>
<td>Sham VMH-sham PDA</td>
<td>9</td>
<td>292.0 ± 3.8</td>
<td>5.0 ± 2.8</td>
<td>4.7 ± 1.9</td>
</tr>
<tr>
<td>Sham VMH-unilateral PDA</td>
<td>8</td>
<td>304.0 ± 5.7</td>
<td>1.4 ± 2.4</td>
<td>32.0 ± 3.8</td>
</tr>
<tr>
<td>VMH-sham PDA</td>
<td>7</td>
<td>291.1 ± 5.5</td>
<td>55.3 ± 4.9</td>
<td>3.5 ± 2.4</td>
</tr>
<tr>
<td>VMH-ipsilateral PDA</td>
<td>7</td>
<td>312.1 ± 7.7</td>
<td>55.8 ± 5.7</td>
<td>0.5 ± 4.9</td>
</tr>
<tr>
<td>VMH-contralateral PDA</td>
<td>8</td>
<td>291.1 ± 5.5</td>
<td>50.7 ± 4.8</td>
<td>32.0 ± 3.5</td>
</tr>
</tbody>
</table>

Values are given as means ± SE. All animals were given sham or unilateral ventromedial hypothalamic (VMH) lesions (day 0) and then were given sham or unilateral posterdorsal amygdala (PDA) lesions on day 20.
no further excess weight gain when the amygdaloid lesions were ipsilateral to the VMH lesions but resulted in additional excess weight gains when the amygdaloid lesions were contralateral to the initial VMH lesions. All rats with unilateral VMH lesions had reached a plateau in body weight by postoperative day 16. The fact that rats with unilateral VMH lesions did not display further excess weight gains after a sham PDA lesion additionally demonstrates that the weight gains in the unilateral VMH-contralateral PDA group during days 20 to 40 were not due to the long-term effects of the VMH lesions. The mean weight gain after PDA lesions in the VMH-contralateral PDA group was nearly identical to that observed after PDA lesions in the sham VMH lesion-contralateral PDA group. Therefore, it is concluded that the effects of the two lesions are not additive and that the weight gain observed after VMH or PDA lesions represents damage at two different points in a common pathway. The mean total weight gain of 82.7 g observed in the unilateral VMH-contralateral PDA group over 40 days is consistent with the typical weight gains of 50–80 g over 20 days previously observed in female rats with bilateral PDA lesions (15, 16, 18, 20; see also 42).

Early studies that used cats and dogs observed both hyperphagia and obesity after the animals were given bilateral lesions of the amygdala (e.g., 7, 10, 26, 34, 35, 52), but a possible role of the amygdala in feeding behavior was largely ignored after little or no effects on food intake or body weight were observed in rats with lesions of the amygdala (see Ref. 42 for a review) or transections of the stria terminalis (2, 3, 36). However, all three studies of the stria terminalis used male rats, but marked hyperphagia and excessive weight gains are observed in female rats when the stria terminalis is cut bilaterally just as it exits the amygdala (22) or at its most dorsal point before it begins to descend toward the hypothalamus (unpublished observation). Transections of the stria terminalis also prevent the suppression of food intake caused by electrical stimulation of the medial amygdala (50). Thus it is proposed that the stria terminalis is the major pathway by which the amygdala’s influence on feeding behavior and body weight is mediated and that the pathway is, for the most part, ipsilateral.

Only a few other studies have observed the effects of combination amygdaloid and VMH lesions on food intake and weight gain, and all used bilateral lesions. Ganaraja and Jeganathan (8) observed that male rats with basolateral amygdaloid (BLA) lesions displayed a modest hyperphagia and weight gain (only 10 g more than controls) but that the lesions reduced the effects of VMH lesions on food intake and weight gain when the two lesions were given sequentially (either BLA + VMH or VMH + BLA). Sclafani et al. (43) reported that female rats with corticomedial amygdaloid (CMA) lesions did not eat more or gain more weight than control animals and that rats with combined CMA + VMH lesions gained no more weight than rats with VMH lesions. In perhaps the most important of these studies, Morgane and Kosman (33) reported that cats with simultaneous electrolytic lesions of the amygdala and VMH + lateral hypothalamus (LH) (LH lesions alone did not cause weight loss) gained weight 3 times faster than had been observed in an earlier study with cats given open suction amygdalecotomies (34, 35). Morgane and Kosman (33) concluded that “the production of amygdalar hyperphagia in the absence of much of the middle hypothalamus strongly suggests that amygdalar effects upon food intake are not mediated through this part of the brain” (p. 1318). However, it is not unreasonable to assume that cats subjected to open amygdalecotomy would have taken longer to recover than cats given much simpler electrolytic lesions. Most importantly, the total weight gain for the amygdala + VMH-LH lesioned cats was the same as had previously been observed for cats with amygdalecotomies (about 24% gain compared with 2.3% for controls). The identical weight gain again suggests that the effects of the two lesions on body weight are not additive.

