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► **To cite this version:**

Daniela Herrera Moro Chao, Matthew Kirchner, Cuong Pham, Ewout Foppen, Raphael G.P. Denis, et al.. Hypothalamic astrocytes control systemic glucose metabolism and energy balance. *Cell Metabolism*, 2022, 34 (10), pp.1532-1547.e6. 10.1016/j.cmet.2022.09.002 . hal-03835771

HAL Id: hal-03835771

<https://hal.science/hal-03835771v1>

Submitted on 4 Oct 2023

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1 **Hypothalamic astrocytes control systemic glucose metabolism and energy balance**

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19 **Summary**

20 The hypothalamus is key in the control of energy balance. However, strategies targeting
21 hypothalamic neurons have failed to provide viable options to treat most metabolic diseases.
22 Conversely, the role of astrocytes in systemic metabolic control has remained largely unexplored.
23 Here we show that obesity promotes anatomically restricted remodeling of hypothalamic astrocyte
24 activity. In the paraventricular nucleus (PVN) of the hypothalamus, chemogenetic manipulation of
25 astrocytes results in bidirectional control of neighboring neuron activity, autonomic outflow,
26 glucose metabolism and energy balance. This process recruits a mechanism involving the
27 astrocytic control of ambient glutamate levels, which becomes defective in obesity. Positive or
28 negative chemogenetic manipulation of PVN astrocyte Ca^{2+} signals respectively worsens or
29 improves metabolic status of diet-induced obese mice. Collectively, these findings highlight a yet
30 unappreciated role for astrocytes in the direct control of systemic metabolism and suggest
31 potential targets for anti-obesity strategy.

32
33 **Key words:** astrocyte, PVN, obesity, glucose metabolism, glutamate.
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50 **Introduction**

51

52 Obesity is a public health concern associated with a higher incidence of type 2 diabetes,
53 cardiovascular disease and other life-threatening comorbidities (Morton et al., 2014; Saklayen,
54 2018). Understanding the mechanisms underlying the onset and progression of the disease is
55 essential to identify potential targets for therapeutic intervention. In mammals, energy
56 homeostasis results from the balance between energy expenditure and nutrient intake. Nervous
57 inputs and circulating factors are integrated by discrete neural circuits in the brain, which, in turn,
58 provide an adaptive behavioral, neuroendocrine and metabolic response (Morton et al., 2014). In
59 the hypothalamus, specialized neuronal populations maintain a strict monitoring of the peripheral
60 metabolic state and regulate food intake and body metabolism by modulating the autonomic
61 nervous system (ANS) (Dietrich and Horvath, 2013; Roh et al., 2016; Timper and Bruning, 2017;
62 Yi et al., 2010). In particular, pre-autonomic neurons located in the paraventricular nucleus (PVN),
63 have emerged as important hypothalamic autonomic/neuroendocrine regulators of the
64 physiology of energy balance. (Licht et al., 2013; Thayer et al., 2010). They modulate feeding,
65 energy expenditure, neurohormonal response and cardiovascular function, through autonomic
66 efferents projecting to the brainstem and spinal autonomic outputs (Betley et al., 2013; Dampney
67 et al., 2018; Li et al., 2019; Munzberg et al., 2016). The activity of hypothalamic neurons is tightly
68 dependent on adequate delivery of energy substrates provided by astrocytes, the major type of
69 glial cells in the brain (Garcia-Caceres et al., 2019; Verkhratsky A, 2018). In physiological and
70 pathological conditions, astrocytes exert a wide spectrum of adaptive functions that can promote
71 and preserve neuronal health, such as synaptogenesis, synaptic efficacy and plasticity within
72 neuronal networks (Freire-Regatillo et al., 2017; Verkhratsky A, 2018). By occupying a strategic
73 position in brain circuits, at the interface between blood vessels and neurons, they participate in
74 the transport and sensing of nutrients and metabolic factors across the blood brain barrier (Allen
75 and Eroglu, 2017).

76 Hypothalamic astrocytes are being recognized as important players in the control of systemic
77 energy metabolism (Caruso et al., 2013; Garcia-Caceres et al., 2016; Garcia-Caceres et al., 2013;
78 Kim et al., 2014) and obesity leads to central low-grade inflammation triggers inflammatory-like
79 response in hypothalamic astrocytes and reactive gliosis in several hypothalamic areas (Horvath
80 et al., 2010), including the PVN (Buckman et al., 2013; Dalvi et al., 2017; De Souza et al., 2005;
81 Milanski et al., 2009; Robb et al., 2020; Thaler et al., 2012). However, the direct contribution of
82 astrocytes as gate keeper of PVN output, as well as their involvement in the control of bodily
83 energy and glucose metabolism remain an open question.

84 In the current study, we took advantage of gain & loss of function approaches allowing for the
85 direct and selective imaging and manipulation of astrocytes to explore the causal role of
86 astrocytes in metabolic response. We first show that while obesity indeed promotes astrocyte
87 calcium (Ca^{2+}) activity remodeling that are anatomically defined to hypothalamic sub-nuclei.
88 Astrocytic response could be mimicked by chemogenetic activation of astrocyte by Gq-coupled
89 Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). *In vivo* chemogenetic
90 activation or inhibition of PVN astrocyte Ca^{2+} signaling exerted bidirectional control over
91 neighboring PVN neuron firing, autonomic outflow, glucose metabolism and energy balance.
92 Finally, we found that the underlying mechanism by which astrocytes gate neural activity in the
93 PVN relies on excitatory Amino-Acid Transporters (EAATs)-dependent control of ambient
94 glutamate. In obese mice, this mechanism was selectively impaired and the chemogenetic
95 increase (Gq) or decrease (Gi) of astrocytic Ca^{2+} led to metabolic aggravation or amelioration,
96 respectively.

97 In summary, we show that PVN astrocytes exert direct control over systemic glucose metabolism
98 and energy balance and support a concept in which obesity-associated diseases might be
99 partially mediated through molecular and signaling modifications in hypothalamic astrocyte-
100 neuron communication.

101 **Results**

102 **Diet-induced obesity induces astrocytic Ca²⁺ signal remodeling in the hypothalamus.**

103 We first assessed how diet-induced obesity (DIO) alters astrocytic Ca²⁺ signaling as a proxy for
104 astrocyte activity (**Figure 1A**). The genetically encoded Ca²⁺ sensor GCaMP6f was expressed in
105 astrocytes by crossing the tamoxifen-inducible *Glast-CreER^{T2}* line and the Cre-dependent
106 GCaMP6f line ([Pham et al., 2020](#)). Astrocyte-specific expression of GCaMP6f after tamoxifen
107 treatment was evidenced by co-localization of the astrocyte marker *s100β* and GCaMP6f in
108 stellated cells characteristic of astrocytes (**Figure 1B**). GCaMP6f-expressing mice were fed either
109 regular diet or high fat high sugar (HFHS, 45% fat and 35% sugar) diet for 2-3-months. Exposure
110 to HFHS diet led to metabolic alteration characterized by increased body weight (**Figure 1C**),
111 decreased glucose tolerance and increased glucose-induced plasma insulin (**Figure S1A, S1B**).
112 Using wide-field fluorescence imaging that enabled the integration of global Ca²⁺ signals, we
113 imaged intrinsic Ca²⁺ activity in hypothalamic astrocytes in acute brain slices. Active regions were
114 identified using the spatio-temporal correlation screening method ([Pham et al., 2020](#)) (**Figure**
115 **1SC, 1SD**). We observed that basal astrocyte Ca²⁺ signals in obese mice showed an overall
116 increase in the PVN (**Figure 1D, Figure S1C, S1D**), the arcuate nucleus (ARC) (**Figure 1E**) and
117 the dorsomedial nucleus of the hypothalamus (DMH) (**Figure S1H**). Interestingly, in the
118 ventromedial nucleus of the hypothalamus (VMH), astrocytes remained relatively protected from
119 diet induced hyperactivity (**Figure 1F**). In the PVN, we quantitatively analyzed the Ca²⁺ imaging
120 data in PVN, the region of focus in the current study and quantified the kinetic parameters between
121 lean and obese animals. The result shows that the duration of Ca²⁺ signaling was reduced in
122 obese animals (**Figure 1SE**) while the frequency and amplitude were significantly enhanced in
123 obese animals (**Figure S1G**). Taken together these results indicate that DIO causes
124 heterogeneous and anatomically restricted remodeling of astrocytic Ca²⁺ activity in metabolically

125 relevant hypothalamic structures. These results are in perfect line with previous observation
126 depicting increased hypothalamic astrocyte reactivity in obese mice (Horvath et al., 2010).

127

128 **PVN astrocytes control systemic glucose metabolism**

129 Given the prominent role of PVN neural substrate in the control of metabolism (Li et al., 2019;
130 Sutton et al., 2016), we explored how direct manipulation of PVN astrocyte activity affects energy
131 homeostasis through cell-specific DREADDs engineering. Mice expressing the Cre enzyme under
132 the astrocyte-specific promoter Aldehyde dehydrogenase family 1, member L1 (Cahoy et al.,
133 2008) (Aldh1L1-Cre mice), received an intra-PVN injection of a mixture of Cre-dependent viruses
134 allowing for the expression of (1) mCherry alone (AAV 2/5.EF1 α .DIO.mCherry, control) or Gq-
135 coupled hM3Dq DREADD fused with mCherry (AAV 2/5.EF1 α .DIO.hM3Dq.mCherry, Gq-
136 DREADD) together with (2) Cre dependent expression of the Ca²⁺ sensor
137 (pAAV5.CAG.Flex.GCaMP6f.WPRE) (Figure 1G). Astrocyte-specific expression of hM3Dq was
138 confirmed by the colocalization of mCherry fluorescent signal with Glial fibrillary acidic protein
139 (GFAP) reactivity (Figure 1H). This combined viral approach allowed us to monitor how selective
140 activation of astrocytic Gq coupled hM3Dq by clozapine-N-oxide (CNO) would affect PVN
141 astrocytic intrinsic Ca²⁺ activity in acute brain slices. Compared to the minor effect in mCherry
142 expressing control mice (Figure S1I, S1J), CNO bath application significantly increased astrocytic
143 Ca²⁺ strength in the PVN as a result of hM3Dq activation (Figure 1I, 1J). Using temporal
144 correlation between Ca²⁺ signals as an evaluation of their synchronization, we observed that in
145 contrast to control mice (Figure S1K), CNO activation of astrocytic hM3Dq drastically increased
146 Ca²⁺ signal synchronization as compared to spontaneous signals (Figure S1L). This result
147 demonstrates that Gq signaling modulation enables selective activation and synchronization of
148 Ca²⁺ signals in PVN astrocytes.

