Interactive report

Posterodorsal amygdala lesions reduce feeding stimulated by 8-OH-DPAT

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Abstract

Injections of the serotonin (5-HT)1A agonist, 8-hydroxy-2(di-n-propylamino)tetralin, (8-OH-DPAT), either systemically or into the midbrain raphe nuclei, elicit food intake in otherwise satiated rats. Lesions of the paraventricular nucleus of the hypothalamus are well known for producing long-term overeating, but past research has excluded this site as a potential locus for short-term 8-OH-DPAT feeding effects. More recent work shows that small lesions of the posterodorsal amygdala (PDA) elicit overeating in their own right. Since this and related regions of the amygdala receive 5-HT innervations from the dorsal raphe nucleus (DRN), we determined if PDA lesions might alter feeding after injecting 8-OH-DPAT into this midbrain region. Adult female rats received either bilateral electrolytic lesions of the PDA or sham lesions. After recording weight gains for over 1 month, all rats were implanted with DRN cannulae, then randomly tested every 3-4 days for 1 h intake of standard lab chow after 0, 0.4, 0.8 or 1.6 nmol injections of 8-OH-DPAT. Additional 90 min measures of intake were also made after 0 vs. 250 mg (760 nmol) 8-OH-DPAT s.c. At the two highest DRN doses tested, lesioned rats showed 50% less intake compared to shams. A similar profile emerged after the single s.c. dose. These results suggest that the PDA may be an important locus at which reduced release of endogenous 5-HT stimulates feeding. Alternatively, the PDA may represent part of a larger brain circuit whose integrity is necessary for eliciting intake in response to a variety of feeding stimuli. © 2000 Elsevier Science B.V.

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1. Introduction

A variety of research has suggested that impairing neurotransmission in the brain’s serotonin (5-hydroxytryptamine or 5-HT) system can lead to overeating [2]. Two experimental techniques that have convincingly demonstrated this in rats using acute perturbations are by: (1) suppressing endogenous 5-HT release through systemic [4,10,12,18] or intra-raphe [6,7,9,14–17] injections of the 5-HT1A agonist, 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT), or (2) blocking brain 5-HT receptors following systemic [11,13,41], intraventricular [5] or intra-cerebral [8] injections of 5-HT antagonists.

Attempts to determine where 8-OH-DPAT’s feeding effects are localized in the brain have implicated dopamine and opiate systems in portions of the caudate-putamen [14,17] and the nucleus accumbens [14,15]. Since the hypothalamus is well known for its involvement in controlling feeding behaviors, and enhancing 5-HT neurotransmission in several of its nuclei can suppress feeding [32], it might be expected that the increased food intake stimulated by 8-OH-DPAT is due to a subnormal release of hypothalamic 5-HT [12,24]. In particular, it might be expected...
that impeding 5-HT release in the paraventricular nucleus (PVN) — a primary site at which lesions induce overeating [19–21,29] and where 5-HT infusions impair feeding induced by food deprivation or infusions of norepinephrine [43] — would also enhance food intake. Contrary to this prediction, feeding produced by intra-raphe injections of 8-OH-DPAT are not reversed by infusing 5-HT directly into the PVN [16] and PVN infusions of the 5-HT antagonist, metergoline, do not induce overeating [5].

Recent work has shown that small lesions localized to the posterodorsal region of the amygdala (PDA) produce reliable overeating and weight gain [28,39]. However, this newer literature has not yet characterized which neurotransmitter system(s) may underlie these effects. Since the PDA and related regions of the amygdala receive 5-HT innervations from the midbrain’s dorsal raphe nucleus (DRN) [1], it is possible that aspects of this PDA lesion effect are mediated by damaging local 5-HT neurocircuity. If that is the case, we predicted that infusing 8-OH-DPAT into the DRN would elicit less feeding in PDA-lesioned rats compared to controls. The results reported here confirm that possibility.

2. Materials and methods

2.1. Animals

Sixteen adult female Long-Evans hooded rats (Harlan Sprague–Dawley, Indianapolis, IN) were used. During all phases of study, animals were housed individually in stainless steel cages with mesh bottoms and had free access to standard lab chow pellets and water.

