Appetite suppressive role of medial septal glutamatergic neurons

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Feeding behavior is controlled by diverse neurons and neural circuits primarily concentrated in the hypothalamus and hindbrain in mammals. In this study, by using chemo/optogenetic techniques along with feeding assays, we investigate how neurons within the medial septal complex (MSc), a brain area implicated in emotion and cognition, contribute to food intake. We find that chemo/optogenetic activation of MSc glutamatergic neurons profoundly reduces food intake during both light and dark periods of the rodent light cycle. Furthermore, we find that selective activation of MSc glutamatergic projections in paraventricular hypothalamus (PVH) reduces food intake, suggesting that MSc glutamatergic neurons suppress feeding by activating downstream neurons in the PVH. Open-field behavioral assays reveal that these neurons do not overtly affect anxiety levels and locomotion. Collectively, our findings demonstrate that septal glutamatergic neurons exert anorexigenic effects by projecting to the PVH without affecting anxiety and physical activities.

medial septum | appetite | suppression | chemo/optogenetics | PVH

For survival, it is essential to maintain adequate levels of food intake to match changing energy demands. Therefore, neural circuits in the brain have evolved to adaptively control feeding in an effort to maintain energy homeostasis (1, 2). For example, neurons and neural circuits in the hypothalamus and hindbrain actively respond to changes in energy states (such as hunger or satiety) to modulate feeding behavior (1, 2). In addition to being regulated by energy states, feeding is also controlled by higher-level cognitive and emotional factors (3–5). For example, brain regions such as the hippocampus and prefrontal cortex regulate learned and/or motivational aspects of food intake (6–11), while the amygdala is thought to be involved in emotional regulation of feeding (8, 12, 13). Compared with the well-studied cell types and neural circuits responsible for hypothalamic and hindbrain control of feeding (2, 14), relatively little is known about the individual cell types and circuits involved in emotional and cognitive control of food intake.

The septal nucleus is a limbic brain structure associated with a variety of cognitive and emotional processes, including stress and aggression, but understudied in the context of feeding behavior (15–17). Recent findings, however, suggest that the lateral portion of septum (LS) is involved in the control of feeding behavior and energy homeostasis. For example, our recent study showed that food intake was reduced by activating hippocampal glutamatergic inputs to the lateral septum (18). The lateral septum in turn regulates feeding, at least in part, by projecting to neurons located in the lateral hypothalamus (LH) (19, 20). Consistently, intra-LS infusions of GABA or acetylcholine increase feeding, while glucagon-like 1 peptide infusions decrease feeding (21–23). Furthermore, the lateral portions of septum are known to be involved in gastric distention (24), suggesting that septal brain regions may control both central and peripheral aspects of energy metabolism. However, compared with the role of lateral septum in the control of food intake and energy metabolism, the role of neighboring medial septum nuclei in feeding behavior remains unknown.

The current study sought to investigate the contribution of the medial portions of the septal complex to food intake. Based on the role of the septal nucleus in emotional behaviors and feeding (15, 25), we hypothesized that medial septal complex (MSc) glutamatergic neurons would reduce food intake. To test this, we took advantage of the cell-type specificity of chemo/optogenetic techniques in conjunction with feeding and anxiety-related behavioral assays to selectively examine how chemo/optogenetic manipulations of MSc glutamatergic neurons affect food intake. We find that activation of MSc glutamatergic neurons suppresses food intake by projecting, at least in part, to the paraventricular hypothalamus (PVH) brain region without affecting anxiety-related behavior and physical activities.

