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Research report

Activation of feeding-related neural circuitry after unilateral injections of muscimol into the nucleus accumbens shell

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Abstract

Chemical inhibition of neurons in the nucleus accumbens shell (AcbSh) elicits intense, behaviorally specific, feeding in satiated rats. We have demonstrated previously that this treatment activates a number of brain regions, most significantly the lateral hypothalamus (LH). This activation could be elicited through a direct neural connection with the AcbSh or secondarily through changes in autonomic activity, stress, or circulating levels of orexigenic or satiety factors. In the present study, we used the immunohistochemical localization of Fos protein to map neuronal activation after unilateral muscimol injections into the AcbSh to determine whether AcbSh-mediated Fos expression remains lateralized in the circuit and whether secondary systemic changes in the rat can be excluded as primary factors in the activation of downstream component nuclei. Rats receiving only saline injections exhibited very little Fos immunoreactivity. In contrast, unilateral injections of muscimol into the AcbSh consistently increased Fos expression in several brain regions. Three distinct patterns of expression were observed. Fos synthesis in the LH was increased only on the side of the brain ipsilateral to the muscimol injection. Fos expression remained primarily ipsilateral to the injection site in the septohypothalamic, paraventricular hypothalamic (PVN), paratenial thalamic, and lateral habenular nuclei, and medial substantia nigra, but was increased bilaterally in the piriform cortex, supraoptic nucleus, central nucleus of the amygdala, and nucleus of the solitary tract. Smaller numbers of Fos-immunoreactive cells were seen unilaterally in the bed nucleus of the stria terminalis, medial ventral pallidum, arcuate nucleus, and ventral tegmental area and bilaterally in the supraoptic and tuberomammillary nuclei. The labeling in the LH, PVN, and other unilaterally labeled structures provides evidence that these brain regions are components of an AcbSh-mediated neural circuit and suggests that they may be involved in the expression of AcbSh-mediated feeding behavior.

Theme: Neural basis of behavior
Topic: Ingestive behaviors

Keywords: Food intake; Feeding behavior; Fos; Lateral hypothalamic area; GABA; Rat

1. Introduction

Data acquired over the past several years suggest that the shell subregion of the nucleus accumbens (AcbSh) may be an important component of a neural system involved in the control of feeding behavior. Inhibiting neurons in the ventromedial AcbSh with local injections of GABA agonists [41,42,50] or glutamate antagonists [31,44] elicits a powerful, but behaviorally specific, feeding response in satiated rats. This feeding is characterized by its short latency and intensity, yet the treatment does not increase water intake, non-ingestive gnawing, or locomotor activity [44]. The fact that large specific increases in food intake are elicited by increasing levels of endogenous GABA or blocking the action of endogenous glutamate in the AcbSh strongly suggests that these neurochemical systems in this particular brain region participate in the physiological control of feeding.

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In order to investigate the neural circuitry underlying the effects of muscimol injected into the AcbSh, previous studies have examined the ability of these injections to induce synthesis of the immediate early gene product Fos, a widely employed marker of neuronal activation [16,37]. These studies have shown that bilateral injections of muscimol into the AcbSh result in pronounced bilateral expression of Fos in several brain regions including the lateral hypothalamic area (LH) and the paraventricular hypothalamic nucleus (PVN) [42]. These results are significant given the substantial evidence indicating that these regions play an important role in the control of food intake [5,13,40]. The AcbSh projects directly to the ipsilateral LH [21,27,53] and can additionally influence this region through projections to the ventral pallidum [21,33], which, in turn, sends ipsilateral efferents to the hypothalamus [19,20]. These findings are all compatible with the possibility that the AcbSh elicits feeding through a neurally mediated influence on the activity of hypothalamic neurons. However, the results on Fos expression do need to be interpreted with caution. For example, studies have shown that Fos synthesis in the LH or PVN can be induced by a variety of stressors [7,10,38,39] and by changes in circulating levels of glucose or a number of hormones [2,6,8,11,17,30,32,34,36,47]. Thus, while it is possible that the hypothalamic Fos expression seen after bilateral intra-AcbSh muscimol injections may reflect alterations in the activity of neural circuits linking the AcbSh and the hypothalamus, it is also quite plausible that this Fos expression might be secondary to AcbSh-mediated changes in autonomic activity, hormone levels, arousal, stress, or other systemically or behaviorally mediated factors.

One approach to the problem of determining whether the AcbSh exerts a direct control over neural activity in the other brain structures is to examine the effects on Fos expression produced by unilateral, as compared to bilateral, injections of muscimol. Under these conditions, one would expect to see bilateral labeling if Fos synthesis reflects alterations in the general physiological or behavioral state of the animal. In contrast, a pattern of unilateral Fos expression would strongly suggest that neurons in the affected regions are part of an uninterrupted neural circuit involving the AcbSh. In the present study, we mapped neuronal activation after unilateral muscimol injections into the AcbSh to determine whether AcbSh-mediated Fos expression remains lateralized in the circuit and whether secondary systemic changes in the rat can be excluded as primary factors in the activation of downstream component nuclei.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley SD rats were obtained from Harlan (Madison, WI). The animals weighed between 280 g and 320 g at the time of surgery. They were housed individually in wire-mesh cages and were maintained on a 12 h:12 h light:dark cycle (lights on at 07:00) in a temperature-controlled environment (~22 °C) with food (Harlan Teklad 7001) 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.

2.2. Surgery

The rats were anesthetized with sodium pentobarbital (50 mg/kg), and bilateral 26-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) were implanted using standard, flat-skull stereotaxic techniques. The guide cannulae were aimed so that they terminated 2.0 mm dorsal to the AcbSh using coordinates anterior–posterior (AP): +1.4, lateral–medial: ±0.75, dorsal–ventral: −6.1 (mm from bregma) and were held in place using stainless steel screws and denture lining material. A stainless steel obturator was inserted into the lumen of each cannula to help maintain patency. Each rat was allowed to recover at least 7 days before the start of testing, during which time the rats were handled daily.

2.3. Intracerebral injections

In order to acclimate the rats to the microinjection procedure, the obturators were removed, and a 33-gauge injection cannula, extending 2.0 mm beyond the ventral tip of the guide, was inserted into each guide cannula on three consecutive days. The obturators were then replaced, and the rats were returned to their home cages. On the final acclimation day, each rat received bilateral 0.5 μl intracerebral injections of sterile 0.15 M saline at a rate of 0.32 μl/min.

2.3.1. Feeding behavior

Forty-eight hours after the final acclimation run, seven rats received simultaneous 0.5 μl injections of muscimol (0, 25, 50, or 100 ng; Sigma, St. Louis, MO) into one AcbSh and the sterile saline vehicle into the opposite AcbSh. After the infusions, the injection cannulae were left in place for an additional 60 s in order to minimize leakage up the cannula track. The order in which the different doses were administered and the side of the brain receiving the muscimol injection were randomized between rats. Following the microinjections, the rats were placed in test cages with a preweighed quantity of the maintenance diet and a graduated bottle containing tap water available. Food intake (corrected for spillage) was calculated at 30, 60, and 120 min. Total water intake was determined at the end of the 120 min test.

2.3.2. Muscimol-induced Fos expression

Rats in Group I (n = 9) received simultaneous 0.5 μl injections of 100 ng muscimol into one AcbSh and the