Published in final edited form as:

Eur J Neurosci. 2013 February ; 37(4): 648–653. doi:10.1111/ejn.12077.

Lateral hypothalamic involvement in feeding elicited from the ventral pallidum

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Abstract

Intense feeding can be elicited by injections of the GABA_A receptor antagonist bicuculline into the medial ventral pallidum (VPm), a basal forebrain structure anatomically interposed between two other feeding-related brain regions, the nucleus accumbens shell and the lateral hypothalamus (LH). To determine whether the VPm effects changes in feeding behavior through actions on the LH, we examined feeding following unilateral injections of bicuculline into the VPm made either ipsilateral or contralateral to a unilateral excitotoxic lesion of the LH in nondeprived rats. We found that lesions of the LH significantly attenuated feeding induced from the ipsilateral VPm, as compared to sham-operated controls. In striking contrast, unilateral LH lesions significantly potentiated the feeding response elicited by injections of bicuculline into the contralateral VPm. The “ipsilateral/contralateral disruption design” we used makes it extremely unlikely that our findings could have resulted from nonspecific effects of the lesions. These results suggest that the LH is causally involved in mediating the ingestive effects produced by activation of the VPm and provide an important insight into the functional circuitry by which basal forebrain structures control food intake in mammals.

Keywords

food intake; rats; GABA; bicuculline; hypothalamus

INTRODUCTION

The medial ventral pallidum (VPm) has been demonstrated to exert a major influence on food intake. Injections of either GABA_A antagonists or μ -opioid agonists into the VPm induce intense feeding in nondeprived rats (Stratford *et al.*, 1999; Smith & Berridge, 2005). Conversely, lesions within the VPm reduce food intake and body weight (Cromwell & Berridge, 1993). It has been proposed that the VPm is an integral component of a basal forebrain feeding circuit incorporating the nucleus accumbens shell (AcbSh) and the lateral hypothalamus LH (Stratford *et al.*, 1999; Stratford, 2007). This suggestion is based on the fact that the VPm is anatomically interposed between these two feeding-related brain regions, receiving a dense GABAergic projection from the AcbSh (Nauta *et al.*, 1978; Churchill *et al.*, 1990; Zahm & Heimer, 1990; Heimer *et al.*, 1991) and sending a substantial projection to the LH (Haber *et al.*, 1985; Groenewegen *et al.*, 1993). Importantly, it has been shown recently that lesions of either VPm or the LH disrupt the feeding behavior evoked by injections of muscimol into the AcbSh (Stratford & Wirtshafer, 2012), a result supporting

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The authors have no conflict of interest to disclose.

the idea that the VPM may act to relay information from the AcbSh to the LH. No data are currently available, however, which directly address the possibility that the VPM may control food through an effect on the LH. One prediction of this hypothesis is that destruction of the LH should attenuate the feeding induced by stimulation of the VPM.

Most studies examining the role of the LH in ingestive responses elicited from other structures have examined the effects of bilateral LH lesions or inactivation (e.g., (Maldonado-Irizarry *et al.*, 1995; Stratford & Kelley, 1999; Stratford & Wirtshafter, 2000)). This approach suffers from the potential pitfall that LH manipulations could suppress feeding indirectly, by inducing some effect that interferes with the feeding response, such as sedation, motor problems, or alterations in gustatory processing. It is possible to render these alternative explanations much less likely through the use of what we have referred to as an “ipsilateral-contralateral disruption” (ICD) design (Stratford & Wirtshafter, 2012). In this approach, experimental subjects receive unilateral lesions of the LH and are then tested following manipulations of the target structure on either the ipsilateral or contralateral side of the brain. If the effects of the unilateral LH lesions were due to nonspecific or generalized effects, one would expect that they would suppress feeding to the same extent whether it was elicited from the same or the opposite side of the brain. In contrast, if the LH were an essential component of a lateralized circuit underlying the elicited feeding, one would expect that ipsilateral lesions would produce a greater suppression than contralateral ones. The use of this design requires that connections between the target structure and the LH be predominantly ipsilateral, a condition which is fulfilled in the case of connections between the VPM and the LH (Haber *et al.*, 1985; Groenewegen *et al.*, 1993).

MATERIALS AND METHODS

Subjects

Seventeen male Sprague-Dawley rats (Charles Rivers) weighing between 340 and 360 g at the time of surgery served as subjects. The rats were housed individually in plastic cages on a 12 h light:12 h dark cycle at a constant room temperature (~ 21° C) with food (Harlan Teklad) and tap water available *ad libitum*, except as noted below. All experiments conformed to the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee.