149 We next explored how direct manipulation of PVN astrocytes could affect systemic metabolism.
150 Aldh1L1-Cre^{+/-} mice received bilateral PVN injections of viruses expressing either mCherry

151 (AAV5-EF1a-DIO-mCherry; Aldh1L1-Cre^{+/-}:PVN^{mCherry}) or hM3Dq (AAV5-EF1a-DIO-hM3Dq-
152 mCherry; Aldh1L1-Cre^{+/-}:PVN^{hM3Dq}) (**Figure 2A**). To evaluate potential unspecific metabolic
153 consequences of peripheral CNO injections ([Gomez et al., 2017](#)), we also injected AAV5-EF1a-
154 DIO-hM3Dq-mCherry in Aldh1L1-Cre^{-/-} mice (Aldh1L1-Cre^{-/-}:PVN^{hM3Dq}). CNO-mediated activation
155 of PVN astrocytes had no consequence on overall whole body energy expenditure (EE), food
156 intake or energy balance in control mice (Aldh1L1-Cre^{+/-}:PVN^{mCherry} and Aldh1L1-Cre^{-/-}:PVN^{hM3Dq}),
157 nor in hM3Dq expressing mice (Aldh1L1-Cre^{+/-}:PVN^{hM3Dq}; **Figure S2A-I**). However, chemogenetic
158 stimulation of PVN astrocytes by peripheral CNO administration decreased glucose clearance
159 (**Figure 2B**), increased plasma insulin levels (**Figure 2C**) and corresponding insulinogenic index
160 (**Figure 2D**), revealing increased insulin release after an oral glucose tolerance test in Aldh1L1-
161 Cre^{+/-}:PVN^{hM3Dq}. No significant effect was found in control animals (**Figure S2J-Q**).

162 Given this striking result, we sought to reproduce this phenomenon in an alternative genetic
163 background. To this aim, C57BL/6 mice received bilateral intra-PVN injection of AAV5-GFAP-
164 hM3Dq-mCherry (C57Bl6j:PVN^{hM3Dq}) or control vector AAV5-GFAP-mCherry
165 (C57Bl6j:PVN^{mCherry}). While ineffective in control C57Bl6j:PVN^{mCherry} (**Figure S3A-D**), CNO-
166 mediated activation of PVN astrocytes in C57Bl6j:PVN^{hM3Dq} resulted in higher glucose-induced
167 insulin release (**Figure S3F**), leading to a trend of altered insulin sensitivity as assessed by
168 insulinogenic index (**Figure S3G**). These results suggest that chemogenetic manipulation of Gq
169 GPCR pathway in PVN astrocytes readily impairs peripheral glucose metabolism and insulin
170 release, both being common outcomes preceding insulin resistance states.

171 We next probed the reciprocal phenomenon by specifically triggering Gi GPCR pathway in PVN
172 astrocytes. Aldh1L1-Cre^{+/-} mice received bilateral injection of AAV5 virus with a modified
173 backbone (pAAV5-EF1a-DIO-HA-KORD-Citrine) to achieve astrocyte, Cre-dependent expression
174 of kappa-opioid receptor (KORD), a recently developed chemogenetic GPCR that can be activated
175 by the pharmacologically inert ligand salvinorin B (SalB) ([Vardy et al., 2015](#)) (**Figure 2A**). After

176 PVN injection of a mixture of virus allowing the co-expression of KORD and GCaMP6f, we could
177 assess astrocyte Ca²⁺ signals before and after KORD activation in acute hypothalamic slices.
178 Bath application of SalB led to a decrease in Ca²⁺ signal strength (**Figure 2E, 2F**) and global
179 synchronization (**Figure 2G**) in KORD expressing mice, while these effects appeared marginal in
180 control mCherry expressing mice (**Figure S4A-C**). Accordingly, the decrease in intracellular Ca²⁺
181 release was more pronounced after KORD activation compared to mCherry expressing mice
182 (**Figure S4D**). Astrocyte-specific KORD expression was corroborated by the colocalization of
183 mCitrine and GFAP reactivity in PVN astrocytes (**Figure 2H**).

184 We further assessed *in vivo* the metabolic output arising from decreasing Ca²⁺ activity *in vivo* in
185 PVN astrocytes. Aldh1L1-Cre^{+/-} or control Aldh1L1-Cre^{-/-} (mice received bilateral injections of
186 AAV5-EF1a-DIO-HA.KORD-mCitrine in the PVN. In both control (Aldh1L1-Cre^{-/-}:PVN^{KORD})
187 (**Figure S4E-L**) and Aldh1L1-Cre^{+/-}:PVN^{KORD} mice (**Figure 2I-K**), peripheral SalB administration
188 did not affect glucose clearance, plasma insulin or insulinogenic index after an oral glucose load.

189 This result was similar when GFAP-driven expression of hM4Di was achieved in C57Bl6J mice
190 (C57Bl6j:PVN^{hM4Di}) (**Figure S5A-D**). However, given the potency difference between hM4Di and
191 KORD-mediated Gi intracellular signaling ([Vardy et al., 2015](#)) and the potential discrepancy
192 between Aldh1L1-CRE and C57Bl6J genetic background, we further explored the metabolic
193 consequence of KORD-mediated Gi signaling in GFAP positive astrocytes. C57Bl6j mice received
194 intra-PVN injection of a mixture of AAV5-GFAP-Cre virus and AAV5-EF1a-DIO-HA.KORD-
195 mCitrine allowing for KORD expression in GFAP-expressing cells upon Cre-mediated
196 recombination (**Figure S3I**). KORD expression was visualized by human influenza hemagglutinin
197 (HA) tag presence in GFAP positive astrocytes (**Figure S3J**). Here again, Gi signaling initiation
198 through SalB-mediated KORD activation in C57Bl6j:PVN^{KORD} mice did not affect any of the
199 aforementioned parameters (**Figure S3K-N**), in line with our observations in Aldh1L1-Cre^{+/-} mice.

200 Similarly, metabolic efficiency analysis revealed that while peripheral injection of SalB had no
201 effect the control groups (Aldh1L1-Cre^{-/-}:PVN^{KORD}; Aldh1L1-Cre^{+/-}:PVN^{mcherry} **Figure S4M-T**),
202 KORD/Gi-mediated modulation of PVN astrocytes did not modify EE (**Figure 2L**) but induced a
203 decrease in food intake (**Figure 2M**) and RER (**Figure 2N**) while increasing FA oxidation (**Figure**
204 **2O**) during the light period. A similar output was observed in C57Bl6j:PVN^{KORD} mice (**Figure 2P-**
205 **S**). Accordingly, SalB administration to Aldh1L1-Cre^{+/-}:PVN^{KORD} affected energy balance during
206 the light period (**Figure 2T**), with a trends for a decrease in C57Bl6j:PVN^{KORD} mice (**Figure 2U**).
207 No effect of SalB administration was observed in control mice Aldh1L1-Cre^{-/-}:PVN^{KORD} and
208 Aldh1L1-Cre^{+/-}:PVN^{mcherry} (**Figure 2V, 2W**). These results suggest that whereas Gq-coupled
209 hM3Dq DREADD modify glucose metabolism and insulin release, activation of astrocytic Gi-
210 KORD receptor in both Aldh1L1 and GFAP expressing astrocytes in the PVN of lean mice
211 promotes only subtle changes in energy utilization.

212

213 **PVN astrocytes modulate sympathetic output and thermogenesis**

214 Given the prominent role played by pre-autonomic PVN neurons in autonomic control of energy
215 balance and glucose metabolism ([Geerling et al., 2014](#); [Stanley et al., 2019](#); [Stern et al., 2016](#)),
216 we hypothesized that chemogenetic activation of PVN astrocytes could exert control over PVN-
217 mediated sympathetic output. As a proxy of autonomic outflow, we first measured change in urine
218 mono-aminergic content as a consequence of chemogenetic activation (Gq) or inhibition (Gi) of
219 PVN astrocytes. ANOVA main effect probability indicated that while no change was observed
220 following CNO or SalB administration in control Aldh1L1-Cre^{+/-}:PVN^{mCherry} mice (**Figure 3A, 3C**),
221 activation of Gq signaling in PVN astrocytes in Aldh1L1-Cre^{+/-}:PVN^{hM3Dq} mice significantly
222 increased urine monoamine concentrations (**Figure 3B**). In contrast, KORD mediated Gi
223 activation showed the opposite effect, with a decrease in urine norepinephrine (NA), as well as a
224 tendency to decrease dopamine (DA) and Serotonin (5HT) in Aldh1L1-Cre^{+/-}:PVN^{KORD} mice

225 (Figure 3D). We further explored if astrocytes in the PVN participate in the regulation of brown
226 adipose tissue (BAT) activity. We monitored *in vivo* BAT metabolic activity by ultrasound and
227 spectral photoacoustic imaging (Clemmensen et al., 2018; Karlas et al., 2019). We observed that
228 PVN astrocyte activation increased local oxygen saturation in BAT blood supply in Aldh1L1-Cre^{+/-}
229 PVN^{hM3Dq} mice (Figure 3E, 3F). We next assessed the functional connection between PVN
230 astrocyte activation and thermogenic output. Mice were administered the agonist of cold receptor
231 transient receptor potential cation channel subfamily M member 8 (TRPM8) icilin in order to
232 pharmacologically trigger an adaptive autonomic response similar to the one triggered by cold
233 exposure (Clemmensen et al., 2018). We used BAT monoamine content as a readout of
234 sympathetic outflow (Virtue and Vidal-Puig, 2013). While CNO intraperitoneal (ip) injection in
235 control mice had no significant effect on BAT monoamine content (Figure 3G), astrocyte Gq
236 activation in the PVN promoted an increase in adrenaline (AD) and 5HT BAT content in Aldh1L1-
237 Cre^{+/-}:PVN^{hM3Dq} mice (Figure 3H). Using telemetric core body temperature measurements, we
238 next demonstrated that PVN astrocyte activation through chemogenetic Gq activation was
239 coupled to increased whole body temperature in Aldh1L1-Cre^{+/-}:PVN^{hM3Dq} mice (Figure 3I).
240 Finally, we confirmed that Gq signaling activation of PVN GFAP-positive astrocytes in C57Bl6
241 mice (C57Bl6j:PVN^{hM3Dq}; Figure 3J) recapitulated the increase in monoamine content and in BAT
242 (Figure 3K), which were not observed in control C57Bl6j:PVN^{mCherry} mice (Figure 3L). Altogether,
243 these results suggest that PVN astrocytes exert efficient control over ANS output, thermogenesis
244 and possibly peripheral glucose metabolism.