Results

Activation of MSc vGluT2 Neurons Reduces Dark Period Feeding. The medial septal complex (MSc) contains neurons that synthesize glutamate (26). To selectively examine the role of MSc glutamatergic neurons in the control of food intake, we targeted Cre-recombinase–dependent viral vectors to medial septal areas in transgenic mice expressing Cre-recombinase selectively in the neurons containing vesicular glutamate transporter type 2 (vGluT2-Cre mice). Consistent with previous reports of glutamatergic neuron distribution in the septum (16, 26), we observed dense transduced neurons localized in medial portions of the MSc with no apparent viral expression observed in lateral septal subregions (hereafter referred to as MSc vGluT2 neurons; Fig. 1). Although the ventral portions of lateral septum (LSv) are known to express glutamatergic neurons (26), our viral targeting strategy did not appear to target these particular cell populations, as the viral expression

Significance

Feeding behavior is composed of emotional, hedonic, and homeostatic aspects. It is therefore important to dissect neuron populations that control feeding and emotions and determine their interactions. In this study, we report a neuronal population involved in suppressing food intake in the septum, a brain region relaying information encoded in higher-level brain regions to downstream targets in the hypothalamus. We find that the septal glutamatergic neurons exert anorexic effects without overtly influencing locomotion or anxiety behavior, representing a promising cellular entry point for future studies investigating higher-level control of feeding.

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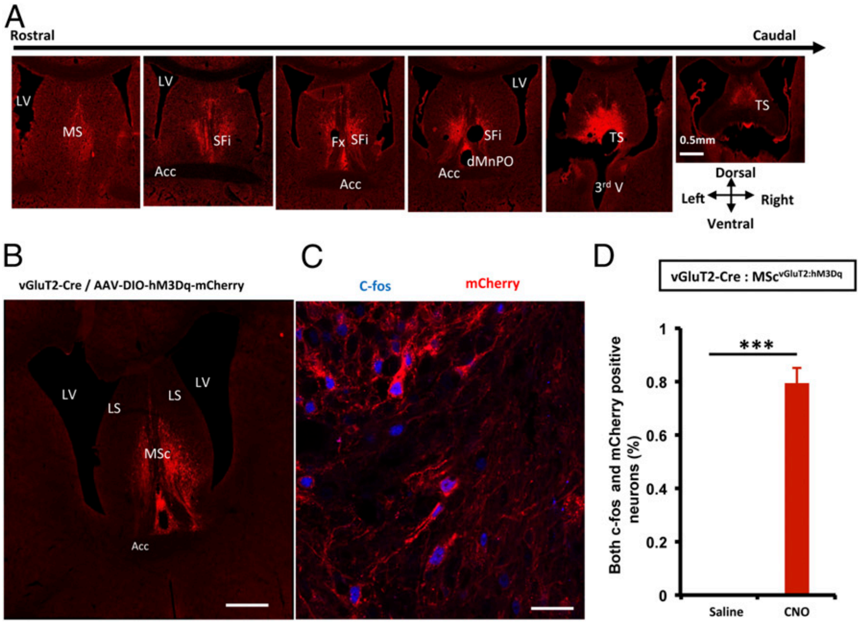

Fig. 1. DREADD-based activation of MSc vGluT2 neurons. (*A*) Representative sample images from rostral to caudal in a mouse transfected with hM3Dq-mCherry in MSc vGluT2 neurons. Viral expression was primarily observed in the septofimbrial nucleus and dorsal median preoptic area. Sparse infection was also observed in the triangular septum and medial septal area. (*B*) Representative image of Cre-dependent expression of hM3Dq in MSc vGluT2 neurons. (*C*) Sample image showing overlap of hM3Dq-mCherry and c-fos in MSc vGluT2 neurons. I.p. injections of CNO were administered before perfusion to selectively activate MSc vGluT2 neurons. (*D*) Quantification of the percentage of c-fos positive neurons that coexpress mCherry following i.p. injections of saline or CNO. CNO injections significantly increased the percentage of c-fos positive cells coexpressing mCherry relative to saline injections ($n = 2$ mice per group, unpaired Student's *t* test). (Scale bars, 200 μm for *A* and *B* and 20 μm for *C*.) Acc, anterior commissure; dMnPO, dorsal median preoptic area; Fx, fornix; LS, lateral septum; LV, lateral ventricle; MS, medial septum; MSc, medial septal complex; SFi, septofimbrial nucleus; TS, triangular septum. Data represent mean ± SEM $***P < 0.001$.

