

1 **Incretin hormones and pharmacomimetics rapidly inhibit AgRP neuron activity to**
2 **suppress appetite**

3 Hayley E. McMorrow^{1,2}, Carolyn M. Lorch^{1,3}, Nikolas W. Hayes^{1,2}, Stefan W. Fleps^{1,4}, Joshua A.
4 Frydman¹, Jessica L. Xia¹, Ricardo J. Samms⁵, Lisa R. Beutler^{1,6}.

5 1. Department of Medicine, Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern
6 University, Chicago, IL 60611, USA

7 2. Interdepartmental Neuroscience Graduate Program, Northwestern University, Chicago, IL, USA

8 3. Driskill Graduate Program in Life Sciences, Northwestern University, Chicago, IL, USA

9 4. Department of Neuroscience, Northwestern University, Chicago, IL, USA

10 5. Diabetes, Obesity and Complications Therapeutic Area, Eli Lilly, Indianapolis, IN, USA

11 6. Lead contact

12

13 **Abstract**

14 Analogs of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent
15 insulinotropic peptide (GIP) have become mainstays of obesity and diabetes management.
16 However, both the physiologic role of incretin hormones in the control of appetite and the
17 pharmacologic mechanisms by which incretin-mimetic drugs suppress caloric intake remain
18 incompletely understood. Hunger-promoting AgRP-expressing neurons are an important
19 hypothalamic population that regulates food intake. Therefore, we set out to determine how
20 incretins analogs affect their activity *in vivo*. Using fiber photometry, we observed that both GIP
21 receptor (GIPR) and GLP-1 receptor (GLP-1R) agonism acutely inhibit AgRP neuron activity in
22 fasted mice and reduce the response of AgRP neurons to food. Moreover, optogenetic stimulation
23 of AgRP neurons partially attenuated incretin-induced feeding suppression, suggesting that AgRP
24 neuron inhibition is necessary for the full appetite-suppressing effects of incretin-based
25 therapeutics. Finally, we found that GIP but not GLP-1 is necessary for nutrient-mediated AgRP
26 neuron inhibition, representing a novel physiologic role for GIP in maintaining energy balance.

27 Taken together, these findings reveal neural mechanisms underlying the efficacy of incretin-
28 mimetic obesity therapies. Understanding these drugs' mechanisms of action is crucial for the
29 development of next-generation obesity pharmacotherapies with an improved therapeutic profile.

30

31 **Main**

32 AgRP neuron activity is necessary and sufficient to promote food intake and critical for maintaining
33 energy homeostasis¹⁻⁵. These neurons integrate external sensory stimuli and interoceptive
34 signals from the gastrointestinal tract to promote adaptive feeding behavior⁶⁻¹⁰. Through
35 incompletely understood mechanisms, AgRP neuron inhibition by recently consumed nutrients
36 reduces subsequent food intake via multiple gut-derived signals and neural circuits^{7,11-15}. Here,
37 we set out to investigate the effects of incretin hormones on AgRP neuron activity.

38

39 We equipped mice for *in vivo* imaging of AgRP neurons using fiber photometry. Intraperitoneal
40 (IP) injection of the GIP analog (D-Ala²)-GIP (DA-GIP) or the GLP-1 analog Exendin-4 (Ex-4)
41 rapidly inhibited AgRP neuron activity (**Fig 1**). This is consistent with *ex vivo* studies showing that
42 GLP-1 analogs inhibit AgRP neurons^{16,17}. The response of AgRP neurons to incretin analogs was
43 dose-dependent (**Fig S1A–H, S2A–H**). Ex-4 induced greater neural inhibition than DA-GIP, and
44 AgRP neuron inhibition in response to the combination of Ex-4 and DA-GIP was stronger than
45 the response to Ex-4 alone (**Fig. 1A–E**).

46

47 In addition to post-ingestive feedback, chow presentation induces rapid, pre-consummatory AgRP
48 neuron inhibition with a magnitude of inhibition that correlates with the quantity of subsequent
49 food intake⁶⁻¹⁰. As previously shown for the long-acting GLP-1R agonist liraglutide¹⁶, pre-
50 treatment with DA-GIP or Ex-4 blunted subsequent chow-induced AgRP neuron inhibition
51 compared to pretreatment with saline in the same mice (**Fig. 2A–H**). Remarkably, when given in

52 combination, Ex-4 and DA-GIP suppressed chow-induced neuron inhibition more than Ex-4 alone
53 (**Fig. 2C, D**). Reduced AgRP neuron responses to chow presentation correlated with feeding
54 suppression induced by DA-GIP, Ex-4 or, DA-GIP + Ex-4 in fasted wildtype mice (**Fig. 2I**).
55 Specifically, consistent with prior findings, acute treatment with DA-GIP modestly inhibited fast re-
56 feeding but significantly potentiated the suppression of food intake induced by Ex-4¹⁸. The effect
57 of Ex-4 on chow-induced AgRP neuron inhibition was dose-dependent (**Fig. S2I–P**), consistent
58 with dose-dependent effects of GLP-1 analogs on food intake¹⁹. By contrast, the effect of DA-GIP
59 on chow-induced AgRP neuron inhibition did not vary significantly with dose (**Fig. S1I–P**), in line
60 with the more subtle acute effects of DA-GIP on food intake. Taken together, the additive effect
61 of GIPR and GLP-1R agonism on AgRP neuron dynamics aligns with mounting evidence for the
62 superior efficacy of combined GIP and GLP-1R activation when compared to selective GLP-1R
63 monoagonism for the treatment of obesity^{20–24}.

64

65 We next used an optogenetic approach to investigate the behavioral relevance of incretin-
66 mediated AgRP neuron inhibition. To determine whether AgRP neuron stimulation can overcome
67 incretin receptor agonist-induced feeding suppression, mice that express channelrhodopsin2
68 (ChR2) selectively in AgRP neurons (AgRP::ChR2 mice) were equipped for optogenetic
69 stimulation of AgRP neuron cell bodies. These mice were fasted for five hours, habituated to
70 feeding chambers for 30 minutes, then systemically treated with saline, Ex-4, or Ex-4 + DA-GIP
71 and immediately re-fed in the absence or presence of light stimulation (**Fig. 3A**). In saline-treated
72 mice, AgRP neuron stimulation significantly increased food intake as expected (**Fig. 3B**). AgRP
73 neuron stimulation partially rescued the anorexia induced by both Ex-4 and Ex-4 + DA-GIP (**Fig.**
74 **3B**). Thus, AgRP neuron inhibition likely contributes to incretin analog-induced appetite
75 suppression.

76

77 These experiments focused on the pharmacologic effects of incretin hormones on AgRP neurons.
78 The physiologic effects of incretins on hypothalamic feeding circuits are also incompletely
79 understood. Specifically, it is unclear whether GIPR and/or GLP-1R signaling are necessary for
80 nutrient-mediated AgRP neuron inhibition^{7,8}. To examine this, mice were equipped for fiber
81 photometry recording from AgRP neurons and intragastric infusion of nutrients⁷, and neural
82 responses to nutrients were measured in the presence versus absence of incretin receptor
83 blockade. To examine the role of GIPR, we first pre-treated mice with a control (non-neutralizing)
84 antibody, then intragastrically administered glucose, lipid, or Ensure on different days. Following
85 intragastric nutrient infusions under control conditions, mice were treated with a long-acting,
86 neutralizing monoclonal murine GIPR blocking antibody (muGIPR-Ab)²⁵, and nutrient infusions
87 were repeated. GIPR blockade attenuated glucose- and Ensure-mediated AgRP neuron
88 inhibition, but not lipid-induced AgRP neuron inhibition (**Fig. 4**). Because muGIPR-Ab is long-
89 acting, the order of pre-treatments could not be counterbalanced. However, control experiments
90 showed that mice maintained consistent neural responses to repeated intragastric nutrient
91 infusions over one to two weeks in the absence of antibody treatment (**Fig. S3**), and multiple prior
92 studies have shown consistent nutrient-mediated AgRP neural responses for several weeks in
93 control mice²⁶⁻²⁸. In contrast to the dramatic effects of GIPR blockade, pretreatment with the GLP-
94 1R antagonist exendin 9-39 (Ex-9) had no effect on nutrient-mediated AgRP neuron inhibition
95 (**Fig. S4**). Finally, neither the GIPR blocking antibody nor Ex-9 dramatically impacted AgRP
96 neuron inhibition in response to food presentation (**Fig. S5 A–G**). This suggests that the blunted
97 responses to gastrointestinal nutrients following muGIPR-Ab are not likely due to a floor effect in
98 the setting of altered baseline AgRP neuron activity. The very small but statistically significant
99 effect of muGIPR-Ab on chow-mediated AgRP neuron inhibition we observed may be related to
100 its previously reported modest effect on food intake (**Fig S5 A, B**)^{25,29}. This newly described
101 physiological function for GIP in mediating nutrient-dependent AgRP neuron inhibition may
102 partially underlie the enhanced weight loss efficacy of dual GIP and GLP-1R agonists when

103 compared to GLP-1R monoagonists, as GIPR activation may recapitulate the post-ingestive
104 effects of glucose.

105

106 Taken together, these findings reveal novel roles for AgRP neurons in incretin pharmacology and
107 physiology. In particular, the physiologic function of GIP in food intake and body weight
108 maintenance has remained elusive. Numerous studies have shown that GIPR agonism reduces
109 food intake^{30,31}. By contrast, other studies have shown that global GIPR knockout mice are
110 protected from both obesity and insulin resistance when fed a high-fat diet³²⁻³⁶, and GIPR
111 antagonism coupled with GLP-1R agonism leads to weight loss in early clinical trials^{37,38} and
112 mouse models^{25,29}. Here, we have identified a clear role for GIP in gut-brain communication with
113 AgRP neurons in a manner that is consistent with the anorexigenic effects of GIPR agonism.
114 Further studies will be required to define which GIPR-expressing neurons are required to elicit
115 this effect, and how obesity impacts gut-brain axis responsiveness to incretin hormones *in vivo*²⁶⁻
116 ²⁸.

117

118 In addition to illuminating a previously unknown physiologic role of GIP, we have also shown that
119 AgRP neurons play a critical role in mediating the anorexigenic effects of pharmacologic incretin
120 receptor agonism. GLP-1 and GIP analogs rapidly inhibit AgRP neurons, and stimulation of AgRP
121 neurons partially restores food intake following treatment with these incretin agonists, suggesting
122 that AgRP neuron inhibition contributes to the anorexigenic effect of incretin-mimetic therapies.
123 While these findings add significantly to our mechanistic understanding of incretin-based anti-
124 obesity agents, many questions remain to be addressed.

125

126 It is unclear what cell types and circuits GLP-1R and GIPR agonists act upon to inhibit AgRP
127 neurons, though based on prior *ex vivo* physiology studies and RNA sequencing findings, this

128 effect is likely indirect^{16,17,39-41}. The GLP-1R is expressed in feeding-related nuclei in the
129 hypothalamus and brainstem^{42,43}, and knockout from glutamatergic but not GABAergic neurons
130 almost entirely abrogates GLP-1R agonist-induced weight loss in obese mice⁴⁴. While
131 hypothalamic or hindbrain knockdown of GLP-1R reduces liraglutide efficacy, no brain region has
132 been shown to be solely responsible for the weight loss efficacy of this drug^{17,45,46}. A recent study
133 described a population of GLP-1R agonist-activated neurons in the arcuate nucleus that inhibit
134 AgRP neurons; however, whether direct GLP-1R activation of this cell type contributes to incretin-
135 mimetic induced weight loss has yet to be illuminated⁴⁷. The GLP-1R is also expressed in a large
136 population of vagal afferent neurons⁴⁸⁻⁵¹, and chemogenetic activation of these distension-sensing
137 nodose ganglion neurons is sufficient to inhibit AgRP neurons⁵⁰. Moreover, central blockade of
138 the GLP-1R does not abrogate the anorexic effects of peripherally administered Ex-4⁵², and GLP-
139 1R deletion from peripheral sensory neurons modestly attenuates the appetite suppressing and
140 weight loss efficacy of GLP-1R agonists in obese mice^{53,54}. Thus, GLP-1-induced appetite
141 suppression and weight loss may be mediated by multiple peripheral and central neural circuits⁵⁵.
142 Alongside prior studies, our data suggest that AgRP neurons are an indirect but critical target of
143 GLP-1-based therapies.

