The Hypothesis and Research on the Regulation of Mouse Feeding Behavior by Astrocytes in the Lateral Hypothalamic Area

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Abstract: Numerous studies have shown that the central nervous system plays a crucial role in regulating feeding behavior [1]. Within the central nervous system, many nuclei and neural circuitry are involved in regulating feeding behavior, such as the ventral tegmental area (VTA), lateral hypothalamus (LH), tuberomammillary nucleus (TN), and amygdala (CeA). Among these, the lateral hypothalamus is a critical target for the regulation of homeostatic feeding. Previous studies on LH regulation of feeding behavior have mostly focused on neurons and neural circuits, with relatively fewer studies on astrocytes.
***Keywords*:** lateral hypothalamus, astrocytes, feeding regulation, calcium channel signaling, immunohistochemistry research

1. Introduction

The Regulatory Role of Astrocytes in Feeding Behavior in the LH Requires Further Investigation.

The lateral hypothalamus (LH) harbors various neurons involved in regulating energy balance, such as melanin-concentrating hormone (MCH) neurons, orexin neurons, and GABAergic cells. MCH neurons promote positive energy balance, as studies have shown that damaging MCH neurons leads to increased activity and energy expenditure in animals [2,3]. Disruption of the orexin system in mice has been found to result in obesity despite consuming less food [4,5]. Recent research also indicates that optogenetic activation of GABAergic neurons in the lateral hypothalamic area's ventral part induces feeding in satiated mice [4,5]. Furthermore, the LH contains numerous astrocytes whose role in regulating feeding behavior warrants further investigation.

2. Research Objectives

(1) To determine the association between astrocytes in the lateral hypothalamus (LH) of mice and feeding behavior.

(2) To investigate whether activation of astrocytes in the mouse hypothalamus promotes feeding.

3. Research Contents

(1) Exploration of the Association between Astrocytes in the Lateral Hypothalamus and Feeding Behavior.

(2) The Regulatory Effect of Chemogenetically Activated LH Astrocytes on Mouse Feeding.

(3) Immunohistochemistry Study.

4. Research Methods

4.1 Experimental Animals

Adult male C57BL6/J mice weighing between 25-30g, approximately 16 weeks old.

4.2 Experimental Methods

4.2.1 Stereotaxic Injection into the Brain

(1) Animal Anesthesia: Anesthetize animals using 3% isoflurane, and monitor their condition closely.

(2) Fixation of Mouse Head: Shave the head of the mouse and secure it in a stereotaxic instrument (RWD, Shenzhen, China). Adjust to the appropriate angle to align the bregma and lambda points on the same horizontal plane.

(3) Preparing for Craniotomy: Maintain the animal's body temperature at around 37°C using a heating pad. Disinfect the skin of the head with 2% iodine or 75% alcohol, then cut the scalp to expose the bregma, lambda, and sagittal sutures.

(4) Determine the Midline: Move the metal positioning needle downward to align with the sagittal suture and then move it anteriorly and posteriorly to position it on the bregma.

(5) Locate the LH Nucleus of the Mouse: We use parameters provided by a mouse brain stereotaxic atlas. The specific

parameters for the plane position of the LH nucleus are: anterior-posterior -1.20mm, medial-lateral ±1.10mm, dorsal-ventral -5.00mm from the bregma. Drill a pair of small holes in the skull at this point.

(6) Virus Injection: Using a microprocessor-controlled microinjector (RWD, Shenzhen, China), draw 200nL of virus into the microinjector, inject into the LH at a rate of 40nL/min, and stay for 10 minutes after injection. Slowly withdraw the needle to prevent virus overflow.The viruses used in the experiment are adenoviruses (AAV) vectors, including rAAV-gfaABC1D-hM3d(Gq)-eGFP, rAAV-gfaABC1D-GCaMP6M, and rAAV-gfaABC1D-eGFP.

4.2.2 Calcium Imaging

Following steps 1 and 2, after mice are injected with rAAV-gfaABC1D-GCaMP6M or rAAV-gfaABC1D-eGFP virus into the LH , the optical fiber is placed in a ceramic sleeve and inserted into the LH through craniotomy. Mice are housed individually for at least 3 weeks to recover. After recovery, fiber photometry experiments are conducted.