was limited to medial portions of the septal complex (Fig. 1). In particular, viral expression was primarily observed in the septofimbrial nucleus (SFi) and dorsal median preoptic area (dMnPO) with weaker expression observed in the triangular septum and medial septum (Fig. 1). I.p. administration of the designer receptor exclusively activated by designer drugs (DREADD) agonist clozapine-*N*-oxide (CNO) (1 mg/kg) to hM3Dq-transduced mice significantly activated MSc vGluT2 neurons, as indicated by increased c-fos levels in MSc vGluT2 neurons relative to control saline conditions (Fig. 1 *B–D*). To assay for changes in food intake in response to MSc vGluT2 neuron activation, we performed free-access feeding assays following i.p. injections of saline or CNO (1 mg/kg). Food intake was dramatically reduced for up to 1 h following *i.p.* CNO injections in the dark period of the rodent

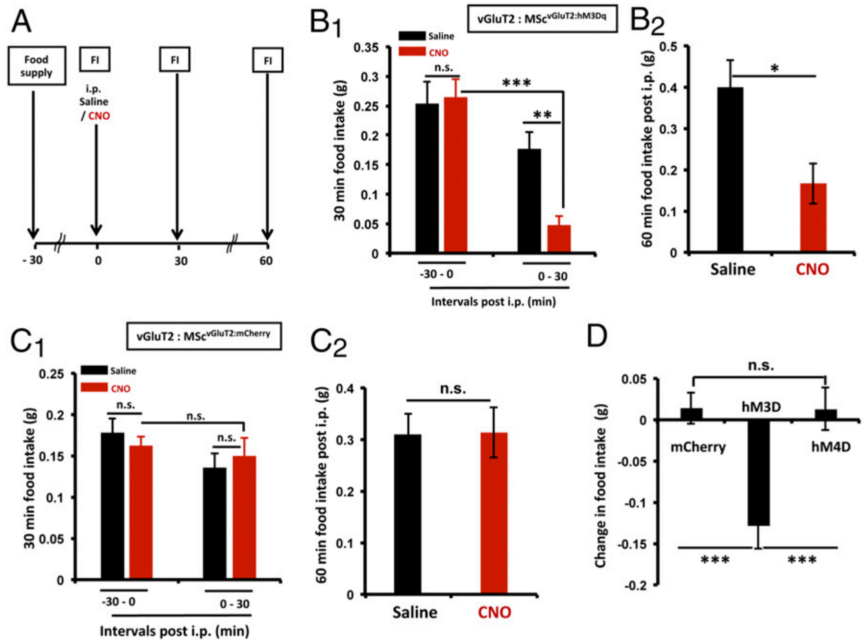

Fig. 2. Activation of MSc vGluT2 neurons suppresses dark period food intake. (*A*) Experimental timeline for feeding behavior experiments. Food was introduced (to ad libitum fed mice) 30 min before i.p. injections of saline or CNO. Thirty-minute food intake (B_1) and 60-min food intake (B_2) was reduced in mice transduced with hM3Dq in MSc vGluT2 neurons following CNO injections, relative to saline-injection conditions ($n = 10$ mice per group). No apparent differences in 30-min food intake (C_1) and 60-min food intake (C_2) were detected between saline and CNO treatments in mice transduced with control fluorescent protein mCherry ($n = 10$ mice per group). (*D*) Change in food intake in vGluT2-Cre mice transduced with control mCherry, hM3Dq, and hM4Di, respectively, in MSc vGluT2 neurons. The change in food intake was calculated by subtracting the average amount of food consumed 30 min following saline injections from the average amount of food consumed 30 min following CNO injections for each mouse tested. Food intake was significantly reduced in mice that expressed hM3Dq vs. mice expressing mCherry or hM4Di ($n = 10$ mice per group). FI, food intake. Data represent mean ± SEM $*P < 0.01$, $**P < 0.01$, $***P < 0.001$; n.s., not significant. Paired Student's *t* tests for B_2 and C_2, repeated measures ANOVA for B_1 and C_1, and one-way ANOVA for *D*.

light cycle, when mice actively consume food (Fig. 2 *A*, B_1, and B_2). Importantly, no differences in food intake were detected when identical experiments were performed on mice transduced with control mCherry (Fig. 2 C_1 and C_2). These results indicate that the decreased food intake was not attributable to nonspecific effects of the DREADD agonist CNO.