144
145 Similarly, the GIPR is expressed in hypothalamic feeding centers, area postrema and NTS but
146 not in hypothalamic AgRP neurons^{40,41}. CNS knockout of the GIPR from GABAergic neurons
147 blocks the modest anorectic effects of long-acting GIPR agonists and abrogates the benefit of
148 dual GLP-1 and GIP receptor agonism when compared to GLP-1R agonism in obese mice^{31,40,56}.
149 Chemogenetic activation of GIPR-expressing cells in the hypothalamus or dorsal vagal complex
150 reduces feeding, but local GIPR knockout in the hypothalamus does not blunt incretin-mimetic
151 induced weight loss^{40,57}. The GIPR is also expressed at low levels in nodose and dorsal root
152 ganglia, but its function in these cell populations has not been examined^{48,57-59}. Recent data
153 support a critical role for spinal afferent neurons in glucose-mediated AgRP neuron inhibition¹²,

154 and it is possible that GIPR activation in peripheral sensory neurons is required for this. Additional
155 studies examining the effect on feeding and neural activity of local, cell-type specific GIPR
156 knockout will be necessary to clarify the physiologic and pharmacologic roles of this hormone.

157
158 In summary, gut hormone receptor agonism has ushered in a new era of obesity management
159 with the efficacy of multi-receptor agonism rivaling that of bariatric surgery. Understanding the
160 molecular and circuit-based mechanisms of hormone-mediated appetite control is critical to refine
161 and more precisely target future therapies to the key cell types mediating the transformative
162 effects of these drugs. Using modern neuroscience and genetic approaches, we have dissected
163 the role of AgRP neurons in incretin-mediated gut-brain communication and elucidated previously
164 unreported physiologic and pharmacologic effects of GLP-1 and GIP on this axis.

165

166 **Acknowledgments**

167 We thank Dr. Joseph Bass and Dr. Jones G. Parker for providing feedback on the manuscript.
168 This work was supported by the American Diabetes Association Pathway to Stop Diabetes Award
169 (12-22-ACE-31) and by NIH grants (P30-DK020595), (K08-DK118188), and (R01-DK128477)
170 (L.R.B.).

171

172 **Competing Interests**

173 R.J.S. is employed by Eli Lilly. Eli Lilly supplied the GIPR blocking antibody used in this study. No
174 compounds used clinically or under investigation for clinical use were employed in this work.

175

176 **Figure Titles and Legends**

177

178 **Figure 1. GIPR and GLP-1R agonists acutely inhibit AgRP neurons**

179 **(A-C)** Calcium signal in AgRP neurons from fasted mice injected with DA-GIP (A), Ex-4 (B), or
180 DA-GIP and Ex-4 (C) compared to saline as indicated. n = 7 mice per group.
181 **(D,E)** Average $\Delta F/F$ in mice from (A-C) 4 minutes (D) and 20 minutes (E) after injection. ((D) one-
182 way ANOVA, $p < 0.0001$; (E) one-way ANOVA, $p = 0.0003$).
183 **(F-I)** Heat maps showing $\Delta F/F$ in individual mice injected with saline (F), DA-GIP (G), Ex-4 (H), or
184 DA-GIP and Ex-4 (I).
185 (A-C) Isosbestic traces for all recordings are shown in gray. (A-C, F-I) Vertical dashed lines
186 indicate the time of injection. (D,E) Lines represent individual mice. Error bars indicate mean \pm
187 SEM. Post-hoc comparisons: * $p < 0.05$, ** $p < 0.01$.

188

189 **Figure 2. GIPR and GLP-1R agonists additively attenuate the AgRP neuron response to**
190 **food presentation and food intake**

191 **(A-C)** Calcium signal in AgRP neurons from fasted mice presented with chow 20 minutes after
192 pre-treatment with DA-GIP (A), Ex-4 (B), or DA-GIP and Ex-4 (C) compared to saline as indicated.
193 n = 7 mice per group.
194 **(D)** Average $\Delta F/F$ in mice from (A-C) 4 minutes after chow presentation. (one-way ANOVA,
195 $p = < 0.0001$).
196 **(E-H)** Heat maps showing $\Delta F/F$ in individual mice from (A-C) after chow presentation.
197 **(I)** Four-hour chow intake following a five-hour fast and incretin or saline injection as indicated in
198 C57BL/6 mice. n = 14 mice per group. (one-way ANOVA, $p = < 0.0001$).
199 (A-C) Isosbestic traces for all recordings are shown in gray. (A-C, E-H) Vertical dashed lines
200 indicate the time of chow presentation. (D,I) Lines represent individual mice. Error bars indicate
201 mean \pm SEM. Post-hoc comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

202

203 **Figure 3. AgRP neuron stimulation partially rescues acute incretin-induced feeding**
204 **suppression**

205 **(A)** Experimental schematic.

206 **(B)** 30-minute chow intake in fasted mice following injection of saline, Ex-4, or DA-GIP and Ex-4
207 in the presence or absence of AgRP neuron stimulation as indicated. n = 10 mice per group. (two-
208 way ANOVA, main effect of hormone treatment $p < 0.0001$, main effect of no stim vs. stim
209 $p < 0.0001$, interaction, $p = 0.0036$). Lines represent individual mice. Error bars indicate mean \pm
210 SEM. Post-hoc comparisons: *** $p < 0.001$, **** $p < 0.0001$.

211

212 **Figure 4. Signaling through GIPR is necessary for nutrient-mediated AgRP neuron**
213 **inhibition**

214 **(A,E,I)** Calcium signal in AgRP neurons from fasted mice during infusion of glucose (A), intralipid
215 (E), or Ensure (I) after pre-treatment with control or muGIPR-Ab as indicated. n = 10-11 mice per
216 group.

217 **(B,F,J)** Average $\Delta F/F$ in mice from (A,E,I) at the end of nutrient infusion. ((B) paired t-test,
218 $p < 0.0001$; (F) paired t-test, $p = 0.0557$; (J) paired t-test, $p = 0.0061$).

219 **(C,D,G,H,K,L)** Heat maps showing $\Delta F/F$ in individual mice from (A,E,I) during nutrient infusion.

220 (A,E,I) Isosbestic traces for all recordings are shown in gray. (C,D,G,H,K,L) Vertical dashed lines
221 indicate the start and end of nutrient infusions. (B,F,J) Lines represent individual mice. Error bars
222 indicate mean \pm SEM. T-tests: ** $p < 0.01$, **** $p < 0.0001$.

223

224 **Figure S1. AgRP neuron responses to GIPR agonists are dose-dependent**

225 **(A-C)** Calcium signal in AgRP neurons from fasted mice injected with DA-GIP at 0.05 mg/kg (A),
226 0.25 mg/kg (B), or 1 mg/kg (C) compared to saline as indicated. n = 7 mice per group.

227 **(D)** Average $\Delta F/F$ in mice from (A-C) 4 minutes after injection. (one-way ANOVA, $p < 0.0001$).

228 **(E-H)** Heat maps showing $\Delta F/F$ in individual mice from (A-C) injected with saline (E), DA-GIP at
229 0.05 mg/kg (F), 0.25 mg/kg (G), or 1 mg/kg (H) as indicated.

230 **(I-K)** Calcium signal in AgRP neurons from fasted mice presented with chow 20 minutes after pre-
231 treatment with DA-GIP at 0.05 mg/kg (I), 0.25 mg/kg (J), or 1 mg/kg (K) compared to saline as
232 indicated. n = 7 mice per group.

233 **(L)** Average $\Delta F/F$ in mice from (I-K) 4 minutes after chow presentation. (one-way ANOVA,
234 $p=0.0738$).

235 **(M-P)** Heat maps showing $\Delta F/F$ in individual mice from (I-K) after chow presentation.

236 (A-C, I-K) Isosbestic traces for all recordings are shown in gray. (A-C, E-H, I-K, M-P) Vertical
237 dashed lines indicate the time of injection or chow presentation. (D,L) Lines represent individual
238 mice. Error bars indicate mean \pm SEM. Post-hoc comparisons: * $p<0.05$, ** $p<0.01$, *** $p<0.001$,
239 **** $p<0.0001$.

240

241 **Figure S2. AgRP neuron responses to GLP-1R agonists are dose-dependent**

242 **(A-C)** Calcium signal in AgRP neurons from fasted mice injected with Ex-4 at 0.02 mg/kg (A), 0.25
243 mg/kg (B), or 1 mg/kg (C) compared to saline as indicated. n = 5 mice per group.

244 **(D)** Average $\Delta F/F$ in mice from (A-C) 4 minutes after injection. (one-way ANOVA, $p=0.0119$).

245 **(E-H)** Heat maps showing $\Delta F/F$ in individual mice from (A-C) injected with saline (E), Ex-4 at 0.02
246 mg/kg (F), 0.25 mg/kg (G), or 1 mg/kg (H) as indicated.

247 **(I-K)** Calcium signal in AgRP neurons from fasted mice presented with chow 20 minutes after pre-
248 treatment with Ex-4 at 0.02 mg/kg (I), 0.25 mg/kg (J), or 1 mg/kg (K) compared to saline as
249 indicated. n = 5 mice per group.

250 **(L)** Average $\Delta F/F$ in mice from (I-K) 4 minutes after chow presentation. (one-way ANOVA,
251 $p=0.0219$).

252 **(M-P)** Heat maps showing $\Delta F/F$ in individual mice from (I-K) after chow presentation.

253 (A-C, I-K) Isosbestic traces for all recordings are shown in gray. (A-C, E-H, I-K, M-P) Vertical
254 dashed lines indicate the time of injection or chow presentation. (D,L) Lines represent individual
255 mice. Error bars indicate mean \pm SEM. Post-hoc comparisons: * $p<0.05$.

256

257 **Figure S3. Nutrient-mediated AgRP neuron inhibition is stable over time in untreated mice**

258 **(A,E,I)** Calcium signal in AgRP neurons from fasted mice during infusion of glucose (A), intralipid
259 (E), and Ensure (I) at baseline and 7-10 days later as indicated. n = 9-10 mice per group.

260 **(B,F,J)** Average $\Delta F/F$ in mice from (A,E,I) at the end of nutrient infusion. ((B) paired t-test,
261 p=0.3471; (F) paired t-test, p=0.1781; (J) paired t-test, p=0.2725).

262 **(C,D,G,H,K,L)** Heat maps showing $\Delta F/F$ in individual mice from (A,E,I) during nutrient infusion.

263 (A,E,I) Isosbestic traces for all recordings are shown in gray. (C,D,G,H,K,L) Vertical dashed lines
264 indicate the start and end of nutrient infusions. (B F,J) Lines represent individual mice. Error bars
265 indicate mean \pm SEM.

266

267 **Figure S4. Signaling through GLP-1R is not necessary for nutrient-mediated AgRP neuron**
268 **inhibition**

269 **(A,E,I)** Calcium signal in AgRP neurons from fasted mice during infusion of glucose (A), intralipid
270 (E), or Ensure (I) after pre-treatment with saline or Ex-9 as indicated. n = 6 mice per group.