Surgery

Surgery was performed using standard, aseptic, flat-skull stereotaxic techniques under sodium pentobarbital (60 mg/kg) anesthesia. In experimental subjects (N=9), a 28-gauge stainless steel injector was lowered into the LH at the following coordinates: AP: -2.4, LM: 1.8, DV: -9.1 (mm from bregma). Fifteen µg of ibotenic acid (10 µg/µl) was then infused into the LH at a rate of 0.33 µl/min after which the injector was allowed to remain in place for 5 minutes to minimize diffusion up the injector path. In order that mechanical damage dorsal to the LH would be equivalent on both sides of the brain, an injector was then lowered to a height of DV: -7.1 on the opposite side of the brain but no infusions were made at this site. Identical procedures were employed in control animals (N=8) except that the phosphate buffer vehicle, rather than ibotenic acid, was infused unilaterally. Immediately after the LH procedures had been performed, bilateral 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA), aimed so as to terminate 2.0 mm dorsal to the VPM, were implanted using the following coordinates: AP: 0.2, LM: ±1.8, DV: -6.8. The guide cannulae were held in place using stainless steel screws and denture lining material and a stainless steel obturator was inserted into the lumen of each cannula to help maintain patency. After surgery, the rats received an injection of carprofen (5 mg/kg, sc) to help

alleviate postoperative pain. Each rat was allowed to recover for at least seven days before the start of behavioral testing.

Test Apparatus

Test chambers consisted of plastic shoebox cages (43 cm X 22 cm) equipped with automated dispensers (Med-Associates, St. Albans, VT) that delivered a single 45 mg pellet of food (Precision Dustless pellets, Bio-Serve, Frenchtown, NJ) whenever the previous pellet was removed from the hopper. In order to acclimate the rats to eating the test chambers, they were placed in them on five consecutive days prior to the start of drug testing. The rats were mildly food-deprived for the first of these trials and non-deprived for the remainder.

Intracerebral Injection Technique

During the intracerebral injections, the rats were restrained gently, the obturators removed, and 28-gauge injection cannulae, extending 2.0 mm beyond the ventral tip of the guide, were inserted into each guide cannula. Injections were made at a rate of 0.33 μ l/min using motor driven microsyringe pumps connected to the injection cannulae with fluid filled polyethylene tubing. After the infusion, the injection cannulae were left in place for an additional 60 seconds in order to minimize leakage up the cannula track. The obturators were then replaced and the rats were placed in the test cages for 120 min. On the last of the acclimation days described above, each rat received bilateral 0.25 μ l intracerebral injections of sterile 0.15 M saline. Test sessions began 48 hours later; on test days, each rat received simultaneous bilateral 0.5 μ l injections at a rate of 0.33 μ l/min. At least 48 hours were allowed between injections.

Experimental Protocol

Each rat first received a series of three injections which were administered in a counterbalanced order. The three conditions were: (1) bilateral saline injections, (2) bicuculline (1(S),9(R)-(-)-bicuculline methbromide, Sigma; 50 ng) ipsilateral to the lesion and saline contralateral and (3) bicuculline contralateral and saline ipsilateral. Following these treatments, animals received two further experimental manipulations consisting of (1) bilateral bicuculline injections and (2) bilateral saline injections, which were again administered in a counterbalanced order. Subjects were then prepared for histological examination as described below.

Perfusion and Immunohistochemistry

When behavioral testing was completed, each of the rats was deeply anesthetized using sodium pentobarbital and perfused transcardially with 50 ml of a 0.15 M saline solution followed immediately by 200 ml of a 10% buffered formalin solution at pH 6.5, then 300 ml of a 10% buffered formalin solution at pH 9.0. The brains were removed and stored in PBS with 20% sucrose for at least two days, after which they were frozen and 35 μ m-thick coronal sections were taken throughout the extent of the AcbSh and the lesion sites. The sections then were processed for the immunohistochemical detection of neuronal nuclear protein (NeuN), a sensitive marker of undamaged neurons that is extremely valuable in evaluating excitotoxic lesions (Jongen-Relo & Feldon, 2002). Briefly, the sections were rinsed in PBS and incubated on a rotary shaker table for 24 hours at 4 °C in a monoclonal mouse anti-NeuN primary antibody (Oncogene Research Products) diluted 1:20,000 with 0.01 M PBS containing 4% normal horse serum and 0.2% Triton X-100. The sections were rinsed again in PBS and incubated in biotinylated horse anti-mouse secondary antibody (diluted 1:200 with PBS containing 4% normal horse serum) for 60 min at room temperature. Sections were then rinsed again in PBS (3 $\times$ 10 min) and incubated in the avidin-