2.2. PDA lesions

Rats were acclimated to food (Harlan Teklad rat diet LM-485) and housing conditions at the University of New Orleans before receiving bilateral electrolytic lesions of the PDA (Lesion: n=8) or sham lesions (Control: n=8) as previously described [39]. Briefly, all rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), placed in a Kopf small animal stereotaxic instrument, and the 0.2 mm uninsulated tips of otherwise insulated no. 0 insect pins positioned at the following coordinates (upper incisor bar level with interaural line): 1.7 mm posterior to bregma, 4.5 mm lateral to midsagittal suture, 8.4 mm below skull surface. Lesions were induced by passing 1.5 mA anodal d.c. for 20 s per hemisphere. Sham surgery consisted of all procedures except that electrodes were lowered to 1.0 mm above target sites and no current was passed. Wounds were sutured closed and rats were returned to their home cages for recovery and assessment of body weights over the next 3 weeks. They were then shipped to Wayne State University for the next phase of investigation.

2.3. DRN implants

Upon arrival, rats were housed in a colony with a 12:12 h light/dark cycle (lights on 0500 h) and controlled temperature (22±2°C) plus ad lib access to lab chow (Rodent Diet 5001; LabDiet, Brentwood, MO) and water. After 2 weeks of adaptation and body weight measurements, all rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), placed in a Kopf small animal stereotaxic instrument, and implanted in the DRN with 26 gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) using methods previously described [6]. With the incisor bar set 3.5 mm below interaural line (to achieve a level skull between lambda and bregma), a single cannula for each animal was positioned at a 20° angle relative to the following vertical coordinates: 1.2 mm anterior to interaural zero, 1.6 mm lateral to midsagittal suture, 7.5 mm below the skull. Each implant was secured with acrylic dental cement that attached to the skull and three stainless steel screws which penetrated it. Guide cannulae were fitted with 33 gauge stainless steel inner styles (Plastics One) to maintain patency.

2.4. Feeding tests

During 7 days of recovery, all rats were handled, weighed and acclimated to mock DRN injections. Then, all were tested for 60 min intake of fresh lab chow at mid-light cycle following 0 (0.15 M saline vehicle; VEH), 0.4, 0.8 and 1.6 nmol 8-OH-DPAT hydrobromide (Research Biochemicals, Natick, MA). Each rat received all four doses once on a semi-randomized schedule across animals, with 3–4 days between sequential injections. All doses were delivered in a volume of 0.4 μl through a 33 gauge microinjector attached to a 5 μl Hamilton syringe. The tip of the microinjector extended 4 mm beyond the tip of the indwelling guide cannula. Injections were infused over 1 min, then injectors left in place for 1 additional min. All feeding tests were conducted in animals’ home cages.

Following the DRN injection series, all remaining rats were tested for 90 min lab chow intake at mid-light cycle after VEH and 250 μg (760 nmol) 8-OH-DPAT injected s.c. with 3–4 days intervening between injections.

2.5. Histology and data analyses

Animals were then sacrificed with an overdose of sodium pentobarbital and brains removed to localize lesions and cannula placements. Body weights and food intakes were analyzed between Lesion and Control groups by Student t-tests (two-tailed) or 2-way analyses of variance (ANOVAs) with post-hoc t-tests.
3. Results

3.1. Post-lesioning weight changes

At the time of PDA lesion surgery, Control rats weighed 298.6±7.0 g (mean±S.E.M.) while Lesion rats weighed somewhat less (275.0±13.4 g) but not significantly so ($t_{14}=1.675$, NS). By 10 days after surgery, Control rats weighed approximately the same (300.3±6.0 g) while Lesion rats weighed 327.6±15.8 g. A $t$-test on weight changes over this time revealed that the average gain of Lesion rats was 52.5±4.5 g, which was highly significant ($t_{14}=10.378$, $P<0.0001$) compared to the 1.6±2.6 g gained by Controls. By 20 days after surgery (just before rats were shipped to Wayne State), group body weights were essentially the same as they were at day 10 (i.e., Control=307.3±6.4 g, Lesion=327.3±14.7 g).