245

246 **PVN astrocytes exert bidirectional control over systemic glucose metabolism and energy** 247 **balance in obese mice**

248 Control C57Bl6j:PVN^{mCherry} mice and C57Bl6j:PVN^{hM3Dq} mice were submitted to 8-weeks of HFHS
249 and assessed for glucose control and metabolic efficiency change induced by PVN astrocyte Gq

250 activation. CNO injection had no effects in obese control mice (**Figure S6A-H**), however, in obese
251 C57Bl6j:PVN^{hM3Dq} mice, it led to an acute and drastic deterioration of glucose tolerance (**Figure**
252 **4A, 4B**) associated with large increases in glucose-induced insulin release (**Figure 4C**),
253 insulinogenic index (**Figure 4D**) and c-peptide release (**Figure 4E**), indicative of acute state of
254 insulin-resistance. PVN astrocyte control of glucose metabolism seemed to operate
255 independently from changes in metabolic efficiency, since neither food intake (**Figure 4F**), RER
256 (**FigureS6I**) nor FA oxidation (**Figure S6J**) were affected by CNO ip injection of obese
257 C57Bl6j:PVN^{hM3Dq} mice. Whole body energy expenditure was reduced during the dark period
258 (**Figure 4G**) and accordingly, showed higher positive energy balance (**Figure 4H**). These results
259 suggest that activation of PVN astrocytes promotes disturbances in systemic glucose metabolism
260 and thereby potentially exacerbate energy storage and metabolic syndrome during obesity.

261 We next probed whether Gi-coupled receptor activation in PVN astrocytes has the opposite effect
262 on metabolism. Gi activation had no impact on glucose tolerance in obese C57Bl6j:PVN^{hM4Di} mice
263 (**Figure S5E**), while KORD activation promoted a beneficial effect in obese C57Bl6j:PVN^{KORD} mice
264 (**Figure 4I and 4J**). In both obese C57Bl6j:PVN^{hM4Di} and C57Bl6j:PVN^{KORD} mice, PVN astrocyte
265 Gi-coupled activation of hM4Di or KORD reduced the level of glucose-induced insulin release
266 during the OGTT (**Figure S5F and Figure S4K**), or insulinogenic index (**Figure S5G and Figure**
267 **S4L**). Yet consistently, plasma c-peptide release was reduced following activation of both
268 receptors (**Figure S5H and Figure S4M**). Given the prominent role of the PVN in the control of
269 hypothalamic-pituitary-adrenal axis we probed whether chemogenetic manipulation of PVN
270 astrocytes would modulate corticosterone level (**Figure S5I-L**). In lean mice chemogenetic
271 activation of Gq or Gi signaling in PVN astrocyte had marginal effect on circulating corticosterone
272 (**Figure S5I-J**). However, CNO-mediated Gq but not Gi activation in PVN astrocytes led to a
273 significant decrease in corticosterone level in obese mice (**Figure S5K-L**). Moreover, SalB
274 injection in C57Bl6j:PVN^{KORD} promoted a decrease in food intake (**Figure 4N**) and RER (**Figure**

275 **S6K**), and an increase in FA oxidation (**Figure S6L**), with no changes in energy expenditure
276 (**Figure 4O**). Finally, contrary to astrocyte Gq receptor activation, KORD-mediated Gi stimulation
277 in PVN astrocytes, promoted negative energy balance during the dark period in obese
278 C57Bl6j:PVN^{KORD} mice (**Figure 4P**). These results indicate that PVN astrocytes exert bidirectional
279 control over systemic glucose metabolism and energy balance.

280

281 **Chemogenetic modulation of astrocyte activity controls PVN parvocellular neuronal firing**

282 We then set out to probe the cellular and molecular mechanism by which PVN astrocytes exert
283 dynamic control of whole-body metabolic outputs. Parvocellular pre-autonomic neurons in the
284 PVN send efferent projections to autonomic centers in the brainstem and spinal cord to generate
285 essential adaptive metabolic responses for proper systemic glucose metabolism and energy
286 balance regulation ([Geerling et al., 2014](#); [Stanley et al., 2010](#); [Stern et al., 2016](#)). We investigated
287 astrocyte-neuron communication by patching parvocellular neurons and recording their activity
288 (**Figure 5A**) before and after chemogenetic manipulation of astrocyte Gq or Gi receptor in lean or
289 obese mice. The recorded neurons displayed membrane properties characteristic of parvocellular
290 pre-sympathetic neurons, namely the presence of a low-threshold spike and absence of a
291 transient outward rectification ([Luther and Tasker, 2000](#); [Stern, 2001](#)). We combined this
292 approach with simultaneous confocal imaging of astrocyte Ca²⁺ signals after expression of
293 GCaMP6f in either GFAP or Aldh1L1 expressing astrocytes. Activation of Gq-coupled hM3Dq
294 expressing PVN astrocytes by CNO significantly increased neuronal firing frequency from lean
295 C57Bl6j:PVN^{hM3Dq} mice (**Figure 5B, 5C**). Conversely, a blunted effect was observed in obese
296 mice (**Figure 5B, 5C**). CNO failed to affect firing frequency in the control group of
297 C57Bl6j:PVN^{mcherry} mice (**Figure 5D**). We also quantified astrocytic Ca²⁺ activity in an event-based
298 manner using AQuA method ([Wang et al., 2019](#)) for both lean and obese C57Bl6j:PVN^{hM3Dq} mice.
299 CNO failed to significantly increase the mean Ca²⁺ event peak in lean or obese C57Bl6j:PVN^{hM3Dq}

300 mice. However, the basal Ca^{2+} peaks in obese mice were significantly higher than lean mice
301 (**Figure 5E, 5F**). On the other hand, CNO significantly prolonged Ca^{2+} event duration, and
302 increased Ca^{2+} event synchrony both in lean and obese C57Bl6j:PVN^{hM3Dq} mice (**Figure 5E, F**).
303 These results are in line with data obtained from wide-field fluorescence imaging (**Figure 1**). We
304 then evaluated the latency between astrocytic Ca^{2+} upregulation and the increase in neuronal
305 spike frequency. We found that CNO-induced Ca^{2+} response appeared first in astrocytes followed
306 by neurons, suggesting that changes in astrocyte Ca^{2+} activity significantly preceded those in
307 neuronal firing discharge. These results confirm a sequence of events in which astrocyte Gq
308 activation promotes changes in neural activity (**Figure 5G**). The latency between activation of
309 astrocytes and upregulation of neuronal spike activity did not differ significantly between lean and
310 obese mice (**Figure 5G, right**).

311 Astrocytes maintain a constant bidirectional communication with neurons, and glutamate is
312 recognized as a key signaling molecule underlying neuro-glial interactions ([Durkee and Araque, 2019](#)).
313 Therefore, we investigated if glutamatergic communication is involved in the excitation of
314 parvocellular neurons following astrocyte chemogenetic activation. Blockade of ionotropic
315 glutamate receptors (KYN, 1 mM) prevented the CNO-induced increase in neuronal activity both
316 in lean and obese C57Bl6j:PVN^{hM3Dq} mice (**Figure 5H, I**). Interestingly, while CNO effects on
317 astrocyte Ca^{2+} event synchrony persisted in the presence of KYN (**Figure 5J, right**), CNO effects
318 on Ca^{2+} event duration were partially blunted (**Figure 5J, left**).

319 We next assessed the effect of astrocyte KORD-Gi-mediated activation on parvocellular neuronal
320 firing. SalB application significantly lowered neuronal activity in acute brain PVN slices of lean
321 Aldh1L1-Cre^{+/-}:PVN^{KORD} mice (**Figure 6A, 6B**) but had no effect in control Aldh1L1-Cre^{+/-}
322 :PVN^{Imcherry} mice (**Figure 6C**). Importantly, KORD-Gi activation also decreased astrocyte
323 intracellular Ca^{2+} activity, which manifested as a reduction in event amplitude, event synchrony,
324 and event frequency (**Figure. 6D, 6E**), while event duration remained unchanged (data not

325 shown). As we observed with chemogenetic activation of Gq in astrocytes, KORD-Gi mediated
326 inhibition of astrocyte Ca^{2+} activity preceded the evoked inhibition of neuronal spike activity
327 (**Figure 6F**). Similar to CNO, we found that KYN prevented the SalB-evoked decrease in neuronal
328 activity in *Aldh1L1-Cre^{+/+}:PVN^{KORD}* mice (**Figure S7A, S7B**), while the decrease in astrocyte Ca^{2+}
329 activities persisted (**Figure S7C**). Taken together, our results indicate that PVN astrocytes exert
330 bidirectional control over parvocellular firing activity, and that both effects involve a glutamate-
331 dependent mechanism.