Next, we performed loss-of-function experiments by transducing MSc vGluT2 neurons with the inhibitory DREADD-hM4Di. However, administration of CNO to the hM4Di-transduced mice did not significantly affect food intake, although food intake was reduced in hM3Dq-transduced mice relative to both control mCherry and hM4Di-transduced mice (Fig. 2*D*).

MSc vGluT2 Neurons Suppress Feeding During the Light Period. To further explore the contribution of MSc vGluT2 neurons to food intake, we performed similar feeding behavior assays during the light period of the rodent light cycle, when mice do not usually readily consume a large amount of food. We observed that activation of MSc vGluT2 neurons also reduced feeding during the light period, compared with vehicle saline-injected mice (Fig. 3*A*). Consistently, food intake was reduced in hM3Dq-transduced mice compared with control mCherry-transduced mice (Fig. 3*B*).

MSc vGluT2 Neurons Do Not Cause Maladaptive Behaviors. To determine whether activation of MSc vGluT2 neurons reduces feeding by altering locomotion or anxiety levels, we next performed open-field behavioral testing on vGluT2-Cre mice transduced with hM3Dq, hM4Di, or control mCherry in the MSc vGluT2 neurons (Fig. 4). No significant differences were detected between the hM3Dq-, hM4Di-, and mCherry-transduced mice in total distance traveled or mean speed (Fig. 4 *A*–*E*). Meanwhile, we did not detect significant differences in anxiety-related behaviors, such as distance traveled in the center of the open field (Fig. 4*F*). Furthermore, a flavor conditioning test suggests that MSc vGluT2 neurons are not aversive, as no apparent changes in flavor preference were detected between initial and postconditioning conditions when activation of MSc vGluT2 neurons was paired with an initially preferred flavor (Fig. S1). Together, these results suggest that MSc vGluT2 neuron manipulations did not cause apparent maladaptive behaviors.

MSc vGluT2 Neural Projections to Lateral Hypothalamus Do Not Affect Food Intake. To dissect downstream brain regions involved in the MSc vGluT2 neural suppression of food intake, we next injected Cre-recombinase–dependent adeno-associated viral (AAV) vectors expressing the blue light-sensitive protein Channelrhodopsin (ChR2) fused to the enhanced yellow fluorescent protein (eYFP) into the medial septal complex of vGluT2-Cre mice (Fig. 5*A*). Interestingly, we observed strong MSc vGluT2 projection fibers in the lateral hypothalamus (Fig. 5 *B* and *C*), a brain region classically implicated in feeding behavior (27, 28). To selectively stimulate MSc vGluT2 projections in the LH, an optic fiber was inserted above the LH, and in vivo photostimulation was applied to stimulate MSc vGluT2 neural inputs in the LH (Fig. 5 *A* and *D*). Unexpectedly, photostimulation of MSc vGluT2 projections in the lateral hypothalamus did not affect subsequent levels of food intake (Fig. 5*E*). These results suggest that MSc vGluT2 neurons suppress feeding by projecting to another downstream target(s). Future studies are needed to test the role of MSc vGluT2 projections to the LH in other critical LH-mediated behaviors, such as arousal, addiction, and motivation (27–30).

Activation of MSc vGluT2 Projections to PVH Reduces Food Intake. In addition to the lateral hypothalamus, we also observed dense MSc vGluT2 neuronal projections in the PVH. As previously described for the lateral hypothalamus, we stimulated MSc vGluT2 neuronal projections in the PVH by expressing ChR2 in MSc vGluT2 neurons and inserting an optic fiber above the PVH (Fig. 6 *A*–*C*). As opposed to stimulation of the LH, photostimulation of the MSc vGluT2 neural projections in the PVH significantly reduced food intake (Fig. 6*D*). Since photostimulation may exert nonspecific effects that could interfere with feeding behavior, we also examined feeding in vGluT2-Cre mice transduced with control eYFP. As expected, food intake did not change following photostimulation in the control eYFP-transduced mice (Fig. 6*E*). Consistently, food intake was reduced in ChR2-transduced mice compared with control eYFP-transduced mice (Fig. 6*F*).