271 **(B,F,J)** Average $\Delta F/F$ in mice from (A,E,I) at the end of nutrient infusion. ((B) paired t-test,
272 p=0.8918; (F) paired t-test, p=0.1314; (J) paired t-test, p=0.2401).

273 **(C,D,G,H,K,L)** Heat maps showing $\Delta F/F$ in individual mice from (A,E,I) during nutrient infusion.

274 (A,E,I) Isosbestic traces for all recordings are shown in gray. (C,D,G,H,K,L) Vertical dashed lines
275 indicate the start and end of nutrient infusions. (B,F,J) Lines represent individual mice. Error bars
276 indicate mean \pm SEM.

277

278 **Figure S5. GIPR and GLP-1R blockade minimally impact AgRP neural response to food**
279 **presentation or water infusion**

280 **(A,C)** Calcium signal in AgRP neurons from fasted mice presented with chow after pre-treatment
281 with muGIPR-Ab (A), Ex-9 (C) or saline as indicated. n = 8 mice per group.

282 **(B,D)** Average $\Delta F/F$ in mice from (A,C) 4 minutes after chow presentation. ((B) paired t-test,
283 $p=0.0258$; (D) paired t-test, $p=0.0792$).

284 **(E,F,G)** Heat maps showing $\Delta F/F$ in individual mice from (A,C) after chow presentation.

285 **(H,J)** Calcium signal in AgRP neurons from fasted mice during water infusion after pre-treatment
286 with muGIPR-Ab (H), Ex-9 (J) or saline as indicated. $n = 8$ mice per group.

287 **(I,K)** Average $\Delta F/F$ in mice from (H,J) at the end of water infusion. ((I) paired t-test, $p=0.9004$; (K)
288 paired t-test, $p=0.3644$).

289 **(L,M,N)** Heat maps showing $\Delta F/F$ in individual mice from (H,J) during water infusion.

290 (A,C,H,J) Isosbestic traces for all recordings are shown in gray. (A,C,E,F,G,L,M,N) Vertical dashed
291 lines indicate chow presentation or the start and end of water infusions. (B,D,I,K) Lines represent
292 individual mice. Error bars indicate mean \pm SEM. T-tests: $*p<0.05$.

293

294 **Methods**

295 **Animals**

296 Experimental protocols were approved by the Northwestern University IACUC in accordance with
297 NIH guidelines for the Care and Use of Laboratory Animals. Mice were housed in a 12/12-hour
298 reverse light/dark cycle with *ad libitum* chow (Envigo, 7012, Teklad LM-495 Mouse/Rat
299 Sterilizable Diet) and water access. Mice were fasted for 5 or 16 hours before experiments, as
300 indicated in the text and figures. During fasting periods, mice had *ad libitum* water access.

301 *AgRP^{tm1(cre)Lowl}* (AgRP-Cre, #012899, Jackson Labs) animals backcrossed onto a C57BL/6J
302 background were used for fiber photometry and nutrient infusion experiments. For optogenetic
303 experiments, AgRP-Cre mice were crossed with B6.Cg-*Gt(ROSA)26Sor^{tm32(CAG-}*
304 *COP4*H124R/EYFP)^{Hze}*/J mice (ROSA26-loxStoplox-ChR2-eYFP, #024109, Jackson Labs), to generate
305 AgRP::ChR2 animals. C57BL/6J mice (wildtype #000664, Jackson Labs) were used to measure
306 food intake following hormone injections. Experiments were performed in male and female mice

307 2-6 months of age unless otherwise indicated. Male and female data were combined. Experiments
308 were performed during the dark cycle in a dark environment.

309

310 **Stereotaxic Surgery**

311 For photometry experiments, AAV expressing Cre-dependent GCaMP6s (100842-AAV9,
312 AAV9.CAG.Flex.GCaMP6s, Addgene) was injected unilaterally above the arcuate nucleus (ARC)
313 of AgRP-Cre mice. During the same surgery, an optical fiber (MFC_400/430-
314 0.48_6.3mm_MF2.5_FLT, Doric Lenses) was implanted unilaterally at the coordinates $x = +0.25$
315 mm, $y = -1.65$ mm, $z = -5.95$ mm from bregma. Mice were allowed 2 weeks for recovery and viral
316 expression before beginning experiments or implanting intragastric catheters.

317

318 For optogenetic experiments, fiberoptic implants (MFC_200/245_0.37_6.1mm_ZF1.25_FLT,
319 Doric Lenses) were placed unilaterally above the ARC of AgRP::ChR2 mice at the coordinates x
320 $= +0.25$ mm, $y = -1.63$ mm, $z = -5.85$ mm from bregma. Mice were allowed 10 days for recovery
321 during which they were habituated to handling, intraperitoneal injection, and tethering to patch
322 cords in feeding chambers before performing experiments.

323

324 Following both surgeries, mice were treated with meloxicam and buprenorphine.

325

326 **Intragastric Catheter Implantation**

327 Surgery was performed as previously described^{7,60}. AgRP-Cre mice with working photometry
328 implants were anesthetized with ketamine/xylazine. An incision was made between the scapula,
329 and the skin was dissected from the subcutaneous tissue. An abdominal incision was made from
330 the xyphoid process caudally. A sterilized catheter was pulled into the abdominal cavity via a
331 small puncture in the abdominal wall. The stomach was externalized, punctured, and the catheter
332 was inserted into the puncture site and sutured in place. The stomach was returned to the

333 abdominal cavity and the abdominal muscle and skin were sutured. Lastly, the catheter was
334 secured at its intrascapular cite using a felt button (VABM1B/22, Instech Laboratories), and the
335 intrascapular skin incision was sutured. Post-operatively, mice were treated with meloxicam,
336 buprenorphine, and a dose of enrofloxacin, and allowed 14 days to recover before experiments.

337

338 **Fiber Photometry**

339 Two photometry processors were used in this study (RZ5P and RZ10X, TDT). For the RZ5P
340 setup, the LEDs and LED driver are separate from the processor (DC4100 (LED driver); M405FP1
341 and M470F3 (LEDs), Thorlabs), while the RZ10X processor has these components integrated.
342 Each mouse was run on the same system using the same patch cord for every recording session
343 to allow for reliable within-mouse comparisons.

344

345 Blue LED (465-470 nm) and UV LED (405 nm) were used as excitation light sources. LEDs were
346 modulated at distinct rates and delivered to a fluorescence minicube (Doric Lenses) before
347 connecting to the mouse implants (MFC_400/430-0.48_6.3mm_MF2.5_FLT, Doric Lenses) via
348 patch cords (MFP_400/430/1100-0.57_2m_FCM-MF2.5_LAF, Doric Lenses). Emissions were
349 collected through the patch cords to photoreceivers (Newport Visible Femtowatt Photoreceiver
350 for the RZ5P system; integrated Lux photosensors in the RZ10X system). Digital signals were
351 demodulated, lock-in amplified, and collected through the processors. Data were collected using
352 Synapse software (TDT).

353

354 During recordings, mice were placed in operant chambers (ENV-307W-CT, Med Associates)
355 within light- and sound-attenuating cubicles (ENV-022MD, Med Associates) with no food or water
356 access unless otherwise indicated. Mice with AgRP signals inhibited less than 20% by chow
357 presentation were considered technical failures and excluded from further experiments.

358

359 **Hormone Injections**

360 Exendin-4 (Ex-4) (HY-13443, MedChemExpress) and (D-Ala²)-GIP (DA-GIP) (4054476, Bachem)
361 were injected intraperitoneally (IP) at the doses indicated in the text and figure legends. Where
362 indicated, Ex-4 and DA-GIP were diluted in the same solution and injected simultaneously. All
363 hormones were dissolved in saline. To monitor AgRP neural response to hormone treatment
364 using fiber photometry, AgRP-Cre mice were habituated to handling, photometry recording
365 chambers and IP injections. For recordings, mice were placed in the chambers for 20 minutes
366 prior to injection. Following injection, the photometry recording continued for 20 minutes. To
367 evaluate the effects of hormones on the response of AgRP neurons to food presentation, we
368 presented mice with chow 20 minutes after hormone injection. Recording continued for 4 minutes
369 following chow presentation.

370

371 To evaluate the effects of Ex-4 and DA-GIP on food intake, wildtype C57BL/6J mice were
372 habituated to handling, IP injection, and individual feeding chambers before undergoing a 5 hour
373 fast at the start of dark cycle. Following the fast, mice received an IP injection of saline, DA-GIP
374 (1 mg/kg), Ex-4 (0.02 mg/kg) or DA-GIP and Ex-4 given simultaneously. Mice were immediately
375 re-fed in feeding chambers and food consumption was measured at 4 hours. Each mouse
376 received all treatments on different days and treatment order was counterbalanced.

377

378 **Optogenetic Feeding Experiments**

379 AgRP::ChR2 mice were group-housed and ranged from 4 to 12 months old. For 10 days during
380 recovery from surgery, mice were habituated to handling, recording chambers, and patch cord
381 tethering. An LED source and TTL pulse generator (D-OG-LED-B/B, Prizmatix) were used to
382 generate blue light (460 nm, 2 s ON/3 s OFF, 10 ms pulse width, 20 Hz, 10-20 mW at the fiber
383 tip). Fiber optic patch cables (500um POF N.A. 0.63 L=75cm, Prizmatix) were connected to the

384 mouse implants (MFC_200/245-0.37_6.1mm_ZF1.25_FLT, Doric Lenses) via a sleeve
385 (MFC_200/245-0.37_6.1mm_ZF1.25_FLT, Doric Lenses).

386

387 On test days, mice were given 30 minutes of habituation without LED stimulation or chow.
388 Following habituation, mice received an IP injection of saline, Ex-4 (0.02 mg/kg), or DA-GIP (1
389 mg/kg) plus Ex-4 simultaneously. After injection, mice were immediately given 30 minutes of
390 access to chow with or without light stimulation. Each experiment was performed in the fasted
391 state (5 hours, beginning at start of dark cycle) in the same mice on different days. Hormone
392 treatment order was counterbalanced.

393

394 **Nutrient Infusions during fiber photometry recording.**

395 Nutrients were infused via intragastric catheters using a syringe pump during fiber photometry
396 recordings as previously described⁷. All infusions were given at 0.1 mL per minute for 10 minutes
397 for a total volume of 1 mL. All infusions were calorie matched at 0.5 kcal. Glucose, intralipid and
398 Ensure were dissolved in deionized water. All photometry experiments involving infusions were
399 performed in overnight-fasted AgRP-Cre mice.

400

401 To determine whether signaling through GIPR is critical for nutrient-mediated AgRP neuron
402 inhibition, mice equipped for fiber photometry recording and intragastric nutrient infusion were
403 given an injection of a control, non-neutralizing antibody at 30 mg/kg IP²⁵ (provided by Eli Lilly)
404 and fasted for 16 hours prior to recordings. At the end of the 16-hour fast, the syringe pump was
405 attached to the intragastric catheter using plastic tubing and adaptors, and mice were habituated
406 to the photometry recording chambers for 20 minutes prior to nutrient infusions. Calorie- and
407 volume-matched infusions of glucose, intralipid, or Ensure were given on different days and
408 recording continued for 10 minutes after the end of infusion. These infusions were completed over
409 the course of 7-10 days, and mice were re-injected with the control antibody every 7 days. After

410 completing these infusions, mice were injected with a previously characterized neutralizing mouse
411 anti-murine GIPR antibody (muGIPR-Ab²⁵) at 30 mg/kg and fasted 16 hours before a second
412 round of nutrient infusions was completed as described above. muGIPR-Ab was dosed weekly
413 based on previously published studies^{25,29}. AgRP neuron inhibition induced by nutrient infusions
414 was compared across the two conditions. The long-lasting effects of GIPR antibody blockade
415 precluded us from balancing treatment order, and thus recordings following muGIPR-Ab were
416 each performed 7-10 days after control recordings. Additionally, given its long-lasting effects, for
417 all mice that received muGIPR-Ab, subsequent nutrient infusion was a final experiment before
418 euthanasia and confirmation of implant placement and viral expression. To control for possible
419 changes in fiber photometry signal strength over time as a possible cause of muGIPR-Ab effects,
420 a separate cohort of untreated mice were given nutrient infusions at the same time points
421 indicated above without antibody administration.