As anticipated, rats lost weight during shipping. By the time of surgery for DRN implants, Control rats had regained weight to levels that were comparable to those seen before shipping (307±5.6 g). Lesion rats’ weights (311±13.8 g) now equaled that of Controls on an absolute basis, but still exceeded them based on pre-surgical weights (+35.6±4.4 g gain for Lesion; +8.4±3.3 g gain for Control; $t_{14}=5.3119$, $P<0.005$). Immediately after surgery, 1 Control rat died. During the course of DRN injections, cannulae became blocked for 2 Control and 2 Lesion rats. Therefore, intake data described below reflect $ns$ ranging from 5 to 7 for Control and 6–8 for Lesion groups. The absolute body weights of rats remaining in these two groups did not differ significantly for the remainder of these investigations.

2.2. Feeding tests

Fig. 1 summarizes the results of 60 min feeding tests for both groups after DRN injections of 8-OH-DPAT. Inspection of this figure shows that neither saline nor the lowest (0.4 nmol) dose elicited substantial feeding. The two higher doses reliably enhanced intake but did so differentially between groups. A 2-way ANOVA (2 Groups×4 Doses) showed significant effects of Group ($F_{1,8}=5.405$, $P<0.05$), Dose ($F_{3,24}=51.522$, $P<0.0001$) and a Group×Dose Interaction ($F_{3,24}=8.501$, $P=0.0005$). These main effects demonstrated that Lesion rats overall ate less than Controls, and that 8-OH-DPAT overall reliably enhanced feeding. However, the Group×Dose Interaction further qualified these main effects. $T$-tests revealed that Lesion rats ate approximately 50% less than Controls only at the two highest doses (0.8 nmol: $t_{12}=3.504$, $P<0.005$; 1.6 nmol: $t_{11}=3.227$, $P<0.01$) — those that significantly enhanced intake in Controls (see Fig. 1).

![Figure 1](image1.png)

**Fig. 1.** Sixty minute food intake of PDA Lesion vs. Control rats after 0, 0.4, 0.8 and 1.6 nmol 8-OH-DPAT infused into the dorsal raphe. Reliable feeding in Controls only occurred at the two highest doses. Lesion rats showed reliable blunting of those responses (i.e., about 50% of Control intakes).

![Figure 2](image2.png)

**Fig. 2.** Ninety minute food intake of PDA Lesion vs. Control rats after 0 and 250 µg (760 nmol) 8-OH-DPAT s.c. Reliable feeding was induced by the drug in Controls. Lesion rats showed approximately a 50% blunted response, but this did not reach statistical significance due to the small numbers of rats tested.
Fig. 2 summarizes the results of the s.c. saline vs. 250 μg (760 nmol) 8-OH-DPAT test in the remaining 4 Control and 5 Lesion rats deemed healthy enough to participate. Due to the small numbers tested, a 2-way ANOVA (2 Groups × 2 Doses) showed only a main effect of Dose ($F_{1,7} = 7.118, P < 0.05$) with no significant effect of Group or Group × Dose Interaction. However, inspection of Fig. 2 shows that Lesion rats again ate about 50% of Control levels, which is in agreement with the results of the DRN injection study run previously.

2.3. Histology relative to DRN 8-OH-DPAT feeding effects

Fig. 3 depicts the maximal extent of lesion damage for the 7 individual rats tested on the highest (1.6 nmol) dose
of 8-OH-DPAT in the DRN. Shown to the right of each coronal plate is the 60 min intake response for each animal. The two rats that showed essentially no feeding (0 or 0.3 g) had very small, discrete, bilateral injury to the PDA. The three rats that ate 1.0 g as well as one that ate 1.1 g also had fairly small bilateral damage in the PDA but which also compromised adjacent areas. The remaining rat that ate the most (1.3 g) sustained the greatest asymmetrical damage, with injury to only the most posterior extent of the PDA in one hemisphere. Most of the damaged tissue in this animal was asymmetrically localized to the CA3 and CA2 regions of Ammon’s horn.