332

333 **Astrocytic control of extracellular glutamate is impaired in obesity and instrumental to the**
334 **control of PVN neuron firing.**

335 Astrocyte-mediated glutamate signaling to neurons could involve glutamate release and/or
336 modulation of ambient glutamate levels via changes in the activity of astrocyte-selective excitatory
337 Amino-Acid Transporters (EAATs) ([Rose et al., 2017](#)). Thus, to determine whether the glutamate-
338 dependent communication between astrocytes and neurons reported above involve modulation
339 of EAAT activity, we repeated experiments in the presence of the glutamate transport blocker DL-
340 *threo*- β -Benzyloxyaspartic acid (TBOA). In this condition, activation of astrocytes by CNO failed
341 to upregulate neuronal activity both in lean and obese *C57Bl6j:PVN^{hM3Dq}* mice (**Figure 7A, 7C**),
342 while CNO effects on astrocyte Ca^{2+} event synchrony persisted (**Figure 7D**). Similarly, TBOA also
343 prevented the KORD-mediated inhibition of neuronal firing (**Figure S7D, S7E**), without impacting
344 KORD-induced astrocyte Ca^{2+} inhibition (**Figure S7F**). Together, these results suggest that the
345 glutamate-dependent increase and decrease of PVN neuronal activity following astrocyte Gq and
346 Gi receptor activation, respectively, is mediated via a bidirectional control of glutamate transporter
347 activity.

348 Finally, we explore if the function and/or expression of astrocyte EAATs is altered in obese mice.
349 Western blot analysis of PVN samples showed no significant difference in EAAT2 protein
350 expression levels between lean and obese mice (**Figure 7E**). We then tested and compared the

351 effects of TBOA *per se* on membrane excitability and firing activity of PVN parvocellular neurons.
352 In voltage clamp mode, we found that bath-applied TBOA produced a significant inward shift in
353 I_{Holding} in lean mice (**Figure 7F, 7G**), which we previously showed to be due to the slow build-up
354 of glutamate in the extracellular space leading to activation of neuronal extrasynaptic NMDARs
355 (**Fleming et al., 2011; Stern et al., 2016; Zhang and Stern, 2017**). Surprisingly, TBOA application
356 failed to significantly affect I_{Holding} in PVN neurons in obese mice (**Figure 7F, 7G**), resulting in a
357 significantly smaller inward shift in I_{Holding} in obese compared to lean mice (**Figure. 7G, right**).
358 Similarly, we found that while TBOA produced a significant membrane depolarization (**Figure 7H,**
359 **7I**) and increase in spike frequency (**Figure 7J, left**) in neurons in lean mice, these effects were
360 absent in obese mice (**Figure 7H-J**). Taken together, our results show a blunted effect of TBOA
361 on membrane excitability and firing activity in obese mice, supporting the notion that in this
362 condition, astrocyte glutamate uptake activity is severely blunted.

363

364 **Discussion**

365 In the present study, we show that PVN astrocytes exert a direct and reversible control on neuron
366 firing, energy balance, systemic glucose metabolism and insulin sensitivity and that specific
367 targeting of astrocyte activity in the PVN exerts beneficial effects on metabolic disturbances during
368 obesity.

369 Astrocytes, the most prevalent glial cells in the CNS, express a wide arrange of neuropeptide,
370 hormonal and nutrient receptors and exhibit a specific type of excitability mediated by ionic
371 signaling and fluctuations of intracellular Ca^{2+} concentration (**Nedergaard et al., 2010**). Using Ca^{2+}
372 signals as a proxy of cellular activity, we show that obesity is associated with hyperactivity in
373 hypothalamic astrocytes, recalling the previous association described between high-fat feeding
374 and astrocyte reactivity (**Horvath et al., 2010**). In that regard, hypothalamic inflammation and
375 gliosis have been suggested to underlie hypothalamic circuit dysfunction which can lead to

376 hyperphagia, insulin and leptin resistance and consequent obesity (De Souza et al., 2005;
377 Douglass et al., 2017; Reis et al., 2015; Thaler et al., 2012). In addition, abnormal Ca²⁺ glial
378 signaling has been associated with the pathophysiological function of astrocytes and regulation
379 of reactive astrogliosis during brain injury and neuropathology (Denisov et al., 2021; Kanemaru
380 et al., 2013; Nedergaard et al., 2010). We show here that obesity affected hypothalamic astrocyte
381 activity in a heterogenous manner, characterized by increased activity in the ARC, DMH and PVN
382 but sparing the VMH. This supports the idea that astrocytes are not a functionally homogeneous
383 cellular population throughout the brain, but rather exhibit inter and intra-regional differences in
384 molecular and cellular profiles (Ben Haim and Rowitch, 2017; Hill et al., 1996; Khakh and
385 Sofroniew, 2015).

386 **In this study, we focused our interest on the PVN, which contains key neuroendocrine neurons**
387 **controlling systemic metabolism.** Strikingly, manipulating PVN astrocytic intracellular signaling
388 cascade and Ca²⁺ waves through chemogenetic Gq/Gi engineering respectively aggravated (Gq)
389 or improved (KORD-Gi) the metabolic condition in obese mice. In the same line we found that
390 activation of astrocytic Gq , but not Gi signaling led to change in stress-induced corticosterone
391 level in obese mice (**Supplemental Figure 5K-L**) indicating that both intracellular cascade
392 (Gq/Gi) and nutritional condition (lean/obese) affect astrocyte-neuron coupling in PVN neural
393 network.

394 Correspondingly, previous evidence supports beneficial consequences of astrocyte Gi receptor
395 activation, as it corrects astrocyte atrophy and behavioral outputs observed during
396 neurodegenerative diseases (Douglass et al., 2017; Yu et al., 2020). **We here reveal** that PVN
397 astrocytes exert a direct and reversible control of energy balance, systemic glucose metabolism
398 and insulin sensitivity. Astrocyte-mediated control of PVN neuron activity might even be an
399 essential component of a recently-described functional PVN-pancreas transneuronal circuits

400 directly connecting the PVN to autonomic control beta cell function and glucose metabolism
401 ([Papazoglou et al., 2022](#)).

402 Our data **show** that chemogenetic control and nutritional modulation of astrocyte networks within
403 the PVN control neighboring parvocellular neuron firing activity. In all cases, changes in astrocytic
404 Ca²⁺ events preceded the one observed in neuronal firing, further establishing that changes in
405 PVN neuron firing were a consequence of DREADD-mediated activation of astrocytic Ca²⁺ events.
406 At the mechanistic level, astrocyte-evoked effects on PVN firing activity were prevented by
407 glutamate receptor blockade, supporting the notion that glutamate signaling is a core component
408 of astrocytic control of PVN neuronal activity. Glutamate-mediated astrocyte-neuron crosstalk
409 could involve active vesicular and non-vesicular glutamate release ([Araque et al., 1998](#); [Bezzi et al., 1998](#);
410 [Fellin et al., 2004](#); [Innocenti et al., 2000](#)) as well as the control of ambient glutamate
411 levels of via the activity of potent glutamate transporters, particularly GLT1.

412 In our hands, selective blockade of glutamate transporters-prevented the change in neuron firing
413 initiated by either Gq or Gi chemogenetic manipulation of PVN astrocytes.

414 Moreover, the delayed firing response of PVN neurons following astrocytic manipulations is
415 consistent with a slow buildup of glutamate in the extracellular space. These results align with
416 previous studies pointing at astrocyte GLT1 as a key regulator of astrocyte-neuronal
417 communication in the PVN and supraoptic nucleus (SON) ([Fleming et al., 2011](#); [Gordon et al., 2009](#);
418 [Zhang et al., 2017](#)).

419 Similarly, angiotensin II activation of Gq AT1 receptors on astrocytes inhibited GLT1 transporter
420 activity, leading to an elevation of extracellular glutamate levels, activation of extrasynaptic N-
421 methyl-D-aspartate receptor (NMDARs) and increased firing of PVN neurons ([Stern et al., 2016](#)).

422 Hence, it is possible that Gq DREADD-mediated increase of astrocyte Ca²⁺ activity-**firing recruits**
423 **the similar pathways to increase parvocellular neuron activity** ([Fleming et al., 2011](#); [Zhang et al., 2017](#))
424 and could mediate the bimodal regulation of PVN neurons by local astrocytes.

425 Given that 80% of glutamate released undergo active reuptake by astrocyte GLT-1 and EAAT1
426 transporters, a critical role for astrocyte glutamate transport in PVN region would have been
427 expected. Our study provides a shift in paradigm by furnishing experimental evidence that,
428 beyond the buffering of glutamate at the multipartite synapse, astrocytes act as gatekeepers of
429 neuronal activity. Targeting astrocyte activity, therefore, may offer upstream control of PVN
430 output, thereby coping with obesity-associated metabolic defects.

431 We found indeed that obesity leads to a disruption in astrocyte-neuronal communication in the
432 PVN. Principally, CNO failed to upregulate neuronal firing in obese mice, despite the fact that
433 DREADDs-induced modulation of astrocytic Ca^{2+} activity persisted. Thus, we propose that the
434 blunted CNO effect on PVN firing activity is a consequence of a steady-state inhibition of GLT1
435 activity by basally enhanced astrocyte Ca^{2+} activity in obese mice. This is supported by our results
436 showing an almost completely blunted/occluded effect of TBOA *per se* on the extrasynaptic
437 glutamate tonic current and firing activity of PVN neurons in obese mice. The fact that no changes
438 in GLT1 protein levels were observed further corroborates a blunted activity of GLT1 in astrocytes
439 in this condition. The mechanisms underlying the exacerbated basal astrocyte Ca^{2+} activity and
440 blunted GLT1 activity in obese mice remain unknown at present. We also acknowledge that the
441 Ca^{2+} related events we recorded might parallel other unknown important intracellular adaptive
442 mechanisms.

443 Given the pivotal role of the ANS in the control of virtually every metabolically active tissue, a
444 profound mal-adaptation of astrocyte-dependent control of these neurons will inevitably result in
445 a chronic perturbation of peripheral organ activity, a feature that has been proposed as a possible
446 central cause of the metabolic syndrome (Buijs et al., 2006; Marina et al., 2016; Zhang and Stern,
447 2017). One would indeed expect that in the complex neuron-glia interplay within PVN astrocyte-
448 neurons share a high degree of intricacy as it has been very recently demonstrated in feeding-
449 related neurons of the ARC nucleus (Varela et al., 2021a; Varela et al., 2021b). Unfortunately,

450 the tools currently available to finely dissect astrocyte function in vivo with respect to their diversity
451 are still scarce

452 In sum, our study demonstrates that PVN astrocytes exert a direct and reversible control of
453 systemic glucose metabolism, insulin sensitivity and energy balance suggesting that future anti-
454 obesity strategies leverage astrocyte function. Further studies are however warranted to identify
455 the genetic and metabolic signatures that **specify** sub-populations of astrocytes in discrete
456 hypothalamic nuclei. The latter being a requisite to harness the full benefit of astrocyte-directed
457 approaches to cope with metabolic diseases.