biotin complex solution (Vectastain Elite ABC kit, Vector Laboratories) for 60 min. Following another series of rinses in PBS, the peroxidase was visualized by incubating the tissue for 5 min in the nickel-enhanced chromogen solution from a Vector 3,3'-diaminobenzidine tetrahydrochloride peroxidase substrate kit. The sections were then mounted on chrome-alum coated slides, air-dried, cleared in xylene, and coverslipped with Eukitt mounting medium. The lesion boundaries were mapped and the VPm injection sites were examined for placement accuracy and evidence of excessive damage. In five animals with ibotenic lesions of the LH, analogous methods were used to stain sections through the striatum for tyrosine hydroxylase-like immunoreactivity using a monoclonal mouse anti-TH serum (TE 101; Eugene Tech International, Allendale, NJ).

RESULTS

Histology

Histological analysis revealed that all cannula placements were located in the VPm within the region depicted in Fig. 1.

The ibotenic acid lesions produced here were very similar to those we have described and illustrated in a previous paper which used an identical lesioning method (Stratford & Wirtshafter, 2012). The lesions (Fig. 2a) were centered in the peduncular region of the LH at the level of the perifornical nucleus and extended approximately 2.0–2.5 mm in the anteroposterior plane. The smallest lesions were confined largely to the tuberal and peduncular regions of the LH (Fig. 3). The larger lesions additionally damaged the perifornical region, the dorsal aspect of the LH, and the most ventral part of the medial zona incerta. None of the lesions studied extended into the VPm. No obvious relations were apparent between damage to structures outside of the intended target area and the observed behavioral effects. In the subjects in which tyrosine hydroxylase-like immunoreactivity was examined, staining intensity in the rostral striatum appeared identical on the two sides of the brain, suggesting that dopaminergic fibers of passage were not damaged by the lesions (Fig. 2b).

Ingestive responses

Responses to unilateral injections of bicuculline into the VP are shown in the left-hand panel of Fig. 4. It can be seen that in control animals, bicuculline injections induced a robust increase in food intake, which was almost identical when the injections were made ipsilateral or contralateral to the side of sham lesions. In marked contrast, in subjects with excitotoxic lesions, much smaller increases in intake were observed when the unilateral bicuculline injections were made ipsilateral to the lesion than when they were placed on the opposite side. These impressions were supported by a 2 X 3 (sham/lesion X injection condition (bilateral saline/ipsilateral bicuculline/contralateral bicuculline) analysis of variance (ANOVA), with repeated measures on the injection condition factor, which demonstrated a significant main effect of injection condition ($F(2,30)=36.7$, $p<0.001$) but not of lesion ($F<1$). The lesion X injection condition interaction was also significant ($F(2,30)=12.2$, $p<0.001$) reflecting the fact that side of injection influenced response magnitude after excitotoxic, but after not sham, lesions. Examination of simple main effects further demonstrated that whereas LH lesions reduced the response to ipsilateral bicuculline injections relative to those seen in sham operated animals ($F(1,15)=6.8$, $p<0.02$), these lesions actually *potentiated* the response to contralateral injections, again relative to sham operated animals ($F(1,15)=7.1$, $p<0.02$). Feeding was however still slightly increased even when injections were made on the ipsilateral side ($F(1,8)=6.8$, $p<0.05$).

Responses to bilateral injections of bicuculline, and their matched saline control condition, are shown in the right-hand panel of Fig. 4. The figure suggests that unilateral LH lesions did not alter the feeding produced by bilateral injections of bicuculline into the VPm. This impression was confirmed by means of a 2 X 2 (sham/lesion X bicuculline) ANOVA indicated a significant effect of bicuculline ($F(1,15)=80.6$, $p<0.001$), but not of lesion or of the lesion X bicuculline interaction ($F<1$ in both cases).