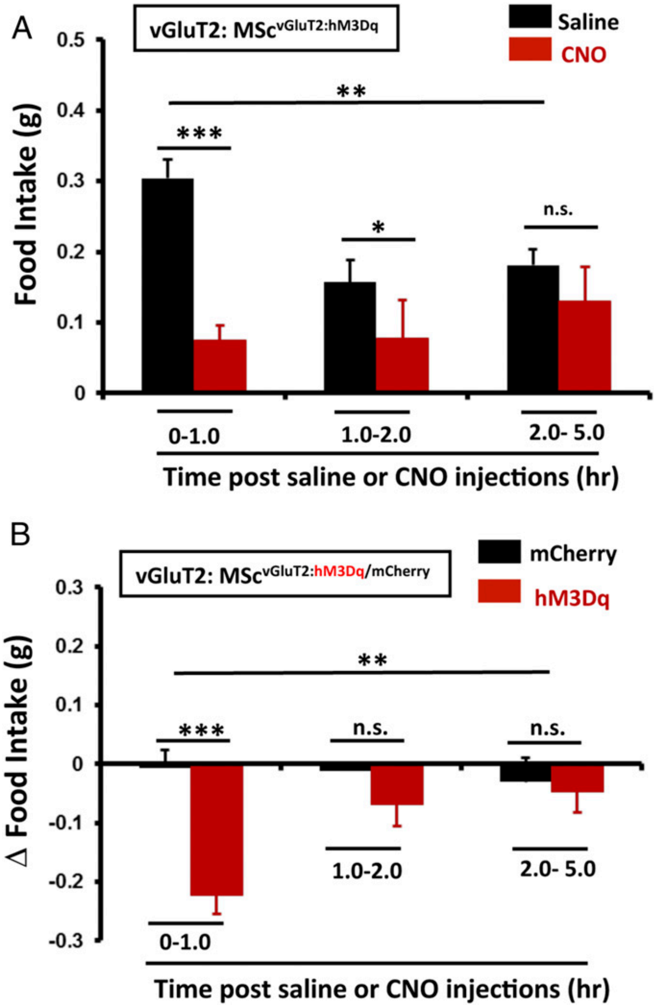

Fig. 3. Activation of MSc vGluT2 neurons reduces daytime food intake. (*A*) Food intake postinjection of saline or CNO, respectively, to vGluT2-Cre mice transduced with hM3Dq in the MSc. Food intake was decreased from 0 to 1 h and 1 to 2 h, but not from 2 to 5 h post-CNO administration, relative to saline treatment ($n = 6$ mice per group; repeated measures ANOVA). (*B*) Changes in food intake relative to saline injections for mice transfected with control mCherry or hM3Dq in MSc vGluT2 neurons. Food intake was reduced in hM3Dq mice compared with mCherry-transduced mice ($n = 6$ mice per group; mixed ANOVA). Change in food intake was calculated by subtracting the average food intake for each mouse following saline injections from the average food intake for each mouse following CNO injections. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; n.s., not significant. Data represent mean ± SEM.

Discussion

Feeding behavior is primarily orchestrated by homeostatic neural circuits located in the hypothalamus and hindbrain that respond to peripheral energy state cues to adaptively modulate food intake (1, 2). Higher-order cognitive and emotional brain regions,