458

459 **Limitations of Study**

460 Due to the lack of current investigating tools allowing to finely dissect sub categories of astrocyte,
461 our study does not provide a complete readout of which subset(s) of astrocytes are involved in
462 the described mechanism.– Also, while change in intracellular Ca²⁺ waves are indicative of
463 astrocytic response they might not recapitulate to the full extent the complex response of
464 astrocytes to change in nutrient availability and obesogenic environment. Finally, we used **ex-vivo**
465 electrophysiology to identify extrasynaptic glutamate signaling as a key component of adaptive
466 astrocytic response, however our study does not provide *in vivo* confirmation for this observation.
467 Further studies are needed to fully flush out how controlling ambient glutamate allows astrocyte
468 to operate control over energy homeostasis.

469

470 **Acknowledgements**

471 This work was funded by the French National Research Agency/Agence Nationale pour la
472 Recherche (ANR) grant # ANR-15-CE14-0030-01, ANR-15-CE14-0030-02 and ANR-15-CE14-

473 0030-03 “Nutripathos” and the ANR-20-CE14-0025-01 “AstrObesity”. We acknowledge funding
474 supports from the Centre National de la Recherche Scientifique (CNRS), The Université Paris
475 Cité and the Fondation pour la Recherche Médicale (FRM). J.E.S was supported from National
476 Heart, Lung, Blood Institute Grant NIH HL090948, National Institute of Neurological Disorders
477 and Stroke Grant NIH NS094640, and funding provided by the Center for Neuroinflammation and
478 Cardiometabolic Diseases (CNCD) at Georgia State University. M.K.K. was supported from
479 National Heart, Lung, Blood Institute Grant F32 HL158172-01. EM was supported by the FRM.
480 CGC was supported from the European Research Council ERC (STG grant AstroNeuroCrosstalk
481 # 757393), the German Research Foundation DFG under Germany’s Excellence Strategy within
482 the framework of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy–ID 390857198)
483 and Helmholtz Excellence Network. MHT was supported from ERC AdG HypoFlam, 695054,
484 European Research Council Executive Agency (ERCEA), DFG Excellence Cluster SyNergy EXC
485 2145 SyNergy – ID 390857198, German Research Foundation (DFG) and ExNet-0041-Phase2-
486 3, Initiative and Networking Fund of the Helmholtz Association. We thank Giuseppe Gangarossa
487 for scientific and technical expertise. We thank Olja Kacanski for administrative support, Isabelle
488 Le Parco, Ludovic Maingault, Angélique Dauvin, Aurélie Djemat, Magguy Boa and Daniel Quintas
489 for animals’ care and Florianne Michel for genotyping. Telemetry experiments were supported by
490 “The Continuous Glucose Telemetry Award 2018” obtained by Raphaël G.P. Denis and
491 sponsored by Data Sciences International. We acknowledge the technical platforms Functional
492 and Physiological Exploration (FPE) and Bioprofiler of the Université Paris Cité, CNRS, Unité de
493 Biologie Fonctionnelle et Adaptative, F-75013 Paris, France, the viral production facility of the
494 UMR INSERM 1089 and the animal core facility “Buffon” of the Université Paris Cité/Institut
495 Jacques Monod. We thank the animal facility of IBPS of Sorbonne Université, Paris. Finally, we
496 would like to thank Xuelong Mi at Virginia Tech for his critical technical support using
497 the AQUA astrocytic calcium analysis.

498

499

500 **Author Contributions**

501 [D.H.M.C. initiated and developed the project, and critically participate in the overall project design.](#)

502 D.H.M.C performed and M.K.K contributed to designing research, performing experiments,
503 analyzing data, interpreting results of experiments, preparing figures, and writing of the
504 manuscript. C.P., E.F., R.D., J.C., C.M., E.M., R.H, L.B., J.R., C.M. and O.L., contributed to
505 performing experiments and data analysis. C.G.C. and M.H.T. contributed to funding and critical
506 analysis of experimental plan and conception. [D.H.M.C](#), D.L, C.M., J.E.S. contributed to
507 conception of the research project, writing of the manuscript, and approving the final version of
508 the manuscript. S.H.L supervised the whole project, secured funding, provided guidance and
509 designed the initial experimental plan and finalized the manuscript with the help of the co-authors.

510

511 **Declaration of interest**

512 [The authors declare no conflict of interest and MHT has no conflict of interest directly related to](#)
513 [the content of this manuscript.](#)

514

515 **Figure Legends**

516 **Figure 1. Hypothalamic astrocytes display Ca²⁺ hyperactivity in a heterogeneous manner**
517 **during diet-induced obesity. (A)** Experimental setting. **(B)** Representative confocal
518 photomicrograph for GCaMP (green) and s100β (red) immunoreactivity in the PVN of GCaMP6f-
519 Glast-CreER^{T2} mice. **(C)** Body weight gain of GCaMP6f-Glast-CreER^{T2} mice after 4-month chow
520 (lean, n=6) or HFHS (obese, n=6) diet consumption. **(D-F)** Pseudo-images (top), overall Ca²⁺
521 strength (middle) and time-course Ca²⁺ signal traces of spontaneous astrocyte activity with under-
522 curve area shaded (bottom) in **(D)** PVN (lean n=195, obese n=1619), **(E)** ARC (lean n=295, obese

523 n=220) or **(F)** VMH (lean n=216, obese n=1428) of lean or obese GCaMP6f-Glast-CreER^{T2} mice.
524 Scale bar: 20 μ m. **(G)** Experimental setting for viral-mediated approaches in the PVN and
525 representative confocal image of corresponding DREADD-mCherry signal (red). Cell nuclei are
526 shown in DAPI (blue), scale bar: 50 μ m. **(H)** Representative confocal photomicrograph of double
527 immunofluorescence of GFAP (green) and mCherry (red) in the PVN of DREADD injected mice.
528 Cell nuclei are labeled with DAPI (blue). Scale bar: 20 μ m. **(I)** Pseudo-images, **(J)** overall Ca²⁺
529 strength (left) and representative Ca²⁺ signal traces (right) before (n=263) and after (n=422) CNO
530 bath application in PVN astrocytes. Scale bar: 20 μ m. Histogram data are expressed as mean +/-
531 SEM. See also Figure S1. * P<0.05 by Student's t-test (C-F,J). For statistical details, see Data
532 S1.

533

534 **Figure 2. PVN astrocytes regulate glucose metabolism and energy balance. (A)**
535 [Experimental design for chemogenetic approaches.](#) **(B)** Blood glucose (Vehicle=6, CNO=6) and
536 corresponding area under the curve (AUC, top right), **(C)** plasma insulin change (Vehicle=7,
537 CNO=7) and **(D)** insulinogenic index (Vehicle=6, CNO=6) after Vehicle or CNO ip administration
538 followed by an OGTT in Aldh1L1-Cre^{+/-}:PVN^{hM3Dq} mice. **(E)** Pseudo-images, **(F)** overall Ca²⁺
539 strength (left, basal=723, SalB=1446) and time-course Ca²⁺ signal traces (right) of GCaMP
540 signals, **(G)** distribution of temporal correlations of Ca²⁺ responses of all paired active domains
541 (as an estimation of global synchronization) before (n=22530) and after (n=49352) SalB bath
542 application to PVN slices of Aldh1L1-Cre mice expressing KORD receptor. Scale bar: 20 μ m.
543 Overall Ca²⁺ strength data are expressed as mean +/- SEM. **(H)** Representative confocal
544 photomicrograph of double immunofluorescence of GFAP (red) and mCitrine (green) in the PVN
545 of Aldh1L1-Cre^{+/-}:PVN^{KORD} mice. Cell nuclei are labeled with DAPI (blue). **(I)** Blood glucose
546 (Vehicle=9, SalB=9) and corresponding AUC (top right), **(J)** plasma insulin change (Vehicle=5,
547 SalB=6) and **(K)** insulinogenic index (Vehicle=5, SalB=6) after Vehicle or SalB ip administration

548 followed by an OGTT in Aldh1L1-Cre^{+/+}:PVN^{KORD} mice. **(L-W)** Energy expenditure (EE), food
549 intake, respiratory exchange ratio (RER) and fatty acid oxidation (FAox) after Vehicle or SalB ip
550 injection to **(L-O)** Aldh1L1-Cre^{+/+}:PVN^{KORD} (Vehicle n=8, SalB n=8) and **(P-S)** C57Bl6j:PVN^{KORD}
551 (Vehicle n=5, SalB n=5) mice. **(T-W)** Energy balance after Vehicle or SalB ip administration in **(T)**
552 Aldh1L1-Cre^{+/+}:PVN^{KORD} (Vehicle n=8, SalB n=8), **(U)** C57Bl6j:PVN^{KORD} (Vehicle n=5, SalB n=5),
553 **(V)** Aldh1L1-Cre^{-/-}:PVN^{KORD} (Vehicle n=4, SalB n=4) and the control mice **(W)** Aldh1L1-Cre^{+/+}
554 :PVN^{mCherry} (Vehicle n=4, SalB n=4) mice. Data are expressed as mean +/- SEM. See also Figure
555 S2-4. *P<0.05 by 2-way ANOVA (B-C,I-W) and Student's t-test (B,D,F,I,K). For statistical details,
556 see Data S1.