Feeding Fiber Photometry Experiment: After viral expression, mice are individually acclimated to the experimental diet for 3 days before each food experiment. They are fasted for 8 hours before the experiment. The ceramic sleeve on their heads is connected to the interface of the fiber optic recording system. The food is placed behind the mice, and mice in a hungry state will voluntarily feed. After the mice feed, the food is removed, and the experiment is repeated five times. At this point, the calcium ion concentration within the astrocytes in the LH of mice can be monitored in real-time. Each day, one type of food is tested. During the experiment, the positions of the food are constantly changed to prevent the mice from anticipating the food location and affecting the experimental results.

4.2.3 Behavioral Experiments

Chemogenetic Feeding Experiment: After the injection of rAAV-gfaABC1D-hM3d(Gq)-eGFP and expression, the following experiment is conducted with the mice. Before and after the experiment, the mice have access to food freely in their Homecage. The mice accustomed to free feeding are placed in an open rectangular enclosure (90X30cm) for two days. The enclosure contains food trays, water dishes, and black wooden boxes (lined with padding). The mice have free movement. The black wooden boxes and food trays are placed diagonally opposite each other, while the water dishes and food trays are placed opposite each other. On two consecutive days at 9 a.m., equal amounts of saline or CNO (1mg/kg) are injected. Infrared cameras above the enclosure observe and record the movement of the mice. Before and after the experiment, the weight of the food trays is measured using a scale to calculate the amount of food consumed by the experimental mice. The feeding amount and feeding time of the mice are manually analyzed .

4.2.4 Immunofluorescence

Brain sections are incubated with primary antibodies, either Chicken-anti-GFAP antibody or Mouse-anti-NeuN antibody, followed by secondary antibodies (Chicken-anti-GFAP antibody or Mouse-anti-NeuN antibody).

First, incubate brain sections overnight at room temperature with 100 ng/mL AC-X in PBS, then wash three times with PBS for 5 minutes each time. Place AC-X-treated brain sections in a monomer solution (2 M NaCl, 8.625% sodium acrylate, 2.5% acrylamide, 0.15% N,N'-methylenebisacrylamide, PBS) and incubate for 20 minutes in a cold room. Apply a drop of vacuum grease at each corner of a coverslip, then transfer the brain sections to the center of the coverslip.

Prepare gelation solution: Ammonium persulfate (APS) and N,N,N',N'-Tetramethylethylenediamine (TEMED) are at a concentration of 10% (w/w). 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (4-HT) is at a concentration of 1% (w/w). APS and TEMED are diluted to 0.2% (w/w) in the monomer solution, and 4-HT is diluted to 0.01% (w/w) in the monomer solution for gelation, followed by the addition of the initiator (APS).

Add the gelation solution to the samples. Cover with a coverslip and gently press to ensure tight contact with the sample, removing excess gel solution. After gelation, gently lift the coverslip with forceps (the coverslip can be separated at this point, but premature separation may cause gel folding, so it is advisable to retain it). Digest the brain sections in digestion buffer containing 8 U/mL proteinase K (40 mM Tris, pH 8, 1 mM EDTA, 0.5% Triton, 0.8 M NaCl) for 6-8 hours. After digestion, separate the brain sections from the coverslip and wash with 10×PBS for 5 minutes. Transfer each brain section to a 9 cm dish, incubate with ddH2O for 3 times, 2 hours each time, and overnight.

4.2.5 Image Acquisition and Analysis

Transfer the processed brain sections to a poly-L-lysine-coated confocal imaging dish. Capture expanded images using an FV3000 microscope equipped with 4x or 10x air objectives. To calculate the expansion factor, measure the gel size before and after expansion based on gel images. All data processing and analysis are performed using ImageJ and Imaris.

5. Research Results

(1) Calcium signaling in LH astrocytes increases during feeding.

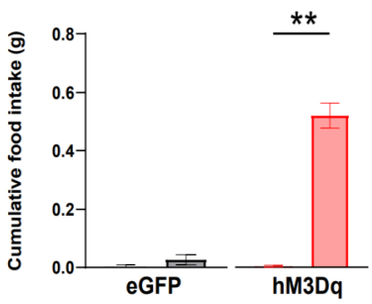

Figure 1. Quantitative analysis of calcium signaling changes in LH astrocytes during standard diet feeding experiments. (n=4, 5 trials per experiment, P < 0.001).

(2) Chemogenetic activation of LH astrocytes promotes feeding behavior.