DISCUSSION

Data obtained in the current study confirm previous reports that blockade of GABA_A receptors in the VPm with bicuculline greatly increases food intake in satiated rats (Stratford *et al.*, 1999; Smith & Berridge, 2005) and provide the first demonstration that feeding can be elicited by unilateral as well as bilateral bicuculline injections. More importantly, we found that unilateral lesions of the lateral hypothalamus (LH) have opposite effects on VPm-mediated feeding behavior depending on whether the bicuculline was injected on the side ipsilateral or contralateral to the lesion. Relative to the response seen in sham lesioned control animals, unilateral LH lesions located ipsilateral to VPm injections significantly decreased the amount of food consumed after blockade of VPm GABA_A receptors. In contrast, unilateral LH lesions located contralateral to the VPm bicuculline injections significantly increased VPm-mediated food intake, a phenomenon we will refer to as “contralateral potentiation” of feeding behavior. This pattern of “side specific” results virtually eliminates the possibility that the effects of the LH lesions could have been due to “generalized” or “nonspecific” effects of the lesions.

The fact that VPm-mediated feeding was inhibited only by ipsilateral LH lesions indicates that the suppression is likely to be a result of specifically disrupting the VPm-LH circuit. These results are consistent with anatomical data indicating that the VPm projects heavily to the LH (Haber *et al.*, 1985; Groenewegen *et al.*, 1993), and with numerous studies supporting the involvement of the latter structure in the control of ingestive behavior (Bernardis & Bellinger, 1996). Although our unilateral lesions significantly reduced the response to VPm injections by a mean of about 55% as compared to control animals, they did not completely eliminate it, and every lesioned subject showed slightly higher intakes after ipsilateral bicuculline injections than they did after bilateral saline. While it is possible that this residual response might reflect partial mediation of VPm feeding through structures other than the LH, or a minor involvement of the contralateral LH, the simplest explanation is that our lesions may not have completely destroyed the relevant cell populations within the region of the LH itself. In all subjects, there was some sparing of LH cells rostral and caudal to the center of the lesion as well as in the perifornical region, especially in its medial zone. Further studies will be required to evaluate this hypothesis, but our results provide unequivocal support for the notion that the ingestive effects of VPm manipulations are at least partially mediated through the LH. It is also possible the aphagia which has been reported to follow bilateral lesions of the VPm (Cromwell & Berridge, 1993) may also result from dysregulation of the LH, although the presence of certain differences between these two syndromes suggests that other mechanisms are also involved.

Since feeding is usually associated with excitatory manipulations of the LH (Stanley *et al.*, 2011), induction of feeding by disinhibition of VPm cells could most easily be accounted for if activation of the VPm were to lead to an increase in LH activity. This possibility is supported by reports of Fos expression in the LH following electrical stimulation of the VPm (Panagis *et al.*, 1997), but would seem to be inconsistent with the observation that most cells within the VPm are GABAergic. Transmitters other than GABA have, however, been identified in some VPm cells (Kalivas *et al.*, 1993; Manns *et al.*, 2001; Hur & Zaborszky, 2005), and it is possible that VPm influences on the LH may be mediated

primarily through these neurons. It is also possible that VPM projections to the LH are primarily inhibitory, but that they terminate chiefly on inhibitory, GABAergic, neurons within the LH (Mugnaini & Oertel, 1985; Hassani *et al.*, 2010) whose activation would be associated with a disinhibition of other neurons in this area. Further studies will be necessary to investigate these possibilities.

We were surprised to find that unilateral lesions of the LH resulted in a significant potentiation of the ingestive response to injections of bicuculline into the contralateral VPM, as compared to the response seen in sham lesioned animals. A somewhat similar phenomenon has been reported by Trojniar and Staszewska (1994) who found that the feeding response to electrical stimulation of the ventral tegmental area (VTA), which lies just caudal to the LH, is potentiated by electrolytic lesions of the contralateral VTA. More recent work has suggested that this latter effect is secondary to lesion-induced depletions of dopamine (Maliszewska-Scislo & Trojniar, 2000), whereas the immunocytochemical data obtained in the current study indicate that our excitotoxic LH lesions did not damage dopaminergic fibers of passage. Additionally, we found in previous studies that LH lesions produced using a technique similar to that employed here, did not alter dopamine levels in striatal homogenates (Stratford & Wirtshafter, 2000). These results suggest that the contralateral potentiation seen here was not dependent on unilateral dopamine depletion. While the neural mechanisms underlying this phenomenon are not yet known, one interesting explanation is suggested by observations that projections from the LH on both sides of the brain converge bilaterally in certain downstream structures, such as the nucleus of the solitary tract (NTS) (Hosoya & Matsushita, 1981; Cho *et al.*, 2003). It is plausible that damage to LH projections arising from one side of the brain might result in sprouting of afferents to downstream structures arising from the intact LH (Deller *et al.*, 2006), or in some other type of plastic sensitization. Such an exaggeration of the functional connectivity between the intact LH and bilaterally innervated structures such as the NTS, might be expressed behaviorally as a potentiated hyperphagic response to activation of the intact LH following bicuculline injections into the VPM on that side. In evaluating this possibility, it would be of interest to see whether acute inactivation of the LH on one side of the brain potentiated the response to stimulation of the contralateral LH, or whether contralateral potentiation is dependent on a chronic disruption of function. Regardless of whether this explanation is correct, the phenomenon of contralateral potentiation does provide further support for the concept that the LH plays an important role in the circuitry responsible for VPM effects on feeding.