557

558 **Figure 3. PVN astrocytes regulate sympathetic output and thermogenesis. (A-D)** Urine
559 mono-aminergic noradrenaline (NA), dopamine (DA) and serotonin (5HT) concentrations in **(A)**
560 Aldh1L1-Cre^{+/+}:PVN^{mCherry} (Vehicle n=3, CNO n=3) and **(B)** Aldh1L1-Cre^{+/+}:PVN^{hM3Dq} (Vehicle n=3,
561 CNO n=3) mice after Vehicle or CNO ip injection, or in **(C)** Aldh1L1-Cre^{+/+}:PVN^{mCherry} (Vehicle n=3,
562 SalB n=3) and **(D)** Aldh1L1-Cre^{+/+}:PVN^{KORD} (Vehicle n=3, SalB n=3) mice after Vehicle or SalB ip
563 administration. **(E)** Time-course local oxygen consumption (as percentage of blood saturated
564 oxygen (sO₂)) in BAT (left) and as AUC (right) after Vehicle (n=4) or CNO (n=7) ip injection to
565 Aldh1L1-Cre^{+/+}:PVN^{hM3Dq} mice. **(F)** Representative images of photoacoustic recording of BAT 40
566 minutes after Vehicle (left) or CNO (right) ip administration in Aldh1L1-Cre^{+/+}:PVN^{hM3Dq} mice. **(G-**
567 **H)** BAT mono-aminergic (NA, adrenaline (AD), DA, 5-hydroxyindolacetic acid (HIAA) and 5HT
568 content in **(G)** Aldh1L1-Cre^{+/+}:PVN^{mCherry} (Vehicle n=3, CNO n=3) and **(H)** Aldh1L1-Cre^{+/+}:PVN^{hM3Dq}
569 (Vehicle n=3, CNO n=3) mice after Vehicle or CNO ip administration. **(I)** Time-course change in
570 core body temperature of Aldh1L1-Cre^{+/+}:PVN^{hM3Dq} mice after Vehicle (n=7) or CNO (n=6) ip
571 injection. **(J)** Representative image of viral delivery of GFAP-hM3Dq in the PVN of C57BL/6 mice.
572 **(K-L)** BAT mono-aminergic (NA, AD, DA, HIAA and 5HT) content in **(K)** C57Bl6j:PVN^{hM3Dq}

573 (Vehicle n=4, CNO n=4) and **(L)** C57Bl6j:PVN^{mCherry} (Vehicle n=5, CNO n=5) mice after Vehicle
574 or CNO ip administration. AU refers to arbitrary units. Data are expressed as mean +/- SEM. *
575 P<0.05 by 2-way ANOVA (A-E,G-L) and Student's t-test (E). For statistical details, see Data S1.
576

577 **Figure 4. Astrocytes in the PVN exert bidirectional control over systemic glucose**
578 **metabolism and energy balance in obese mice. (A)** Blood glucose (Vehicle=12, CNO=12), **(B)**
579 glucose AUC, **(C)** plasma insulin change (Vehicle=6, CNO=6) and corresponding AUC (top right),
580 **(D)** insulinogenic index (Vehicle=6, CNO=6) and **(E)** plasma c-peptide (Vehicle=8, CNO=8)
581 change after Vehicle or CNO ip administration followed by an OGTT in obese C57Bl6j:PVN^{hM3Dq}
582 mice. **(F)** Food intake, **(G)** EE and **(H)** energy balance after Vehicle or CNO ip injection to obese
583 C57Bl6j:PVN^{hM3Dq} (Vehicle=7, CNO=7) mice. **(I)** Blood glucose (Vehicle=7, SalB=8), **(J)**
584 corresponding AUC, **(K)** plasma insulin change (Vehicle=6, SalB=7) and corresponding AUC (top
585 right), **(L)** insulinogenic index (Vehicle=6, SalB=7) and **(M)** plasma c-peptide change (Vehicle=7,
586 SalB=7) after Vehicle or SalB ip administration followed by an OGTT in obese C57Bl6j:PVN^{KORD}
587 mice. **(N)** Food intake, **(O)** EE and **(P)** energy balance after Vehicle or SalB ip injection to obese
588 C57Bl6j:PVN^{KORD} (Vehicle=7, SalB=7) mice. AU refers to arbitrary units. Data are expressed as
589 mean +/- SEM. See also Figure S5 and Figure S6. * P<0.05 by 2-way ANOVA (A,C,F-I,K,N-P)
590 and Student's t-test (B-E,J-M). For statistical details, see Data S1.
591

592 **Figure 5. Chemogenetic activation of Gq-coupled receptor in PVN astrocytes enhances**
593 **parvocellular neuronal firing in lean mice. (A)** Representative image of a patched parvocellular
594 neuron labelled with Alexa 555 (red) in the PVN of C57Bl6j:PVN^{hM3Dq} mice expressing GCaMP6
595 (green). **(B)** Firing activity traces in parvocellular PVN neurons in response to CNO in lean (top)
596 and obese (bottom) C57Bl6j:PVN^{hM3Dq} mice. **(C)** Parvocellular PVN neuron spike frequency in

597 response to CNO in lean (left, n=10) and obese (right, n=9) C57Bl6j:PVN^{hM3Dq} mice. **(D)** %Change
598 in the parvocellular PVN neuron action potential firing frequency after CNO bath application in
599 lean C57Bl6j:PVN^{hM3Dq} (Gq^{+/+}, n=8) or C57Bl6j:PVN^{mCherry} (Gq^{-/-}, n=7) mice. **(E)** photomicrograph
600 of Ca²⁺ event analysis overlay in PVN slices of C57Bl6j:PVN^{hM3Dq} mice, in the presence of CNO
601 in both lean (left) and obese (right) animals. Scale bar: 25 μ m. **(F)** Summary data of astrocyte
602 Ca²⁺ activity. Ca²⁺ strength (left, Lean/Baseline n=3036, Lean/CNO n=3009, Obese/Baseline
603 n=5132 Obese/CNO n=5016), event duration (middle, Lean/Baseline n=3036, Lean/CNO
604 n=3009, Obese/Baseline n=5132 Obese/CNO n=5016) and event synchrony (right,
605 Lean/Baseline n=618, Lean/CNO n=543, Obese/Baseline n=815, Obese/CNO n=541) of GCaMP
606 signals in PVN of lean or obese C57Bl6j:PVN^{hM3Dq} mice. **(G)** (Left) Latency of astrocytic Ca²⁺
607 activity response to CNO application and the upregulation of PVN neuronal spiking activity in lean
608 (n=7) and obese (n=11) C57Bl6j:PVN^{hM3Dq} mice. (Right) Difference between the chemogenetic
609 Gq-induced upregulation of Ca²⁺ activity in astrocytes and parvocellular neuronal firing (Lean n=7,
610 Obese n=11). **(H)** PVN parvocellular firing activity traces recorded from lean (top) and obese
611 (bottom) C57Bl6j:PVN^{hM3Dq} mice, combining bath application of CNO and glutamate channel
612 blocker Kynurenic Acid (KYN, 1 mM). **(I)** Parvocellular PVN neuron spike frequency in the
613 presence of KYN and CNO, in both lean (solid, n=6) and obese (striped, n=4) C57Bl6j:PVN^{hM3Dq}
614 mice. **(J)** Summary data of AQuA astrocyte Ca²⁺ activity analysis. Ca²⁺ event duration (left,
615 Lean/Baseline n=128, Lean/CNO n=124, Obese/Baseline n=2158, Obese/CNO n=1885) or event
616 synchrony (right, Lean/Baseline n=429, Lean/CNO n=282, Obese/Baseline n=429, Obese/CNO
617 n=257) in the presence of CNO/KYN. Data are expressed as mean +/- SEM. * P<0.05, ** P<0.01,
618 *** P<0.001, **** P<0.0001 by 2-Way ANOVA (C, I), Student's t-test (D, G), and Brown-Forsythe
619 ANOVA (F, J). For statistical details, see Data S1.

620

621 **Figure 6. Astrocyte KORD-Gi receptor chemogenetic activation inhibits PVN parvocellular**
622 **neuronal firing. (A)** Firing activity traces in parvocellular PVN neurons in response to SalB in
623 Aldh1L1-Cre^{+/+}:PVN^{KORD} mice. **(B)** Parvocellular PVN neuron spike frequency (left) and
624 percentage of decrease in firing frequency (right) in the presence of SalB in Aldh1L1-Cre^{+/+}
625 :PVN^{KORD} mice (n=5). **(C)** %Change in the parvocellular PVN neuron action potential firing
626 frequency after SalB bath application in Aldh1L1-Cre^{+/+}:PVN^{KORD} (KORD^{+/+}, n=7) or Aldh1L1-Cre^{+/+}
627 :PVN^{mCherry} (KORD^{-/-}, n=3) mice. **(D)** Photomicrograph of Ca²⁺ event analysis overlay in PVN slices
628 of Aldh1L1-Cre^{+/+}:PVN^{KORD} mice, in the presence of SalB. Scale bar: 25 μm. **(E)** Summary data of
629 astrocyte Ca²⁺ activity. Ca²⁺ strength (left, Control n= 3646, SalB n=2620), event synchrony
630 (middle, Control n= 3646, SalB n=2620) and event frequency (right, n=5) of GCaMP signals in
631 PVN of Aldh1L1-Cre^{+/+}:PVN^{KORD} mice. **(F)** (Left) Latency of astrocytic Ca²⁺ activity response to
632 SalB application and the upregulation of PVN neuronal spiking activity in Aldh1L1-Cre^{+/+}:PVN^{KORD}
633 mice. (Right) Difference between the Gi-induced downregulation of Ca²⁺ activity in astrocytes and
634 parvocellular neuronal firing. Data are expressed as mean +/- SEM. See also Figure S7. * P<0.05,
635 ** P<0.01, *** P<0.0001 by paired Student's t-test (B, E_{right}), unpaired Student's t-test (C), and
636 Welch's t-test (E_{left, middle}). For statistical details, see Data S1.