422

423 To determine whether signaling through GLP-1R is critical for nutrient-mediated AgRP neuron
424 inhibition, mice were habituated to the photometry recording chamber for 20 minutes then pre-
425 treated with the GLP-1R antagonist Exendin (9-39) (Ex-9, 1 mg/kg) (HY-P0264,
426 MedChemExpress) or vehicle (saline) 5 minutes prior to infusion of glucose, intralipid or Ensure
427 on separate days. Neural recordings were continued for 10 minutes after the end of infusions.
428 Neural responses to infusions following Ex-9 versus vehicle pretreatment were measured 7-10
429 days apart for each nutrient, and treatment order was counterbalanced across mice.

430

431 **Quantification and statistical analysis**

432 *Photometry analysis*

433 Photometry data were analyzed with custom Python scripts
434 (<https://github.com/nikhayes/fibphoflow>), and statistical analyses and data visualizations were
435 generated with Python and Prism. Photometry recordings included emissions from 470nm

436 stimulation and from 405nm stimulation, which were smoothed and downsampled to 1 Hz.
437 Normalization of responses to stimuli relative to baseline was performed on each these signals
438 via the formula: $\Delta F/F = (F_t - F_0) / F_0$, where F_t represents fluorescence at time (t), and F_0
439 represents the average fluorescence during the five-minute baseline period preceding the
440 stimulus start time (time zero). To determine statistical significance, the average $\Delta F/F$ was
441 calculated for the time frames indicated in the legend for Figures 1, 2, 4, and S1-S5.

442

443 *Behavioral data analysis*

444 To determine chow consumption during fast re-feeding and optogenetic experiments, chow was
445 weighed manually at the specified time points.

446

447 *Statistical analysis*

448 Fiber photometry data were collected and analyzed as previously described^{7,26,28}. For photometry
449 traces shown in Figures 1, 2, 4, and S1-S5, $\Delta F/F$ (%) refers to the mean $\Delta F_t/F_0 * 100$. For bar
450 graphs quantifying neural responses to chow presentation (Figures 2, S1, S2, and S5), the
451 average $\Delta F/F$ over a 1-minute period 3-4 minutes following chow presentation was calculated.
452 For bar graphs quantifying neural responses to nutrient or water infusion (Figures 4, S3, S4, and
453 S5), the average $\Delta F/F$ over a 1-minute period at the end of nutrient infusion (9-10min) was
454 calculated. For bar graphs quantifying neural responses to hormone injection (Figures 1, S1, and
455 S2), the average $\Delta F/F$ over a 1-minute period 3-4 minutes or 19-20 minutes after injection was
456 calculated as indicated in the figures.

457

458 The effects of experimental manipulation versus controls were analyzed with a one-way,
459 repeated-measures ANOVA (Figures 1, 2, S1, S2) or paired T-test (Figures 4, S3, S4, S5) as
460 appropriate for photometry experiments. Fast re-feeding in wildtype mice after treatment with
461 saline or incretin agonists (Figure 2) was analyzed with a one-way, repeated-measures ANOVA.

462 Food intake in the presence or absence of light stimulation following saline or hormone
463 administration (Figure 3) was analyzed with a 2-way, repeated-measures ANOVA. The Holm-
464 Šídák multiple comparisons test was used as appropriate. Prism was used for all statistical
465 analyses, and significance was defined as $p < 0.05$. Sample sizes are indicated in the figure
466 legends for each experiment. Where multiple technical replicates of an experiment were
467 performed, trials from the same animal were averaged and handled as a single biological replicate
468 for data analysis and visualization.

469 References

- 470 1 Aponte, Y., Atasoy, D. & Sternson, S. M. AGRP neurons are sufficient to orchestrate
471 feeding behavior rapidly and without training. *Nat Neurosci* **14**, 351-355,
472 doi:10.1038/nn.2739 (2011).
- 473 2 Cai, J. *et al.* AgRP neurons are not indispensable for body weight maintenance in adult
474 mice. *Cell Rep* **42**, 112789, doi:10.1016/j.celrep.2023.112789 (2023).
- 475 3 Gropp, E. *et al.* Agouti-related peptide-expressing neurons are mandatory for feeding.
476 *Nat Neurosci* **8**, 1289-1291, doi:10.1038/nn1548 (2005).
- 477 4 Krashes, M. J. *et al.* Rapid, reversible activation of AgRP neurons drives feeding
478 behavior in mice. *J Clin Invest* **121**, 1424-1428, doi:10.1172/JCI46229 (2011).
- 479 5 Luquet, S., Perez, F. A., Hnasko, T. S. & Palmiter, R. D. NPY/AgRP neurons are
480 essential for feeding in adult mice but can be ablated in neonates. *Science* **310**, 683-
481 685, doi:10.1126/science.1115524 (2005).
- 482 6 Betley, J. N. *et al.* Neurons for hunger and thirst transmit a negative-valence teaching
483 signal. *Nature* **521**, 180-185, doi:10.1038/nature14416 [doi] (2015).
- 484 7 Beutler, L. R. *et al.* Dynamics of Gut-Brain Communication Underlying Hunger. *Neuron*
485 **96**, 461-475 e465, doi:10.1016/j.neuron.2017.09.043 (2017).
- 486 8 Su, Z., Alhadeff, A. L. & Betley, J. N. Nutritive, Post-ingestive Signals Are the Primary
487 Regulators of AgRP Neuron Activity. *Cell Rep* **21**, 2724-2736,
488 doi:10.1016/j.celrep.2017.11.036 (2017).
- 489 9 Chen, Y., Lin, Y. C., Kuo, T. W. & Knight, Z. A. Sensory detection of food rapidly
490 modulates arcuate feeding circuits. *Cell* **160**, 829-841, doi:10.1016/j.cell.2015.01.033
491 [doi] (2015).
- 492 10 Mandelblat-Cerf, Y. *et al.* Arcuate hypothalamic AgRP and putative POMC neurons show
493 opposite changes in spiking across multiple timescales. *Elife* **4**, doi:10.7554/eLife.07122
494 (2015).
- 495 11 Buchanan, K. L. *et al.* The preference for sugar over sweetener depends on a gut sensor
496 cell. *Nat Neurosci* **25**, 191-200, doi:10.1038/s41593-021-00982-7 (2022).
- 497 12 Goldstein, N. *et al.* Hypothalamic detection of macronutrients via multiple gut-brain
498 pathways. *Cell Metab* **33**, 676-687 e675, doi:10.1016/j.cmet.2020.12.018 (2021).
- 499 13 Gribble, F. M. & Reimann, F. Function and mechanisms of enteroendocrine cells and gut
500 hormones in metabolism. *Nat Rev Endocrinol* **15**, 226-237, doi:10.1038/s41574-019-
501 0168-8 (2019).
- 502 14 Kaelberer, M. M., Rupprecht, L. E., Liu, W. W., Weng, P. & Bohorquez, D. V. Neuropod
503 Cells: The Emerging Biology of Gut-Brain Sensory Transduction. *Annu Rev Neurosci* **43**,
504 337-353, doi:10.1146/annurev-neuro-091619-022657 (2020).
- 505 15 Liu, W. W. & Bohorquez, D. V. The neural basis of sugar preference. *Nat Rev Neurosci*
506 **23**, 584-595, doi:10.1038/s41583-022-00613-5 (2022).
- 507 16 Dong, Y. *et al.* Time and metabolic state-dependent effects of GLP-1R agonists on
508 NPY/AgRP and POMC neuronal activity in vivo. *Mol Metab* **54**, 101352,
509 doi:10.1016/j.molmet.2021.101352 (2021).
- 510 17 Secher, A. *et al.* The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-
511 dependent weight loss. *J Clin Invest* **124**, 4473-4488, doi:10.1172/JCI75276 (2014).
- 512 18 NamKoong, C. *et al.* Central administration of GLP-1 and GIP decreases feeding in
513 mice. *Biochem Biophys Res Commun* **490**, 247-252, doi:10.1016/j.bbrc.2017.06.031
514 (2017).
- 515 19 Turton, M. D. *et al.* A role for glucagon-like peptide-1 in the central regulation of feeding.
516 *Nature* **379**, 69-72, doi:10.1038/379069a0 (1996).
- 517 20 Baggio, L. L. & Drucker, D. J. Glucagon-like peptide-1 receptor co-agonists for treating
518 metabolic disease. *Mol Metab* **46**, 101090, doi:10.1016/j.molmet.2020.101090 (2021).

- 519 21 Frias, J. P. *et al.* Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor
520 agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active
521 comparator-controlled phase 2 trial. *Lancet* **392**, 2180-2193, doi:10.1016/S0140-
522 6736(18)32260-8 (2018).
- 523 22 Wilding, J. P. H. *et al.* Once-Weekly Semaglutide in Adults with Overweight or Obesity.
524 *New England Journal of Medicine* **384**, 989-1002, doi:10.1056/NEJMoa2032183 (2021).
- 525 23 Jastreboff, A. M. *et al.* Tirzepatide Once Weekly for the Treatment of Obesity. *New*
526 *England Journal of Medicine* **387**, 205-216, doi:10.1056/NEJMoa2206038 (2022).
- 527 24 Finan, B. *et al.* Unimolecular Dual Incretins Maximize Metabolic Benefits in Rodents,
528 Monkeys, and Humans. *Science Translational Medicine* **5**, 209ra151-209ra151,
529 doi:10.1126/scitranslmed.3007218 (2013).
- 530 25 Killion, E. A. *et al.* Anti-obesity effects of GIPR antagonists alone and in combination with
531 GLP-1R agonists in preclinical models. *Sci Transl Med* **10**,
532 doi:10.1126/scitranslmed.aat3392 (2018).
- 533 26 Beutler, L. R. *et al.* Obesity causes selective and long-lasting desensitization of AgRP
534 neurons to dietary fat. *Elife* **9**, doi:10.7554/eLife.55909 (2020).
- 535 27 Mazzone, C. M. *et al.* High-fat food biases hypothalamic and mesolimbic expression of
536 consummatory drives. *Nat Neurosci* **23**, 1253-1266, doi:10.1038/s41593-020-0684-9
537 (2020).
- 538 28 Lorch, C. M. *et al.* Sucrose overconsumption impairs AgRP neuron dynamics and
539 promotes palatable food intake. *Cell Rep* **43**, 113675, doi:10.1016/j.celrep.2024.113675
540 (2024).
- 541 29 Killion, E. A. *et al.* Chronic glucose-dependent insulinotropic polypeptide receptor (GIPR)
542 agonism desensitizes adipocyte GIPR activity mimicking functional GIPR antagonism.
543 *Nat Commun* **11**, 4981, doi:10.1038/s41467-020-18751-8 (2020).
- 544 30 Mroz, P. A. *et al.* Optimized GIP analogs promote body weight lowering in mice through
545 GIPR agonism not antagonism. *Mol Metab* **20**, 51-62, doi:10.1016/j.molmet.2018.12.001
546 (2019).
- 547 31 Zhang, Q. *et al.* The glucose-dependent insulinotropic polypeptide (GIP) regulates body
548 weight and food intake via CNS-GIPR signaling. *Cell Metab* **33**, 833-844 e835,
549 doi:10.1016/j.cmet.2021.01.015 (2021).
- 550 32 Bates, H. E. *et al.* Gipr is essential for adrenocortical steroidogenesis; however,
551 corticosterone deficiency does not mediate the favorable metabolic phenotype of Gipr(-/-)
552 mice. *Diabetes* **61**, 40-48, doi:10.2337/db11-1060 (2012).
- 553 33 Hansotia, T. *et al.* Extrapancreatic incretin receptors modulate glucose homeostasis,
554 body weight, and energy expenditure. *J Clin Invest* **117**, 143-152, doi:10.1172/JCI25483
555 (2007).
- 556 34 Miyawaki, K. *et al.* Glucose intolerance caused by a defect in the entero-insular axis: a
557 study in gastric inhibitory polypeptide receptor knockout mice. *Proc Natl Acad Sci U S A*
558 **96**, 14843-14847, doi:10.1073/pnas.96.26.14843 (1999).
- 559 35 Miyawaki, K. *et al.* Inhibition of gastric inhibitory polypeptide signaling prevents obesity.
560 *Nat Med* **8**, 738-742, doi:10.1038/nm727 (2002).
- 561 36 Ugleholdt, R. *et al.* Transgenic rescue of adipocyte glucose-dependent insulinotropic
562 polypeptide receptor expression restores high fat diet-induced body weight gain. *J Biol*
563 *Chem* **286**, 44632-44645, doi:10.1074/jbc.M111.311779 (2011).
- 564 37 Hammoud, R. & Drucker, D. J. Beyond the pancreas: contrasting cardiometabolic
565 actions of GIP and GLP1. *Nat Rev Endocrinol* **19**, 201-216, doi:10.1038/s41574-022-
566 00783-3 (2023).
- 567 38 Véniant, M. M. *et al.* A GIPR antagonist conjugated to GLP-1 analogues promotes
568 weight loss with improved metabolic parameters in preclinical and phase 1 settings.
569 *Nature Metabolism* **6**, 290-303, doi:10.1038/s42255-023-00966-w (2024).