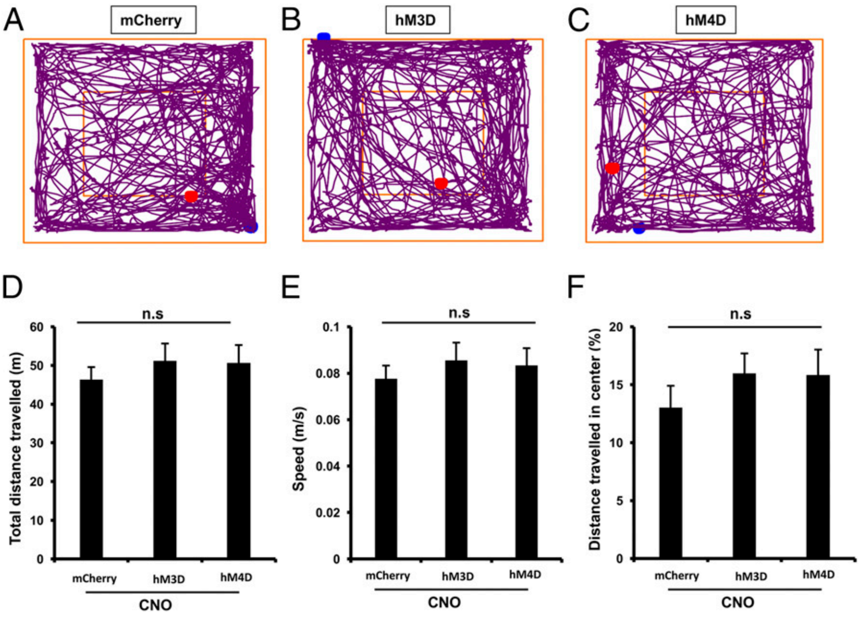

Fig. 4. MSc vGluT2 neurons do not alter locomotion and anxiety-related behaviors. Representative open-field behavioral tracks of vGluT2-Cre mice transduced with control mCherry (*A*), hM3Dq (*B*), and hM4Di (*C*) with CNO treatment (1 mg/kg). Open-field behavioral tests were performed in vGluT2-Cre mice transfected with hM3Dq ($n = 10$ mice), hM4Di ($n = 10$ mice), or control mCherry ($n = 8$ mice) in MSc vGluT2 neurons. No significant differences in total distance traveled (*D*), mean speed (*E*), and distance traveled in the center of open field (*F*) were detected between the mice transduced with control mCherry ($n = 8$), hM3Dq ($n = 10$), and hM4Di ($n = 10$), during 10 min of open-field exploration. All mice were injected with CNO 10–20 min before open-field experiments. One-way ANOVA was used to analyze all panels. n.s., not significant. Data represent mean ± SEM. For *A*–*C*, blue dots represent start point and red dots represent end point.

such as hippocampus and prefrontal cortex, also modulate feeding by providing cognitive and/or emotional valence to feeding behavior (4–13). However, the precise cell types and neural circuits within cognitive and emotional brain regions that contribute to feeding behavior remain underexplored. Here, we investigate the contribution of medial septal brain regions, an area of the brain involved in emotion, cognition, and locomotion, to feeding behavior (31–33). We find that vGluT2-expressing