637
638 **Figure 7. Astrocyte glutamatergic transport signaling contributes to PVN astrocyte-neuron**
639 **interaction. (A)** PVN parvocellular firing activity traces recorded from lean (left) and obese (right)
640 C57Bl6j:PVN^{hM3Dq} mice, combining bath application of CNO and glutamate transporter blocker
641 DL-*threo*-β-Benzyloxyaspartic acid (TBOA, 1 μM). **(B)** Representative images of AQuA Ca²⁺ event
642 analysis overlay of GCaMP signals in PVN slices of lean (left) or obese (right) C57Bl6j:PVN^{hM3Dq}
643 mice, demonstrating the strength of Ca²⁺ signals at baseline activity and in the presence of SalB
644 and TBOA. Each image is an average of 10 frames. Scale bar: 25 μm. **(C)** PVN parvocellular
645 neuron spike frequency at baseline activity and in the presence of CNO and TBOA in both lean
646 (solid, n=6) and obese (striped, n=4) C57Bl6j:PVN^{hM3Dq} mice. **(D)** Summary data of astrocyte Ca²⁺

647 activity in the presence of CNO/TBOA. Ca²⁺ event duration (left, Lean/Baseline n=352, Lean/CNO
648 n=339, Obese/Baseline n=4903, Obese/CNO n=4332) and event synchrony (right, Lean/Baseline
649 n=457, Lean/CNO n=312, Obese/Baseline n=1338, Obese/CNO n= 818) of GCaMP signals in
650 PVN of lean or obese C57Bl6j:PVN^{hM3Dq} mice. **(E)** Protein quantification and representative
651 western blot (inset) of glutamate transporter GLT1 (EAAT2) in lean (solid, n=5 biological
652 replicants, 1 technical replicant each) and obese (striped, n=5 biological replicants, 1 technical
653 replicant each) C57BL/6 mice. **(F)** Membrane and firing properties of parvocellular neurons during
654 voltage clamp, after TBOA (1 μM) bath application to PVN slices of lean (top) and obese (bottom)
655 C57BL/6 mice. Holding current (I_{Holding}). **(G)** Summary graphs representing the shifts in I_{Holding} in
656 lean (left, n=8), obese (middle, n=7), and their difference (right). **(H)** Parvocellular neuron
657 membrane potential (mV) and spike frequency (Hz) in current clamp, during TBOA bath
658 application to PVN slices of lean (top) and obese (bottom) C57BL/6 mice. **(I)** Summary graphs
659 representing the shifts in membrane potential in lean (left, n=6), obese (middle, n=6), and their
660 difference (right). **(J)** Summary graphs representing the shifts in spike frequency in lean (left, n=6),
661 obese (middle, n=6), and their difference (right). Data are expressed as mean +/- SEM. * P<0.05,
662 ** P<0.01, *** P<0.0001 by 2-Way ANOVA (C), Brown-Forsythe ANOVA (D), unpaired Student's
663 t-test (E, G_{right}, I_{right}, J_{right}), and Repeated Measures ANOVA (G_{left, middle}, I_{left, middle}, J_{left, middle}). For
664 statistical details, see Data S1.

665

666 **STAR Methods**

667 **RESOURCE AVAILABILITY Lead contact**

- 668
- 669 • Further information and requests for resources and reagents should be directed to and
will be fulfilled by the Lead Contact, Serge Luquet (serge.luquet@u-paris.fr).

670 **Materials Availability**

- 671
- This study did not generate new unique reagents.

672

673 **Data and code availability**

- 674 • Data reported in this paper will be shared by the lead contact upon request.
- 675 • Original data for creating all graphs in the paper and the corresponding statistical details
676 are provided in Data S1.
- 677 • This study did not report original code.
- 678 • Any additional information required to reanalyze the data reported in this study is available
679 from the lead contact upon request.

680 **Experimental models and subject details**

681 **Animals**

682 Unless noted otherwise, littermates of the same sex were randomly assigned to experimental
683 groups. All animal protocols were approved by the Animal Care Committee of the University of
684 Paris (APAFIS # 2015062611174320), Institut Biologie Paris Seine of Sorbonne University (C75-
685 05-24) or the Georgia State University IAUCUC protocols. Twelve to fifteen-week-old male Aldh1-
686 L1-Cre (Tg(Aldh111-cre)) JD1884Htz, Jackson laboratory, Bar Harbor, USA), male C57BL/6J
687 (Janvier, Le Genest St-Isle, France) or male GCaMP6f/Glast-CreER^{T2} (Pham et al., 2020) mice
688 were individually housed at constant temperature (23± 2°C) and submitted to a 12/12h light/dark
689 cycle. All mice had access to regular chow diet (Safe, Augy, France) and water *ad libitum*, unless
690 stated otherwise. Additionally, age matched C57BL/6J or GCaMP6f/Glast-CreER^{T2} mice groups
691 were fed with either chow diet or high-fat high-sugar diet (HFHS, cat n. D12451, Research Diets,
692 New Brunswick, USA) for twelve to sixteen weeks. Body weight was measured every week. Body
693 weight gain was calculated as the difference between week one, twelve-sixteen weeks of HFHS
694 diet exposure.

695

696 **Method details**

697 **Viral constructs**

698 Designer receptor exclusively activated by designer drugs (DREADD) and GCaMP6f viruses were
699 purchased from <http://www.addgene.org/>, unless stated otherwise. pAAV-EF1 α -DIO-hM3Dq-
700 mCherry (2.4×10^{12} vg/ml, working dilution 1:6, Addgene plasmid #50460-AAV5;
701 <http://www.addgene.org/50460/>; RRID:Addgene_50460), pAAV-EF1 α -DIO-hM4Di-mCherry
702 (2.4×10^{12} vg/ml, working dilution 1:6, Addgene plasmid #50461-AAV5;
703 <http://www.addgene.org/50461/>; RRID:Addgene_50461), pAAV-EF1 α -DIO-mCherry (3.6×10^{12}
704 vg/ml, working dilution 1:4, Addgene plasmid #50462-AAV5; <http://www.addgene.org/50462/>;
705 RRID:Addgene_50462), pAAV-GFAP-hM3Dq-mCherry (9.1×10^{12} vg/ml, working dilution 1:2,
706 Addgene plasmid #50478-AAV5; <http://www.addgene.org/50478/>; RRID:Addgene_50478) and
707 pAAV-GFAP-hM4Di-mCherry (3.2×10^{11} vg/ml, working dilution 1:1.5, Addgene plasmid #50479-
708 AAV5; <http://www.addgene.org/50479/>; RRID:Addgene_50479) were a gift of Bryan Roth. pAAV-
709 GFAP104-mCherry (6.4×10^{12} vg/ml, working dilution 1:1.5, Addgene plasmid #50479-AAV5;
710 <http://www.addgene.org/50479/>; RRID:Addgene_50479) was a gift of Edward Boyden. pAAV-
711 GFAP-Cre.WPRE (2.2×10^{12} vg/ml, working dilution 1:10, Addgene plasmid #105550-AAV5;
712 <http://www.addgene.org/105550/>; RRID:Addgene_105550) was a gift from James M Wilson.
713 pAAV-CAG-Flex.GCaMP6f.WPRE (3.15×10^{13} vg/ml, working dilution 1:10, Addgene plasmid
714 #100835-AAV5; <http://www.addgene.org/100835/>; RRID:Addgene_100835) was a gift of Douglas
715 Kim and GENIE Project. pAAV-GfaACC1D.Lck-GCaMP6f.SV40 (1.53×10^{13} vg/ml, working
716 dilution 1:5, Addgene plasmid #52925-AAV5; <http://www.addgene.org/52295/>;
717 RRID:Addgene_52925) was a gift of Baljit Khak. In order to produce the pAAV-EF1 α -DIO-KORD-
718 mCitrine, the NheI/Ascl fragment containing the HA-KORD-Citrine cassette from addgene#65417
719 was subcloned in Nhe1/Ascl backbone pAAV-EF1 α -DIO-hM3D(Gq)-mCherry (addgene#50460)
720 to produce pAAV-EF1 α -DIO-HA-KORD-Citrine and subsequent adeno-associated virus 5HType

721 2/5 (2.4×10^{12} vg/ml, working dilution 1:6) were produced by the viral production facility of the UMR
722 INSERM 1089 (Nantes, France).

723 **Surgical procedures**

724 For all surgical procedures, mice were rapidly anesthetized with isoflurane (3%), followed by
725 intraperitoneal (ip) injection of analgesic Buprenorphine (Buprecare, 0.3 mg/kg, Recipharm,
726 Lancashire, UK) and Ketoprofen (Ketofen, 10 mg/kg, France) and maintained under 1.5%
727 isoflurane anesthesia throughout the surgery.

728 *Stereotaxic surgery.* Male *Aldh1-L1-Cre^{+/-}*, *Aldh1-L1-Cre^{-/-}* and male C57BL/6J mice were
729 anaesthetized with isoflurane (3.5% for induction, 1.5% for maintenance) and received 10 mg.kg-
730 1 intraperitoneal injection (i.p.) of Buprécare® (Buprenorphine 0.3 mg) diluted 1/100 in NaCl 0.9%
731 and 10 mg/kg of Ketofen® (Ketoprofen 100 mg) diluted 1/100 in NaCl 0.9%, and placed on a
732 stereotactic frame (Model 940, David Kopf Instruments, California) During surgery, body
733 temperature was maintained at 37°C. Mice received bilateral viral injections (0.3 µl) in the PVN
734 (stereotaxic coordinates: AP -0.8mm, L +0.3mm, V -4.9mm) at a rate of 0.1 µl/min. The injection
735 needle was carefully removed after 5 minutes waiting at the injection site and 2 minutes waiting
736 half way to the top (Berland et al., 2020). Mice recovered for at least 3 weeks after the surgery
737 before being involved in experimental procedures.

738 *Continuous body temperature telemetry implantation.* Two-weeks after stereotaxic delivery of viral
739 vectors, additional groups of male C57BL/6J mice were implanted with telemetric devices (HD-
740 XG, Data Sciences International (DSI), Minnesota, USA) in the abdominal cavity. After a seven-
741 day period recovery time, the implanted mice were then installed on the DSI receiver. Data were
742 collected using a Ponemah acquisition system (DSI).

743 **Photoacoustic BAT imaging.**

744 Male Aldh1L1-Cre^{+/-} mice previously injected in the PVN with DREADD viruses were anesthetized
745 under isoflurane and placed on a heated platform. Respiration and cardiac rhythm were constantly
746 monitored. Hair was removed above the interscapular BAT and optic gel was applied before
747 positioning the transducer to ensure a good transmission of the signal. Image acquisition, before
748 and after vehicle (0.9% Sterile sodium chloride solution (Fisher Scientific, Illkrich, France) or
749 Clozapine-N-oxide dihydrochloride (CNO, 0.6mg/kg, Tocris, Bristol, UK) ip injection, was
750 performed using a Vevo LAZR system (FujiFilm VisualSonics, Toronto, ON, Canada) which
751 combine ultrasound and photoacoustic imaging. Light generated from a tunable laser (680–970
752 nm) is delivered to the tissue through fiber optic bundles integrated into linear-arrays mounted on
753 the ultrasonic transducer (LZ400, fc = 30 MHz). Spectral unmixing analyses were performed with
754 Vevo®Lab software.