- 570 39 He, Z. *et al.* Direct and indirect effects of liraglutide on hypothalamic POMC and
571 NPY/AgRP neurons - Implications for energy balance and glucose control. *Mol Metab*
572 **28**, 120-134, doi:10.1016/j.molmet.2019.07.008 (2019).
- 573 40 Adriaenssens, A. E. *et al.* Glucose-Dependent Insulinotropic Polypeptide Receptor-
574 Expressing Cells in the Hypothalamus Regulate Food Intake. *Cell Metab* **30**, 987-996
575 e986, doi:10.1016/j.cmet.2019.07.013 (2019).
- 576 41 Smith, C. *et al.* A comparative transcriptomic analysis of glucagon-like peptide-1
577 receptor- and glucose-dependent insulinotropic polypeptide receptor-expressing cells in
578 the hypothalamus. *Appetite* **174**, 106022, doi:10.1016/j.appet.2022.106022 (2022).
- 579 42 Cork, S. C. *et al.* Distribution and characterisation of Glucagon-like peptide-1 receptor
580 expressing cells in the mouse brain. *Mol Metab* **4**, 718-731,
581 doi:10.1016/j.molmet.2015.07.008 (2015).
- 582 43 Jensen, C. B. *et al.* Characterization of the Glucagonlike Peptide-1 Receptor in Male
583 Mouse Brain Using a Novel Antibody and In Situ Hybridization. *Endocrinology* **159**, 665-
584 675, doi:10.1210/en.2017-00812 (2018).
- 585 44 Adams, J. M. *et al.* Liraglutide Modulates Appetite and Body Weight Through Glucagon-
586 Like Peptide 1 Receptor-Expressing Glutamatergic Neurons. *Diabetes* **67**, 1538-1548,
587 doi:10.2337/db17-1385 (2018).
- 588 45 Burmeister, M. A. *et al.* The Hypothalamic Glucagon-Like Peptide 1 Receptor Is
589 Sufficient but Not Necessary for the Regulation of Energy Balance and Glucose
590 Homeostasis in Mice. *Diabetes* **66**, 372-384, doi:10.2337/db16-1102 (2017).
- 591 46 Fortin, S. M. *et al.* GABA neurons in the nucleus tractus solitarius express GLP-1
592 receptors and mediate anorectic effects of liraglutide in rats. *Sci Transl Med* **12**,
593 doi:10.1126/scitranslmed.aay8071 (2020).
- 594 47 Webster, A. N. *et al.* Molecular Connectomics Reveals a Glucagon-Like Peptide 1
595 Sensitive Neural Circuit for Satiety. *bioRxiv*, doi:10.1101/2023.10.31.564990 (2023).
- 596 48 Nakagawa, A. *et al.* Receptor gene expression of glucagon-like peptide-1, but not
597 glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. *Auton*
598 *Neurosci* **110**, 36-43, doi:10.1016/j.autneu.2003.11.001 (2004).
- 599 49 Williams, E. K. *et al.* Sensory Neurons that Detect Stretch and Nutrients in the Digestive
600 System. *Cell* **166**, 209-221, doi:10.1016/j.cell.2016.05.011 [doi] (2016).
- 601 50 Bai, L. *et al.* Genetic Identification of Vagal Sensory Neurons That Control Feeding. *Cell*
602 **179**, 1129-1143.e1123, doi:S0092-8674(19)31181-X [pii] (2019).
- 603 51 Krieger, J. P. *et al.* Knockdown of GLP-1 Receptors in Vagal Afferents Affects Normal
604 Food Intake and Glycemia. *Diabetes* **65**, 34-43, doi:10.2337/db15-0973 (2016).
- 605 52 Williams, D. L., Baskin, D. G. & Schwartz, M. W. Evidence that intestinal glucagon-like
606 peptide-1 plays a physiological role in satiety. *Endocrinology* **150**, 1680-1687,
607 doi:10.1210/en.2008-1045 (2009).
- 608 53 Sisley, S. *et al.* Neuronal GLP1R mediates liraglutide's anorectic but not glucose-
609 lowering effect. *J Clin Invest* **124**, 2456-2463, doi:10.1172/JCI72434 (2014).
- 610 54 Varin, E. M. *et al.* Distinct Neural Sites of GLP-1R Expression Mediate Physiological
611 versus Pharmacological Control of Incretin Action. *Cell Rep* **27**, 3371-3384 e3373,
612 doi:10.1016/j.celrep.2019.05.055 (2019).
- 613 55 Brierley, D. I. & de Lartigue, G. Reappraising the role of the vagus nerve in GLP-1-
614 mediated regulation of eating. *Br J Pharmacol* **179**, 584-599, doi:10.1111/bph.15603
615 (2022).
- 616 56 Liskiewicz, A. *et al.* Glucose-dependent insulinotropic polypeptide regulates body weight
617 and food intake via GABAergic neurons in mice. *Nat Metab* **5**, 2075-2085,
618 doi:10.1038/s42255-023-00931-7 (2023).

- 619 57 Adriaenssens, A. *et al.* Hypothalamic and brainstem glucose-dependent insulinotropic
620 polypeptide receptor neurons employ distinct mechanisms to affect feeding. *JCI Insight*
621 **8**, doi:10.1172/jci.insight.164921 (2023).
622 58 Okawa, T. *et al.* Sensory and motor physiological functions are impaired in gastric
623 inhibitory polypeptide receptor-deficient mice. *J Diabetes Investig* **5**, 31-37,
624 doi:10.1111/jdi.12129 (2014).
625 59 Usoskin, D. *et al.* Unbiased classification of sensory neuron types by large-scale single-
626 cell RNA sequencing. *Nat Neurosci* **18**, 145-153, doi:10.1038/nn.3881 (2015).
627 60 Ueno, A. *et al.* Mouse intragastric infusion (iG) model. *Nat Protoc* **7**, 771-781,
628 doi:10.1038/nprot.2012.014 (2012).
629

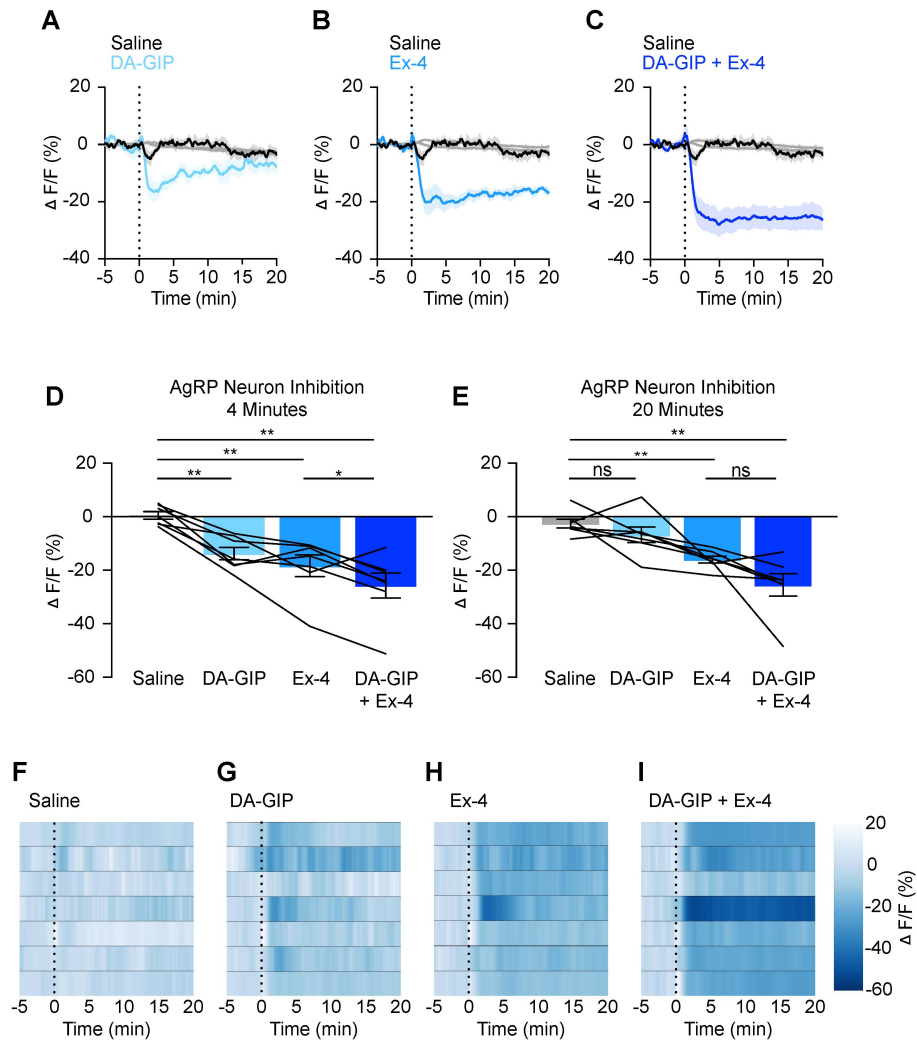


Figure 1. GIPR and GLP-1R agonists acutely inhibit AgRP neurons

(A-C) Calcium signal in AgRP neurons from fasted mice injected with DA-GIP (A), Ex-4 (B), or DA-GIP and Ex-4 (C) compared to saline as indicated. $n = 7$ mice per group.

(D,E) Average $\Delta F/F$ in mice from (A-C) 4 minutes (D) and 20 minutes (E) after injection. ((D) one-way ANOVA, $p < 0.0001$; (E) one-way ANOVA, $p = 0.0003$).

(F-I) Heat maps showing $\Delta F/F$ in individual mice injected with saline (F), DA-GIP (G), Ex-4 (H), or DA-GIP and Ex-4 (I).

(A-C) Isosbestic traces for all recordings are shown in gray. (A-C, F-I) Vertical dashed lines indicate the time of injection. (D,E) Lines represent individual mice. Error bars indicate mean \pm SEM. Post-hoc comparisons: * $p < 0.05$, ** $p < 0.01$.