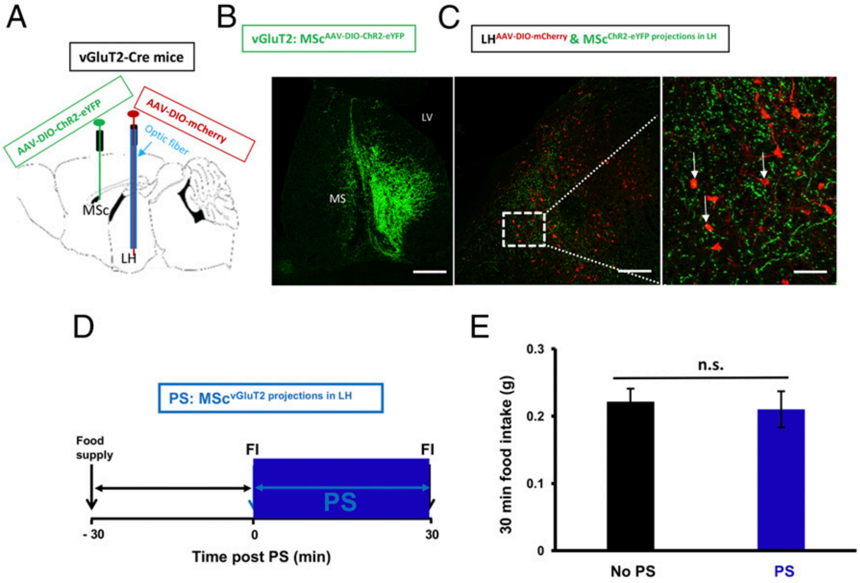

Fig. 5. MSc vGluT2 projections to LH do not affect feeding. (*A*) Schematic illustration of viral vector injection strategy. AAV vectors expressing Cre-dependent ChR2 were injected into the MSc together with a second AAV injection of the Cre-dependent mCherry protein in lateral hypothalamus to visualize putative LH vGluT2 neurons. An optic fiber was placed above the lateral hypothalamus to selectively stimulate MSc vGluT2 inputs to LH. (*B*) Representative image showing expression of ChR2-eYFP in MSc vGluT2 neurons. (*C*) Strong MSc projections of ChR2-eYFP positive fibers were observed in the lateral hypothalamus in the vicinity of LH vGluT2 neurons expressing mCherry. (*D*) Experimental protocol for optogenetic feeding behavior experiments. Food intake was measured in 30-min increments before and during PS, as shown. (*E*) Photostimulation had no effect on food intake relative to before PS (paired Student's *t* test, $n = 6$ mice). [Scale bars, 400 μm for *B*, 200 μm for *C* (*Left*), and 20 μm for *C* (*Right*).] FI, food intake; LH, lateral hypothalamus; LV, lateral ventricle; MS, medial septum; n.s., not significant; PS, photostimulation; SFi, septofimbrial nucleus. Data represent mean ± SEM.

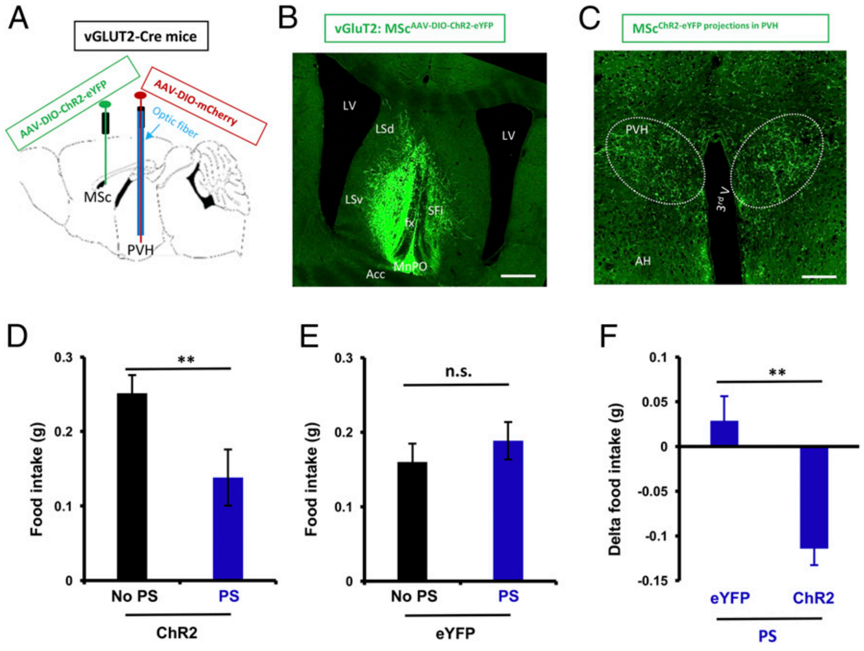

Fig. 6. MSc vGluT2 projections to PVH reduce feeding. (*A*) AAV vector expressing Cre-dependent ChR2 was injected into MSc and an optic fiber was placed above the paraventricular hypothalamus of vGluT2-Cre mice to stimulate MSc vGluT2 projections to the PVH. Representative images showing expression of ChR2-eYFP in MSc vGluT2 neurons (*B*) and ChR2-eYFP–expressing fibers in the PVH (*C*). (*D*) Photostimulation of MSc vGluT2 projections in PVH reduced food intake relative to no photostimulation (paired Student's *t* test, $n = 6$ mice). (*E*) No significant differences in food intake were detected following stimulation of the PVH in vGluT2-Cre mice transduced with control eYFP (paired Student's *t* test, $n = 7$ mice). (*F*) Change in food intake relative to no photostimulation in mice transduced with ChR2 in MSc or control eYFP in MSc. Food intake was reduced following stimulation of MSc vGluT2 projections in the PVH in mice transduced with ChR2 relative to mice transduced with control eYFP. Change in food intake was calculated by subtracting the average amount of food consumed during no photostimulation from the average amount of food consumed with photostimulation for each mouse tested. AH, anterior hypothalamus. (Scale bars, 200 μm for *B* and 100 μm for *C*.) $^{**}P < 0.01$. Data represent mean ± SEM.