It is interesting that in both lesioned and control subjects, the response to bilateral injections of bicuculline was approximately equal to the sum of the responses seen after the unilateral injections. Thus in sham lesioned subjects, equivalent response were seen with injections ipsilateral or contralateral to the sham lesion, and the response to bilateral injections was about twice that seen after unilateral injections. In animals with ibotenate lesions, the response to unilateral injections was much larger from the intact than the lesioned side, but the response to bilateral injections was again almost exactly equivalent to that expected from the sum of the two unilateral injections. In this case, the increase in food intake seen after contralateral VPM bicuculline compensated for the reduced food intake caused by the LH lesion, so while the relative contribution of each LH was changed by the lesion, the total amount of food consumed in lesioned animals was the same as in intact rats.

The ventral pallidum is known to receive a heavy GABAergic input from the nucleus accumbens (Zahm & Heimer, 1990; Heimer *et al.*, 1991; Napier *et al.*, 1995; Usuda *et al.*, 1998; Zahm *et al.*, 1999; Zhou *et al.*, 2003), and projections from the medial shell region of the accumbens (AcbSh), which appears to be especially involved in feeding (Stratford *et al.*, 1998; Kelley, 1999; Stratford, 2007), terminate primarily in the VPM. Stimulation of the

AcbSh produces a bicuculline sensitive inhibition of cells in the VPm (Chrobak & Napier, 1993), suggesting that bicuculline would act in the VP to disinhibit neurons receiving GABAergic projections from the AcbSh. Coupled with the ability of intra-VP bicuculline to induce feeding, these data support the idea that disinhibition of cells in the VPm plays a major role in mediating the influence of the AcbSh on feeding. Data obtained in the current study are consistent with the proposal that the VPm is actually an intervening node in an AcbSh-LH feeding circuit (Stratford et al., 1999; Stratford, 2007), a hypothesis that was given added support by the recent finding that ipsilateral, but not contralateral, lesions of either the VPm or LH significantly suppress AcbSh-mediated food intake (Stratford & Wirtshafter, 2012). Our current finding that LH lesions specifically suppress VPm-mediated feeding, taken together with our previous results, suggests that one way in which the AcbSh controls feeding behavior is by mediating activity in the VPm, which, in turn, modifies activity in the LH.

Acknowledgments

This publication is based upon the work supported by grants R01DK071738 from the National Institute of Diabetes and Digestive and Kidney Diseases and 0641943 from the National Science Foundation, and R03DA020802 from the National Institute for Drug Abuse.

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Figure 1. Schematic illustration of the location of ventral pallidal injection sites in the current study. All cannulae terminated within the shaded regions. Figure modified from (Paxinos & Watson, 2007).

Figure 2.

a) Photomicrograph of a section stained for NeuN taken through the center of an excitotoxic lesion of the lateral hypothalamus. b) Photomicrograph of a section stained for tyrosine hydroxylase taken through the striatum of the rat with the largest LH lesion. Equivalent staining intensity on both sides of the brain suggests that the unilateral LH lesions did not damage ascending dopaminergic fibers. (Calibration bars = 2 mm)

Figure 3.
Illustrations of the largest, smallest, and intermediate lesions of the lateral hypothalamus.
Numbers indicate distance from bregma in mm.

Figure 4.

Panel 1) Cumulative numbers of 45 mg food pellets consumed in the 2 h following bilateral injections of saline, or unilateral injections of bicuculline into the medial ventral pallidum located ipsilateral or contralateral to unilateral lesions of the lateral hypothalamus. (* = $p < 0.05$) Panel 2) Cumulative numbers of 45 mg food pellets consumed in the 2 h following bilateral injections of saline or bicuculline into the VPM.