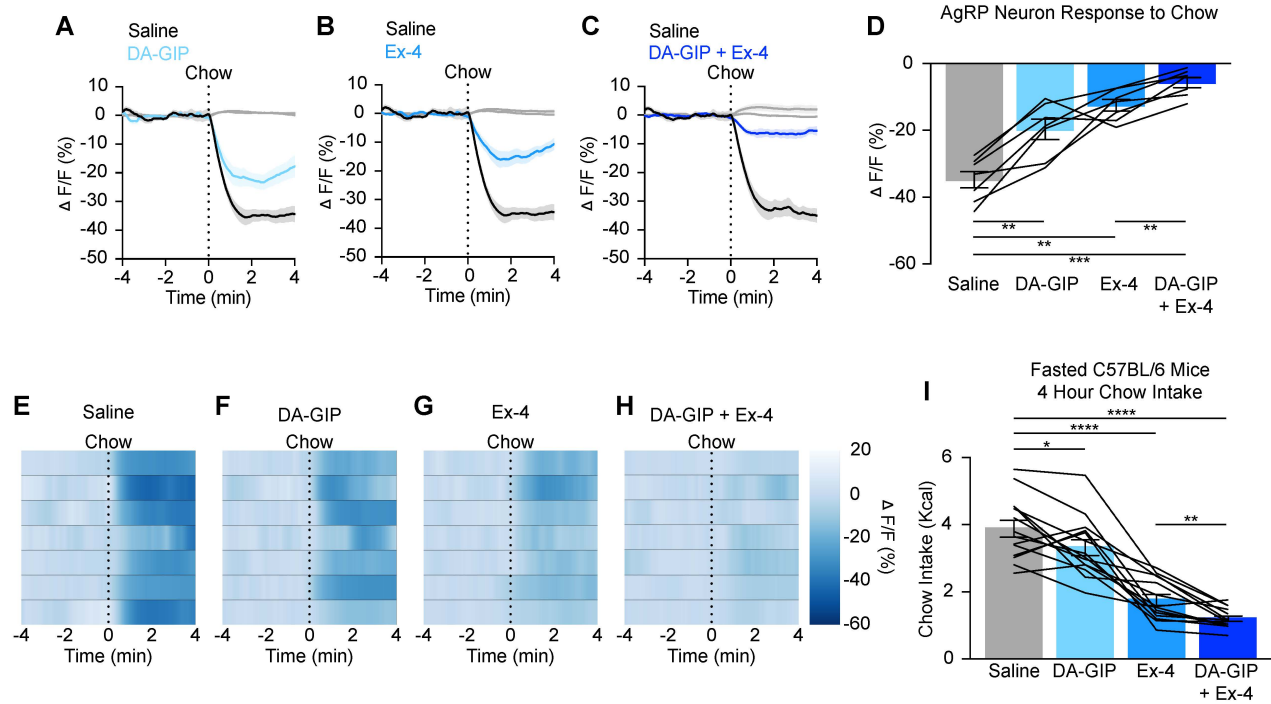


Figure 2. GIPR and GLP-1R agonists additively attenuate the AgRP neuron response to food presentation and food intake

(A-C) Calcium signal in AgRP neurons from fasted mice presented with chow 20 minutes after pre-treatment with DA-GIP (A), Ex-4 (B), or DA-GIP and Ex-4 (C) compared to saline as indicated. $n = 7$ mice per group.

(D) Average $\Delta F/F$ in mice from (A-C) 4 minutes after chow presentation. (one-way ANOVA, $p < 0.0001$).

(E-H) Heat maps showing $\Delta F/F$ in individual mice from (A-C) after chow presentation.

(I) Four-hour chow intake following a five-hour fast and incretin or saline injection as indicated in C57BL/6 mice. $n = 14$ mice per group. (one-way ANOVA, $p < 0.0001$).

(A-C) Isosbestic traces for all recordings are shown in gray. (A-C, E-H) Vertical dashed lines indicate the time of chow presentation. (D, I) Lines represent individual mice. Error bars indicate mean \pm SEM. Post-hoc comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

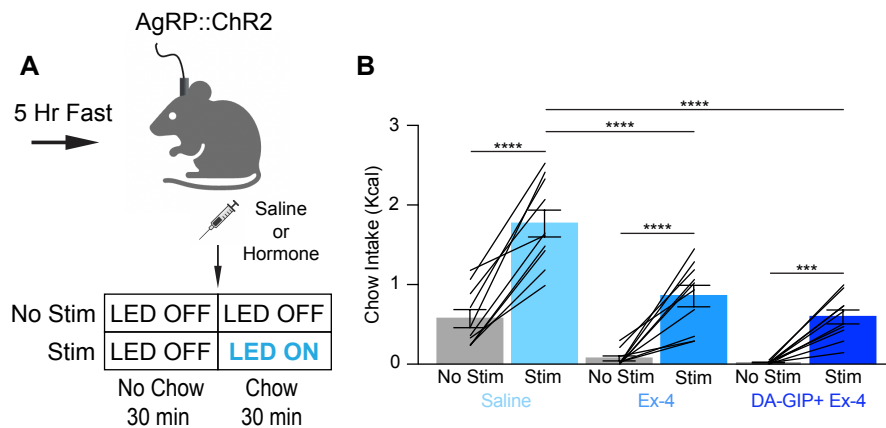


Figure 3. AgRP neuron stimulation partially rescues acute incretin-induced feeding suppression

(A) Experimental schematic.

(B) 30-minute chow intake in fasted mice following injection of saline, Ex-4, or DA-GIP and Ex-4 in the presence or absence of AgRP neuron stimulation as indicated. $n = 10$ mice per group. (two-way ANOVA, main effect of hormone treatment $p < 0.0001$, main effect of no stim vs. stim $p < 0.0001$, interaction, $p = 0.0036$). Lines represent individual mice. Error bars indicate mean \pm SEM. Post-hoc comparisons: *** $p < 0.001$, **** $p < 0.0001$.

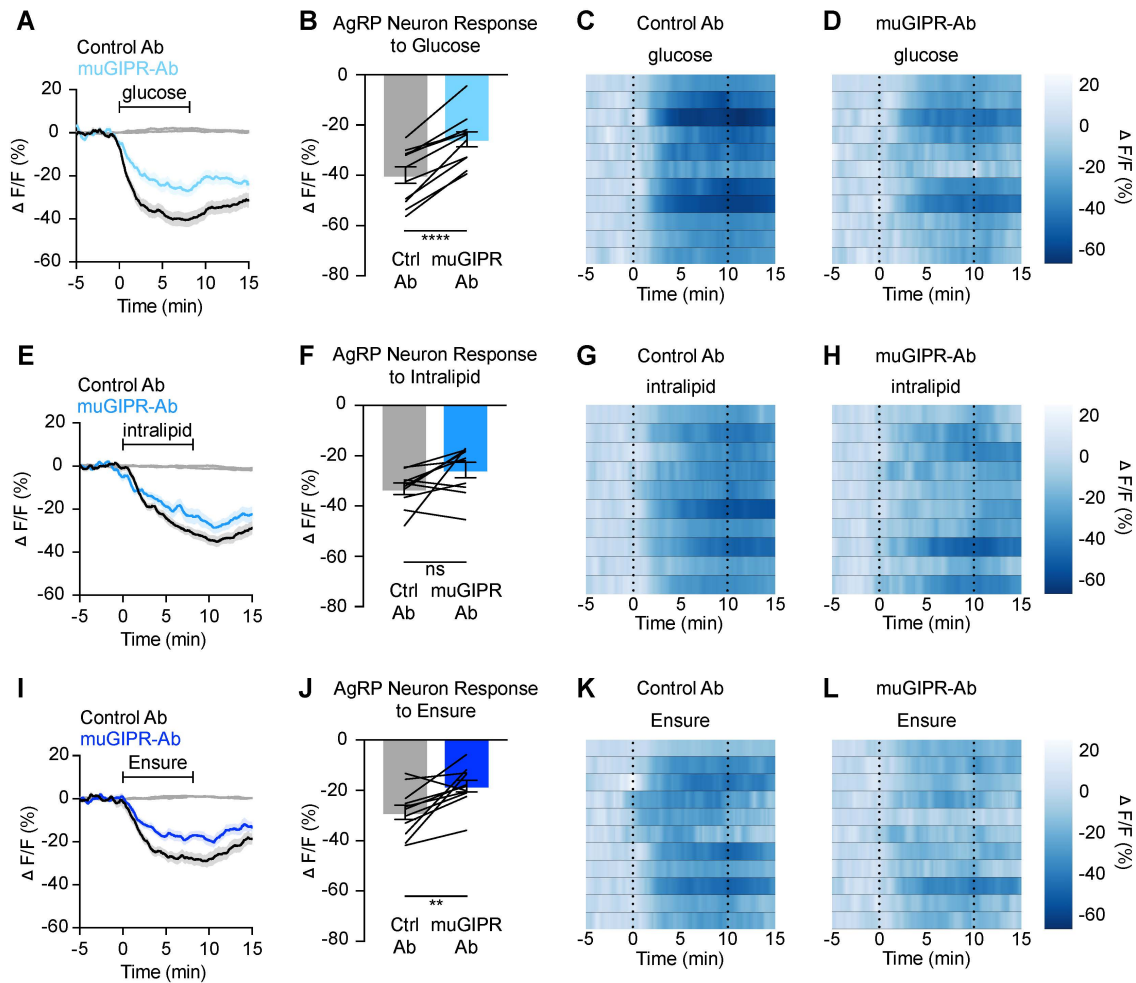


Figure 4. Signaling through GIPR is necessary for nutrient-mediated AgRP neuron inhibition

(A,E,I) Calcium signal in AgRP neurons from fasted mice during infusion of glucose (A), intralipid (E), or Ensure (I) after pre-treatment with control or muGIPR-Ab as indicated. $n = 10-11$ mice per group.

(B,F,J) Average $\Delta F/F$ in mice from (A,E,I) at the end of nutrient infusion. ((B) paired t-test, $p < 0.0001$; (F) paired t-test, $p = 0.0557$; (J) paired t-test, $p = 0.0061$).

(C,D,G,H,K,L) Heat maps showing $\Delta F/F$ in individual mice from (A,E,I) during nutrient infusion.

(A,E,I) Isosbestic traces for all recordings are shown in gray. (C,D,G,H,K,L) Vertical dashed lines indicate the start and end of nutrient infusions. (B,F,J) Lines represent individual mice. Error bars indicate mean \pm SEM. T-tests: ** $p < 0.01$, **** $p < 0.0001$.