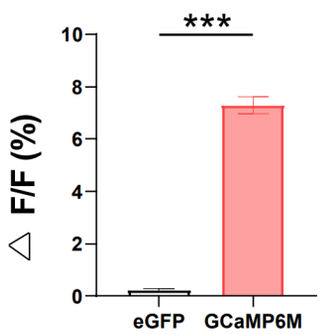

Figure 2. During the period from ZT 2 to ZT 5 after administration, the food intake of the Vehicle group and the CNO group (n=4 each) was measured. In the hM3Dq vehicle vs CNO comparison, the difference was statistically significant. (P < 0.01, P < 0.01).

(3) The results of GFAP and NEUN immunostaining demonstrate the specificity of the virus.

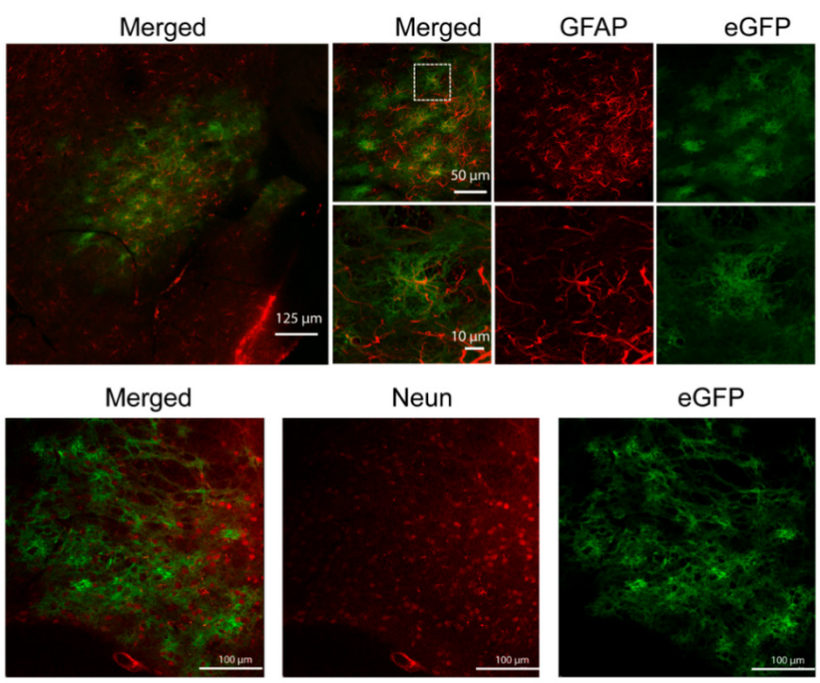

Figure 3. Immunofluorescent staining for GFAP and NEUN.

6. Conclusion

(1) Analysis of fiber optic recordings indicates that during feeding, the experimental group (GCaMP6m) exhibits significantly higher calcium signal activity compared to the control group (eGFP), suggesting a correlation between feeding behavior in mice and the activity of astrocytes in the lateral hypothalamus.

(2) Analysis of chemogenetic results shows that after injecting CNO to activate astrocytes in the lateral hypothalamus, the experimental group (hM3Dq) displays significantly higher feeding behavior compared to the control group (eGFP), demonstrating that activation of astrocytes in the lateral hypothalamus promotes feeding behavior in mice.

(3) Analysis of immunofluorescent staining results confirms the specificity of virus expression in astrocytes in mice.

References

[1] Locke A, Kahali B, Berndt S, et al. Genetic studies of body mass index yield new insights for obesity biology[J], 2015, 518(7538):197-206.

[2] Alon T, Friedman J J T J O N T O J O T S F N. Late-onset leanness in mice with targeted ablation of melanin concentrating hormone neurons[J], 2006, 26(2):389-97.

[3] Fujita A, Zhong L, Antony M, et al. Neurokinin B-Expressing Neurons of the Central Extended Amygdala Mediate Inhibitory Synaptic Input onto Melanin-Concentrating Hormone Neuron Subpopulations[J], 2021, 41(46):9539-9560.

[4] Nieh E, Matthews G, Allsop S, et al. Decoding neural circuits that control compulsive sucrose seeking[J], 2015, 160(3):528-41.

[5] Nieh E, Vander Weele C, Matthews G, et al. Inhibitory Input from the Lateral Hypothalamus to the Ventral Tegmental Area Disinhibits Dopamine Neurons and Promotes Behavioral Activation[J], 2016, 90(6):1286-1298.