neurons within the MSc reduce food intake (Figs. 2 and 3). Importantly, activation of these neurons did not overtly affect anxiety behavior, locomotion, and aversive behavior (Fig. 4 and Fig. S1), indicating that MSc vGluT2 neurons do not reduce feeding merely via a secondary response to maladaptive behaviors. Along these lines, it is noteworthy that chemogenetic inhibition of MSc vGluT2 neurons was not sufficient to increase feeding (Fig. 2*D*). Although we cannot exclude other possibilities, inhibition of MSc vGluT2 neurons may only increase food intake if the basal activity of these neurons is elevated during the period when these neurons are reversibly inhibited. Therefore, future studies are needed to determine the in vivo firing rates of MSc vGluT2 neurons during naturally occurring feeding behaviors to design reversible inhibition experiments that more accurately reflect the function of these neurons in vivo.

Meanwhile, although MSc vGluT2 neurons reduce feeding, the underlying neural circuits responsible for this effect remained elusive. In the current study, we tested the hypothesis that MSc vGluT2 neurons reduce feeding by projecting to the LH (Fig. 5). Importantly, we observed dense MSc vGluT2 neuronal projection fibers in the LH that overlapped with anorexic vGluT2 expressing neurons (Fig. 5*C*). However, photostimulation of MSc vGluT2 neuronal projection fibers in the LH did not affect feeding (Fig. 5*E*), indicating that the LH is likely not a downstream target mediating MSc vGluT2 neural suppression of food intake. This particular projection may have significance for other LH-mediated behaviors, such as arousal, addiction, and motivation (29, 30), which will be further studied in the future.

In addition to the lateral hypothalamus, we observed MSc vGluT2 neuronal fibers in other hypothalamic brain regions involved in feeding, such as PVH. In contrast to stimulation of MSc vGluT2 inputs to the LH, stimulation of MSc vGluT2 inputs to the PVH reduced food intake (Fig. 6). Given the well-described role for the PVH as a brain region that largely suppresses feeding (34, 35), excitatory inputs to the PVH would likely be expected to suppress food intake, as has been previously described for other sources of excitatory inputs to the PVH (36, 37). However, the postsynaptic cell type in the PVH that mediates the role of MSc vGluT2 inputs remains to be determined.

Taken together, we report a neuronal population in the septum involved in suppressing food intake. These neurons exert their anorexic effects without overtly influencing locomotion, anxiety, or aversive behavior, suggesting that they primarily affect the feeding circuitry. Future studies are needed to precisely determine the role of MSc vGluT2 neurons in physiological and/or pathological forms of feeding and to precisely determine the neural circuitry governing the role of these neurons in feeding behavior. Nonetheless, MSc vGluT2 neurons represent a promising cellular entry point for future experiments investigating higher-level control of feeding behavior.

Materials and Methods

All experiments were performed in agreement with the guidelines described by the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the State University of New York Upstate Medical University.

Mice. Both male and female adult mice (5–8 wk old) were used for all experiments. The vGluT2-Cre transgenic mice used in this study were obtained from The Jackson Laboratory. All mice were provided ad libitum access to food (5008 Formulab Diet, LabDiet) and water. Before stereotaxic injections, mice were group housed with three to five mice per cage.

Supporting Information. *Supporting Information* includes viral vectors, viral injections and optic fiber placement, open-field behavioral test, feeding behavior assays, conditioned flavor aversion test, immunofluorescence and imaging, and data analysis.

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