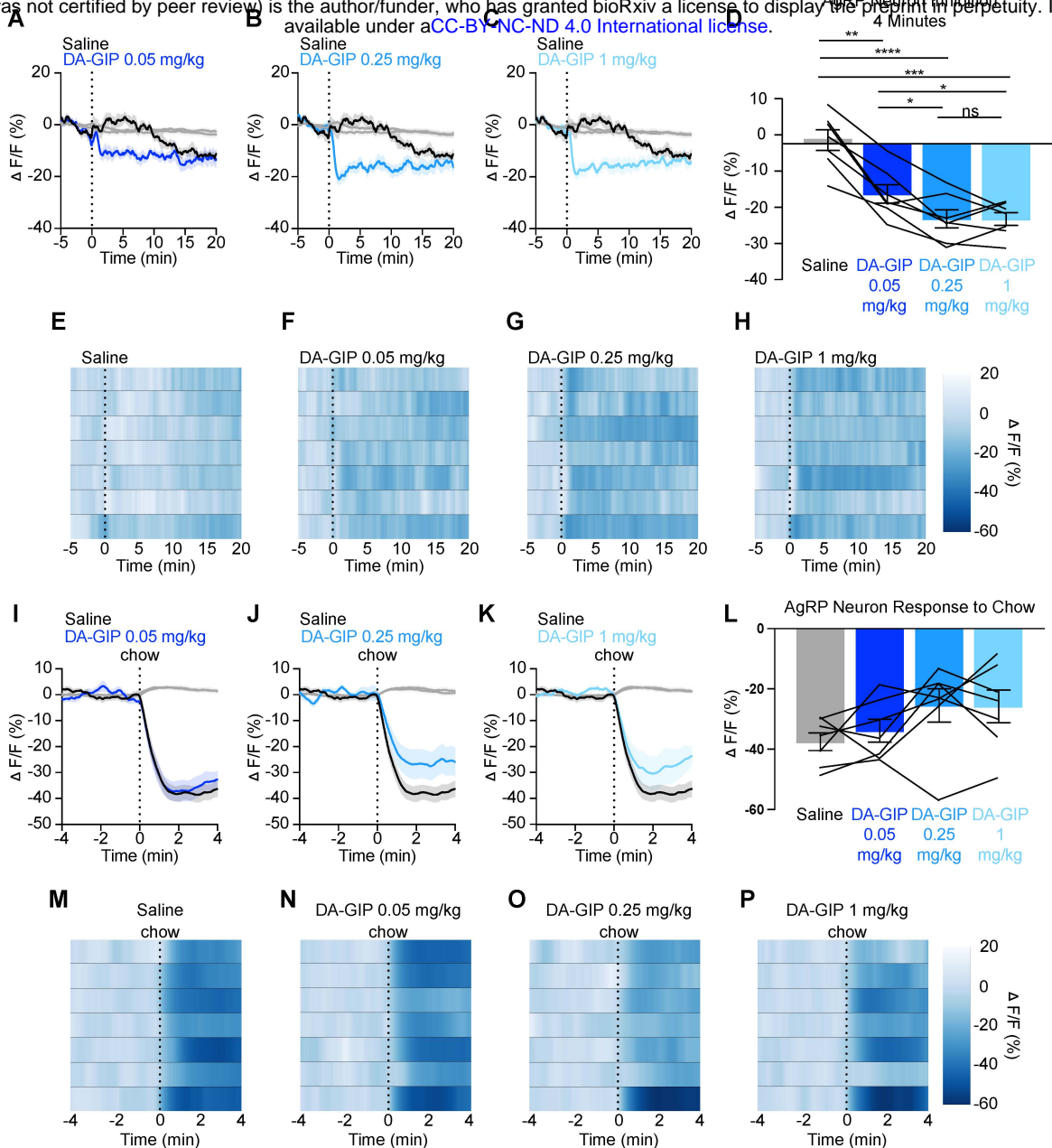


Figure S1. AgRP neuron responses to GIPR agonists are dose-dependent

(A-C) Calcium signal in AgRP neurons from fasted mice injected with DA-GIP at 0.05 mg/kg (A), 0.25 mg/kg (B), or 1 mg/kg (C) compared to saline as indicated. $n = 7$ mice per group.

(D) Average $\Delta F/F$ in mice from (A-C) 4 minutes after injection. (one-way ANOVA, $p < 0.0001$).

(E-H) Heat maps showing $\Delta F/F$ in individual mice from (A-C) injected with saline (E), DA-GIP at 0.05 mg/kg (F), 0.25 mg/kg (G), or 1 mg/kg (H) as indicated.

(I-K) Calcium signal in AgRP neurons from fasted mice presented with chow 20 minutes after pre-treatment with DA-GIP at 0.05 mg/kg (I), 0.25 mg/kg (J), or 1 mg/kg (K) compared to saline as indicated. $n = 7$ mice per group.

(L) Average $\Delta F/F$ in mice from (I-K) 4 minutes after chow presentation. (one-way ANOVA, $p = 0.0738$).

(M-P) Heat maps showing $\Delta F/F$ in individual mice from (I-K) after chow presentation.

(A-C, I-K) Isosbestic traces for all recordings are shown in gray. (A-C, E-H, I-K, M-P) Vertical dashed lines indicate the time of injection or chow presentation. (D, L) Lines represent individual mice. Error bars indicate mean \pm SEM. Post-hoc comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

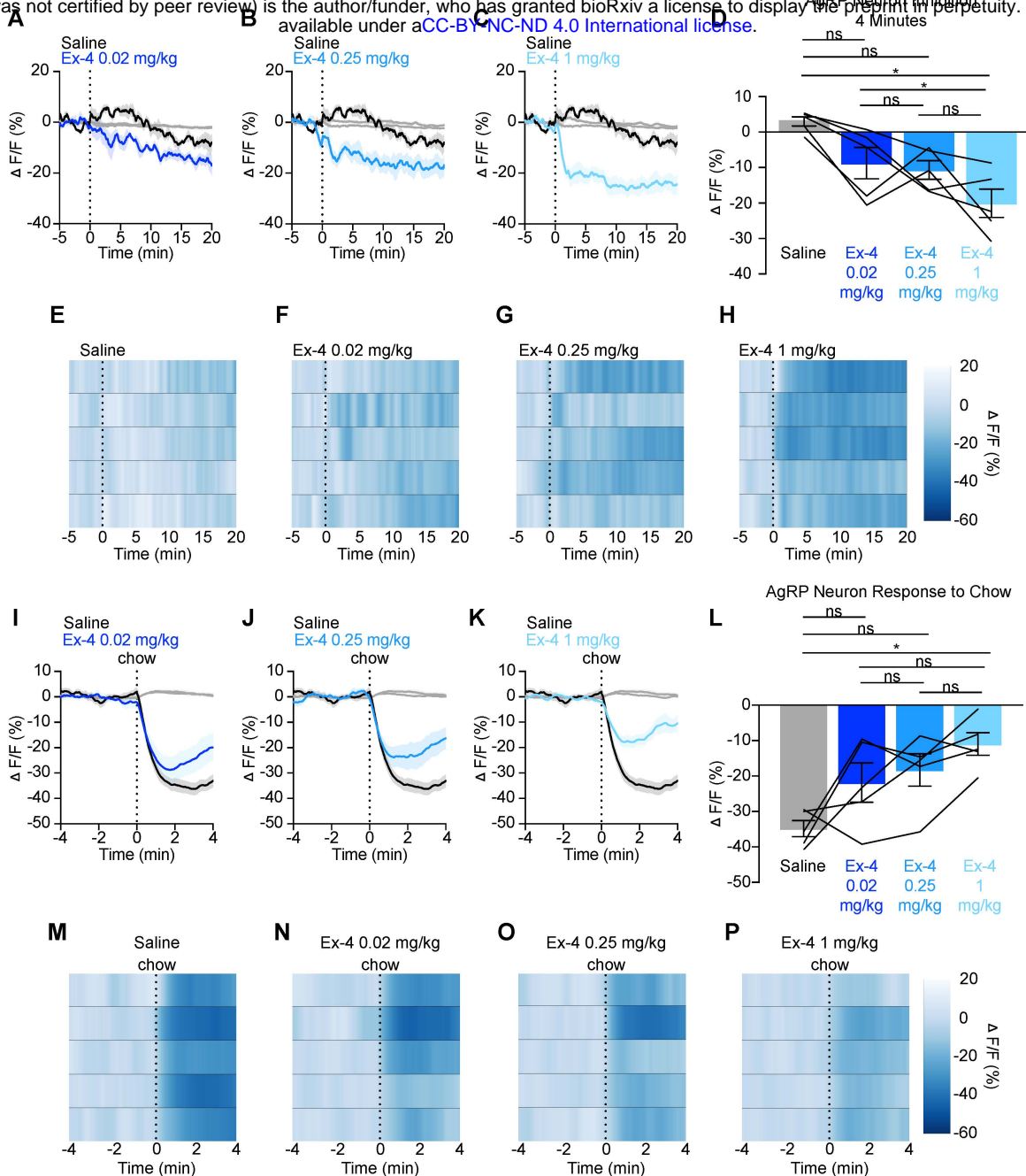


Figure S2. AgRP neuron responses to GLP-1R agonists are dose-dependent

(A-C) Calcium signal in AgRP neurons from fasted mice injected with Ex-4 at 0.02 mg/kg (A), 0.25 mg/kg (B), or 1 mg/kg (C) compared to saline as indicated. $n = 5$ mice per group.

(D) Average $\Delta F/F$ in mice from (A-C) 4 minutes after injection. (one-way ANOVA, $p=0.0119$).

(E-H) Heat maps showing $\Delta F/F$ in individual mice from (A-C) injected with saline (E), Ex-4 at 0.02 mg/kg (F), 0.25 mg/kg (G), or 1 mg/kg (H) as indicated.

(I-K) Calcium signal in AgRP neurons from fasted mice presented with chow 20 minutes after pre-treatment with Ex-4 at 0.02 mg/kg (I), 0.25 mg/kg (J), or 1 mg/kg (K) compared to saline as indicated. $n = 5$ mice per group.

(L) Average $\Delta F/F$ in mice from (I-K) 4 minutes after chow presentation. (one-way ANOVA, $p=0.0219$).

(M-P) Heat maps showing $\Delta F/F$ in individual mice from (I-K) after chow presentation.

(A-C, I-K) Isobestic traces for all recordings are shown in gray. (A-C, E-H, I-K, M-P) Vertical dashed lines indicate the time of injection or chow presentation. (D, L) Lines represent individual mice. Error bars indicate mean \pm SEM. Post-hoc comparisons: * $p < 0.05$.