Previous studies have demonstrated that up to 40% of the weight gain in female rats with electrolytic lesions of the VMH may be due to metallic ion deposits stimulating vagally mediated insulin responses (see Ref. 14). Electrolytic lesions were nevertheless chosen for the present study 1) because they allow for a more direct comparison with previous studies [the large majority of VMH studies and nearly all amygdaloid studies used direct current (DC) lesions], and more importantly, 2) because of the desire to see the effects of PDA lesions on body weight in rats that had already gained the maximum amount of weight possible after VMH lesions. Because of the clear pattern of results (i.e., no further weight gain in rats given a PDA lesion ipsilateral to an initial unilateral VMH lesion), the use of DC lesions does not affect the present conclusions.

Some researchers have dismissed any role of the VMH in feeding behavior. This is the result of a couple of studies that did not observe any hyperphagia or excessive weight gain after VMH lesions (9, 13) and the subsequent discovery that lesions of the nearby paraventricular hypothalamic nucleus (PVN) did result in hyperphagia and obesity (e.g., 1, 29). However, this ignores the hundreds of studies that have observed hyperphagia and obesity after VMH lesions as well as subsequent studies that found major differences between the two lesion-induced obesity syndromes (e.g., 25, 47, 48). It is of interest, therefore, that the weight gain after an initial unilateral PVN-dorsomedial hypothalamic lesion and subsequent ipsilateral lesion of the PDA was additive. The previous study that traced anterograde degeneration after unilateral PDA lesions found little or no degenerating terminals in the PVN (17). Thus the PVN appears
to be involved in a feeding pathway that is separate from the one proposed here.

The presence of an ipsilateral pathway mediating feeding behavior and body weight regulation that includes both the posterodorsal medial amygdala and the VMH has now been demonstrated by both anatomical (17) and behavioral (present study) techniques. A major role for the stria terminalis, the direct pathway between the PDA and VMH, has also been established (22). However, it should be noted that the weight gain typically observed after bilateral VMH lesions is much greater than that observed after bilateral PDA lesions or stria terminalis transections (e.g., 4, 19), and thus the PDA-stria terminalis pathway is most certainly only one of many influences upon the VMH affecting feeding behavior and body weight regulation. The amygdala itself can influence hypothalamic function by means other than the direct pathway of the stria terminalis. Anatomic studies by Petrovich et al. (39) have shown that amygdaloid nuclei also influence hypothalamic function indirectly via the lateral septum, ventral hippocampus, and bed nucleus of the stria terminalis. Unilateral lesions of the PDA also result in dense anterograde degeneration in the ipsilateral lateral septum, medial bed nucleus of the stria terminalis, medial preoptic area, and subparaventricular area (17). It is of interest that lesions in the lateral septum also result in excessive weight gains for female but not male rats (44, 49).

Perspectives

Lesions of the VMH, stria terminalis, and posterodorsal medial amygdala all result in hyperphagia and excess weight gains in female rats, an indication that all three play an inhibitory role in food intake. Although olfactory bulbectomy by itself does not result in hyperphagia or weight gain, bilateral bulbectomy in obese, static-phase, VMH-lesioned rats does result in additional weight gain (28), suggesting that the olfactory bulbs also play an inhibitory role. There is also some evidence that food odors inhibit food intake in humans (12). Because of the well-established input to the CMA nuclei from the olfactory bulb (e.g., 27), it is tempting to speculate about an olfactory bulb-medial amygdaloid-VMH inhibitory pathway for feeding behavior. The comparison with sexual behavior, which also is highly sensitive to olfactory cues, cannot be ignored. The pattern of anterograde degeneration observed after PDA lesions is remarkably similar to that outlined by Winans Newman (51) for sexual behavior (see Ref. 17 for a review), and the VMH is known to receive terminals that mediate both sexual and feeding behavior (see Ref. 17). In fact, the VMH, amygdala, and bed nucleus of the stria terminalis all have a high density of estrogen-binding receptors (e.g., 30, 40, 41), which may account, in part, for the sex differences in weight gain observed after lesions. Thus, in conclusion, the PDA may well be a nodal point for the integration of olfactory, neuroendocrine, and behavioral stimuli involved in feeding and sexual behavior.

REFERENCES