4. Discussion

The results of this experiment provide potentially novel insights into the brain circuitry responsible for 8-OH-DPAT-induced feeding. It has been known for some time that intracerebral infusions of this prototypic 5-HT1A agonist are capable of stimulating food intake in otherwise satiated rats [6,7,9,14–17]. Since 8-OH-DPAT suppresses the release of endogenous 5-HT [24], this feeding effect has been interpreted to result from acute suppression of 5-HT neurotransmission [11,12]. What has remained uncertain is exactly which brain region(s) mediate the capacity of such 5-HT1A agonism to stimulate food intake.

A long-standing literature on brain feeding controls has pointed to the medial hypothalamus as a critical site within which 5-HT and other neurochemical agents exert their feeding effects (e.g. [2,31,44]). While it is abundantly clear that enhancing hypothalamic 5-HT neurotransmission can suppress feeding [8,16,30,32,43], studies specifically designed to impede this function have proven ineffective in enhancing feeding [5,16]. This is of some concern for two important reasons: (1) impaired 5-HT functioning has been thought of as one causal biological mechanism underlying hunger in human eating disorders [27,30,42], and (2) pharmacological treatments for these disorders have been largely targeted to enhancing insufficient 5-HT neurotransmission [22,26].

Previous work from the first author’s laboratory has shown that the capacity of 8-OH-DPAT to induce short-term feeding is not due to its actions in the PVN, either alone [16] or when interacting with local noradrenergic feeding systems [7,9]. Other work has indicated that opioid and dopaminergic mechanisms mediate such feeding in portions of the caudate/putamen and nucleus accumbens [14,15]. Since portions of the amygdala interact with these structures [3,23,25,33–36,40,45] and 5-HT subsystems also innervate this limbic region [1], we performed the present experiments to determine if hyperphagia-inducing lesions in the PDA area might alter 8-OH-DPAT-induced feeding. Clearly, they do.

The most compelling evidence supporting the PDA/8-OH-DPAT/feeding linkage derived from the dose-response data obtained from DRN injections. The lowest 8-OH-DPAT dose was ineffective in enhancing feeding compared to control infusions. However, at the two higher doses, the enhanced feeding seen in Controls was suppressed on average by 50% in Lesion rats. The suppression of feeding following systemic 8-OH-DPAT was of similar magnitude but failed to reach statistical significance. This was almost certainly due to the small numbers of animals that remained testable at this stage of the experiment for two reasons. First, the body weights of rats remaining in the Lesion vs. Control groups did not differ at the time of systemic injections. Therefore, there is no possibility that the results obtained reflect differential dosing of rats in these two groups. Second, since this work was completed, we have confirmed that rats bearing lesions similar to the PDA ones described here do, indeed, show blunted feeding in response to a range of systemic 8-OH-DPAT doses [37].

The importance of selective PDA damage to the blunted 8-OH-DPAT feeding response was reinforced further by qualitatively comparing the magnitudes of amounts eaten by individual rats at the highest DRN dose relative to the loci of their lesions. In two animals where damage was bilateral and restricted to the PDA, 8-OH-DPAT - induced feeding was essentially non-existent. In several others where damage was less localized and less bilaterally symmetrical, sub-normal feeding was observed. Finally, in one rat with asymmetrical lesions beyond the posterior extent of the PDA, 8-OH-DPAT feeding was maximal within the Lesion group. While it has been reported that asymmetric amygdala damage may be as effective as bilateral injury in producing short-term weight gain, lesions to adjacent regions of that structure correlate negatively with weight gain [28]. More recent work has confirmed the importance of selectively damaging the PDA in order to obtain maximal overeating and weight gain effects [39]. Based on the data presented here, the anatomical specificity of such lesion-induced feeding appears equally relevant to its capacity to block 8-OH-DPAT-induced feeding.

5. Implications

This study represents the first evidence that highly circumscribed portions of the amygdala may participate in the feeding stimulated in otherwise satiated animals following 8-OH-DPAT treatment. Additional studies are underway to clarify this implication. At this early stage of investigation, we can only conclude that (a) the PDA axis represents a potentially important nodal point in brain neurocircuitry that specifically mediates 5-HT1A feeding, and/or (b) this brain region is part of a more complex feeding circuit traversed by other as yet unknown feeding-effective stimuli.
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