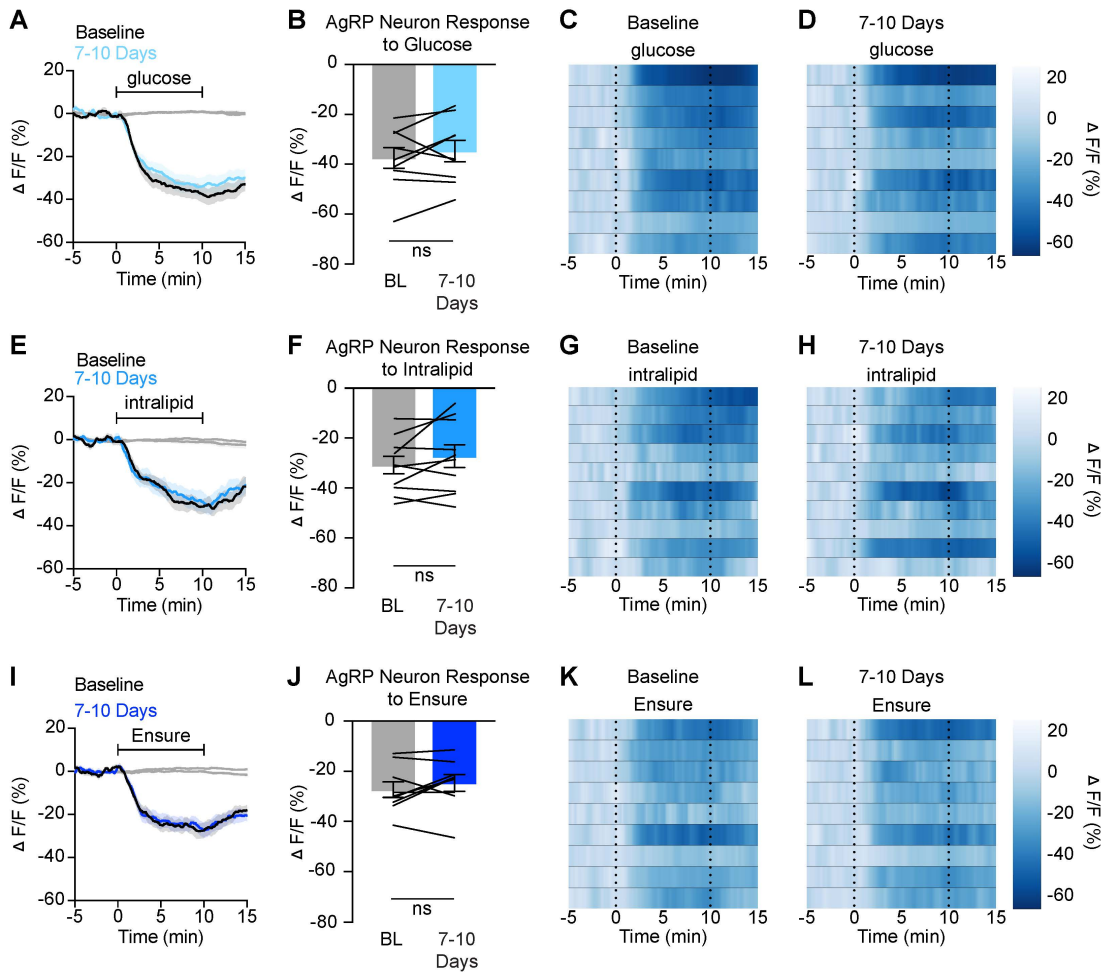


Figure S3. Nutrient-mediated AgRP neuron inhibition is stable over time in untreated mice
(A,E,I) Calcium signal in AgRP neurons from fasted mice during infusion of glucose (A), intralipid (E), and Ensure (I) at baseline and 7-10 days later as indicated. $n = 9-10$ mice per group.
(B,F,J) Average $\Delta F/F$ in mice from (A,E,I) at the end of nutrient infusion. ((B) paired t-test, $p=0.3471$; (F) paired t-test, $p=0.1781$; (J) paired t-test, $p=0.2725$).
(C,D,G,H,K,L) Heat maps showing $\Delta F/F$ in individual mice from (A,E,I) during nutrient infusion. (A,E,I) Isosbestic traces for all recordings are shown in gray. (C,D,G,H,K,L) Vertical dashed lines indicate the start and end of nutrient infusions. (B F,J) Lines represent individual mice. Error bars indicate mean \pm SEM.

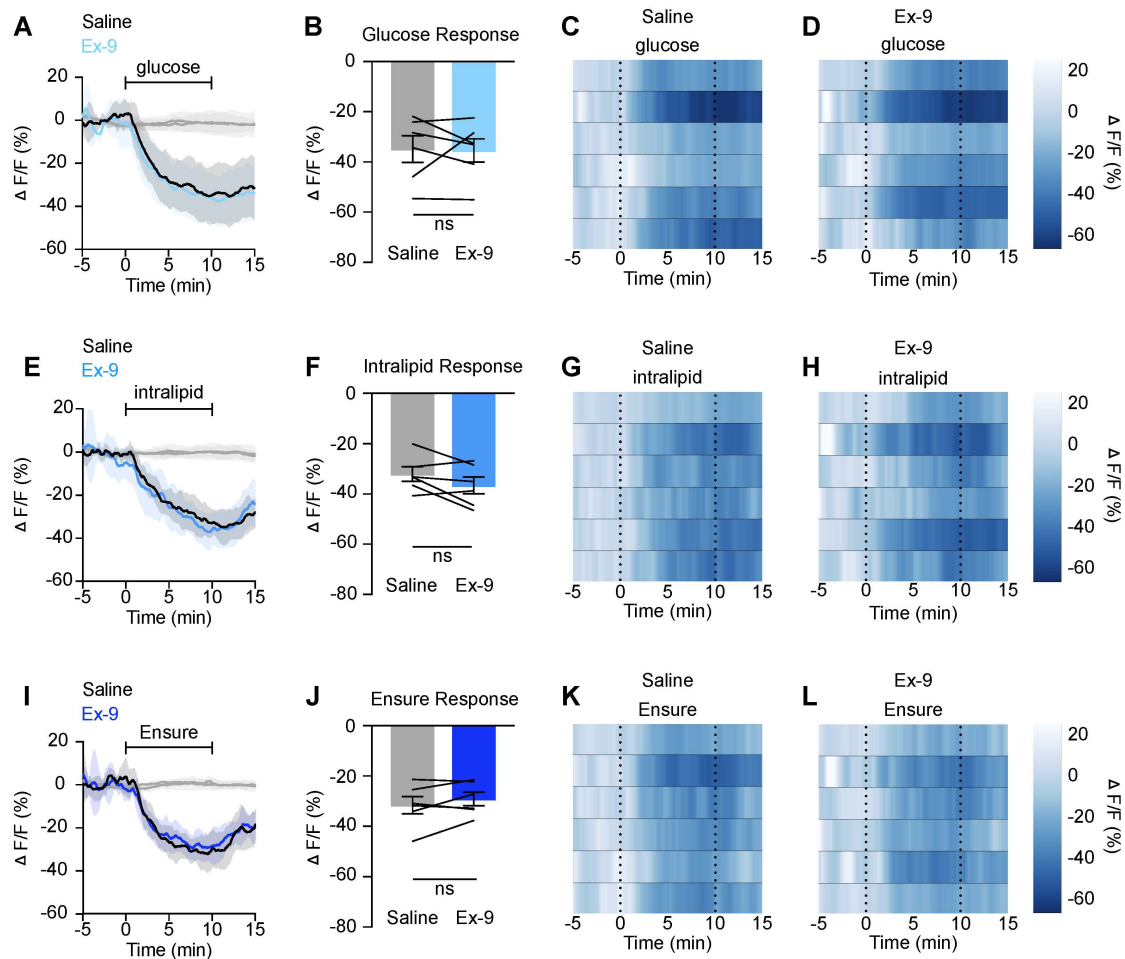


Figure S4. Signaling through GLP-1R is not necessary for nutrient-mediated AgRP neuron inhibition

(A,E,I) Calcium signal in AgRP neurons from fasted mice during infusion of glucose (A), intralipid (E), or Ensure (I) after pre-treatment with saline or Ex-9 as indicated. $n = 6$ mice per group.

(B,F,J) Average $\Delta F/F$ in mice from (A,E,I) at the end of nutrient infusion. ((B) paired t-test, $p=0.8918$; (F) paired t-test, $p=0.1314$; (J) paired t-test, $p=0.2401$).

(C,D,G,H,K,L) Heat maps showing $\Delta F/F$ in individual mice from (A,E,I) during nutrient infusion.

(A,E,I) Isobestic traces for all recordings are shown in gray. (C,D,G,H,K,L) Vertical dashed lines indicate the start and end of nutrient infusions. (B,F,J) Lines represent individual mice. Error bars indicate mean \pm SEM.

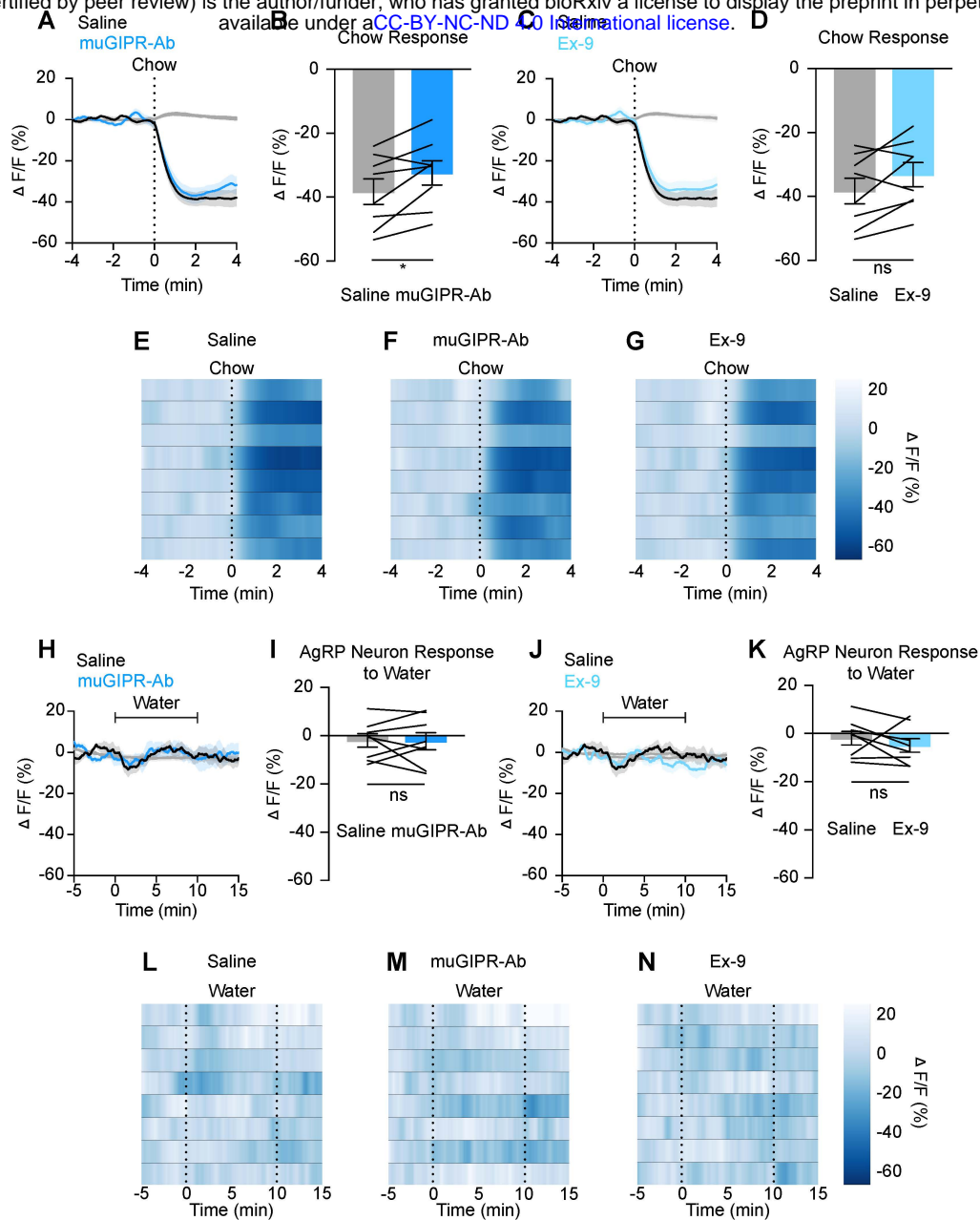


Figure S5. GIPR and GLP-1R blockade minimally impact AgRP neural response to food presentation or water infusion

(A,C) Calcium signal in AgRP neurons from fasted mice presented with chow after pre-treatment with muGIPR-Ab (A), Ex-9 (C) or saline as indicated. $n = 8$ mice per group.

(B,D) Average $\Delta F/F$ in mice from (A,C) 4 minutes after chow presentation. ((B) paired t-test, $p=0.0258$; (D) paired t-test, $p=0.0792$).

(E,F,G) Heat maps showing $\Delta F/F$ in individual mice from (A,C) after chow presentation.

(H,J) Calcium signal in AgRP neurons from fasted mice during water infusion after pre-treatment with muGIPR-Ab (H), Ex-9 (J) or saline as indicated. $n = 8$ mice per group.

(I,K) Average $\Delta F/F$ in mice from (H,J) at the end of water infusion. ((I) paired t-test, $p=0.9004$; (K) paired t-test, $p=0.3644$).

(L,M,N) Heat maps showing $\Delta F/F$ in individual mice from (H,J) during water infusion.

(A,C,H,J) Isosbestic traces for all recordings are shown in gray. (A,C,E,F,G,L,M,N) Vertical dashed lines indicate chow presentation or the start and end of water infusions. (B,D,I,K) Lines represent individual mice. Error bars indicate mean \pm SEM. T-tests: * $p < 0.05$.