755 **Indirect calorimetry analysis.**

756 After stereotaxic surgery and viral delivery, male Aldh1-L1-Cre^{+/-}, Aldh1-L1-Cre^{-/-} and C57BL/6J
757 mice were monitored for metabolic efficiency (Labmaster, TSE Systems GmbH, Bad Homburg,
758 Germany). After an initial period of acclimation in the calorimetry cages, Vehicle (Vehicle matched
759 with CNO injected group: 0.9% Sterile sodium chloride solution (Fisher Scientific, Illkrich, France)
760 or Vehicle matched with SalB injected group: 0.6% DMSO (Sigma-Aldrich, Saint-Louis, USA) in
761 0.9% sterile sodium chloride solution (Fisher Scientific, Illkrich, France)), CNO (0.6mg/kg
762 dissolved in 0.9% Sterile sodium chloride solution, Tocris, Bristol, UK) or Salvinorin B (SalB,
763 10mg/kg, dissolved in DMSO and injected in a 0.6% DMSO final solution, Hellobio, Dunshaughlin,
764 Republic of Ireland) were ip injected with a two-day interval between injections depending on the
765 corresponding DREADD expressing group.

766 Metabolic efficiency was assessed as previously described (Berland et al., 2020). Additionally,
767 fatty acid oxidation (Frayn, 1983; Herrera Moro Chao et al., 2019) and energy balance (Hall et al.,
768 2012) were calculated as previously reported. Briefly, whole energy expenditure (EE), O₂

769 consumption and CO₂ production, respiratory exchange rate (RER=VCO₂/VO₂), fatty acid
770 oxidation (FAO), and locomotor activity using calorimetric cages with bedding, food and water
771 (Labmaster, TSE Systems GmbH, Bad Homburg, Germany) were recorded as previously
772 reported (Berland et al., 2020; Frayn, 1983; Hall et al., 2012; Herrera Moro Chao et al., 2019).
773 Ratio of gases was determined through an indirect open circuit calorimeter (Arch et al., 2006;
774 Even and Nadkarni, 2012). This system monitors O₂ and CO₂ concentration by volume at the inlet
775 ports of a tide cage through which a known flow of air is being ventilated (0.4 L/min) and compared
776 regularly to a reference empty cage. For optimal analysis, the flow rate was adjusted according
777 to the animal body weights to set the differential in the composition of the expired gases between
778 0.4-0.9% (Labmaster, TSE Systems GmbH, Bad Homburg, Germany). The flow was previously
779 calibrated with O₂ and CO₂ mixture of known concentrations (Air Liquide, S.A. France). O₂
780 consumption and CO₂ production were recorded every 15 min for each animal during the entire
781 experiment. Whole energy expenditure (EE) was calculated using the Weir equation for
782 respiratory gas exchange measurements. Food consumption was measured as the instrument
783 combines a set of highly sensitive feeding sensors for automated online measurements. Mice had
784 access to food and water *ad libitum*. To allow measurement of every ambulatory movement, each
785 cage was embedded in a frame with an infrared light beam-based activity monitoring system with
786 online measurement at 100 Hz. The sensors for gases and detection of movements operated
787 efficiently in both light and dark phases, allowing continuous recording.

788 Body mass composition was analyzed using an Echo Medical systems' EchoMRI (Whole Body
789 Composition Analyzers, EchoMRI, Houston, USA), according to manufacturer's instructions.
790 Readings of body composition were given within 1 min. Data analysis was performed on Excel
791 XP using extracted raw values of VO₂ consumed (expressed in ml/h), VCO₂ production
792 (expressed in ml/h), and energy expenditure (kcal/h).

793

794 **Oral glucose tolerance test**

795 Viral injected male *Aldh1-L1-Cre^{+/-}*, *Aldh1-L1-Cre^{-/-}* and C57BL/6J mice and non-injected
796 GCaMP6f/*Glast-CreER^{T2}* mice were fasted for four hours. A small tail cut incision was performed
797 to take blood samples and measure glycemia with a glucometer (A. Menarini Diagnostics,
798 France). The mice were ip injected firstly with Vehicle, CNO (0.6 mg/kg) or SalB (10 mg/kg) and
799 fifteen minutes after received a glucose oral gavage (2 g/kg). Blood glucose levels were measured
800 after 0, 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes and blood samples were only taken at 0,
801 15, 30, and 90 minutes after receiving the glucose oral gavage. Plasma samples were further
802 processed for insulin (mouse ultrasensitive insulin ELISA kit, ALPCO, Salem, USA) and c-peptide
803 (mouse C-peptide ELISA kit, Crystal Chem, IL, USA) measurements. Insulinogenic index was
804 calculated as the area under the curve (AUC) of plasma insulin change during OGTT (in pmol/l)
805 divided AUC of glycaemia during OGTT (in mmol/l) as described ([Singh and Saxena, 2010](#)).

806 **Corticosterone level measurement**

807 Circulating corticosterone was measured in blood draw using the DtetctX® corticosterone
808 chemiluminescent ELISA kits from #K014 ArborAssays™ (source Clinisciences S.A.S Nanterre,
809 France).

810 ***Ex-vivo* epifluorescence calcium imaging**

811 Male *Aldh1-L1-Cre^{+/-}* mice previously injected with GCaMP6f and DREADDs viral constructs and
812 GCaMP6f/*Glast-CreER^{T2}* mice were terminally anaesthetized using isoflurane. Brains were
813 removed and placed in ice-cold oxygenated slicing artificial cerebrospinal solution (aCSF, 30mM
814 NaCl, 4.5mM KCl, 1.2mM NaH₂PO₄, 1mM MgCl₂, 26mM NaHCO₃, and 10mM D-Glucose and
815 194mM Sucrose) and subsequently cut into 300-µm thick PVN coronal slices using a vibratome
816 (Leica VT1200S, Nussloch, Germany). Next, brain slices were recovered in aCSF (124mM NaCl,
817 4.5mM KCl, 1.2mM NaH₂PO₄, 1mM MgCl₂, 2mM CaCl₂, 26mM NaHCO₃, and 10mM D-Glucose)

818 at 37 °C for 60 minutes. Imaging was carried out at room temperature under constant perfusion
819 (~3 ml/min) of oxygenated aCSF. The overall cellular fluorescence of astrocytes expressing
820 GCaMP6f was collected by epifluorescence illumination. A narrow-band monochromator light
821 source (Polychrome II, TILL Photonics, Germany) was directly coupled to the imaging objective
822 via an optical fiber. Fluorescence signal was collected with a 40x 0.8NA or a 63x 1.0NA water
823 immersion objective (Zeiss, Germany) and digital electron-multiplying charge-coupled device
824 (EMCCD Cascade 512B, Photometrics, Birmingham, UK). The imaging system was hosted in an
825 upright microscope (Zeiss Axioskop 50, Germany) and coordinated by MetaMorph software
826 (Molecular Devices, USA) (Pham, 2020). A double-band dichroic/filter set was used to reflect the
827 excitation wavelength (470 nm) to slices and filter the emitted GCaMP6 green fluorescence (Di03-
828 R488/561-t3; FF01-523/610, Semrock). The same filter was used for slices expressing both
829 GCaMP6 and DREADD-mCherry. PVN slices were transferred to the imaging chamber, where 3-
830 minute astrocyte spontaneous activity recordings were performed in slices of GCaMP6f/Glast-
831 CreER^{T2} mice. In the case of PVN slices of Aldh1-L1-Cre^{+/-} mice, we performed a basal
832 epifluorescence recording (60 seconds), followed by a 120 second bath application of CNO
833 (10µM) or SalB (100µM) and 240 seconds recording over the washing of the compounds.

834 The responsive regions displaying Ca²⁺ signals were scrutinized by the three-dimensional spatio-
835 temporal correlation screening method (Pham et al., 2020). Background signal was subtracted
836 from the raw images by using the minimal intensity projection of the entire stack. Ca²⁺ signals of
837 individual responsive regions were normalized as dF/F_0 , with F_0 representing the baseline
838 intensity and quantified using Matlab (The MathWorks, France) and Igor Pro (Wavemetrics, USA).
839 We gauged signal strength of Ca²⁺ traces of single responsive regions by calculating their
840 temporal integration and normalizing per minute. The global temporal synchronization of detected
841 Ca²⁺ signals was determined by the temporal Pearson's correlation coefficients of all
842 combinations between single Ca²⁺ regions (Pham et al., 2020).

843

844 **Simultaneous *ex-vivo* whole-cell patch clamp electrophysiology and confocal Ca²⁺**
845 **imaging**

846 Viral injected male Aldh1-L1-Cre^{+/+} and C57BL/6J mice were anesthetized with pentobarbital (50
847 mg kg⁻¹ ip) and subsequently perfused through the heart with 30 mL of ice cold aCSF sucrose
848 solution with NaCl replaced by equal-osmol sucrose (in mM: 200 sucrose, 2.5 KCl, 1 MgSO₄, 26
849 NaHCO₃, 1.25 NaH₂PO₄, 20 D-Glucose, 0.4 ascorbic acid, and 2.0 CaCl₂; pH 7.2; 300-305
850 mosmol l⁻¹). The brain was subsequently removed, mounted in the chamber of a vibratome (Leica
851 VT1200s, Leica Microsystems, Buffalo Grove, IL, USA) using superglue and the ventral surface
852 pressed firmly against a block of 3% KCl agar. The brain was submerged in sucrose solution and
853 bubbled constantly with 95% O₂/5% CO₂. Coronal slices (240 μm thickness) containing the PVN
854 were cut and placed in a holding chamber filled with aCSF and bubbled with 95% O₂/5% CO₂.
855 The aCSF is identical in composition to the sucrose solution, but with 200 mM sucrose replaced
856 by 119 mM NaCl. The slice chamber was warmed using a water bath at 32°C for 20 minutes
857 before placement at room temperature for a total minimum of 60 minutes rest before proceeding
858 with the experiment.

859 Slices were placed into a chamber on the stage of a microscope and perfused constantly (~3
860 ml/min) with aCSF bubbled continuously with 95% O₂/5% CO₂ and warmed to 32°C. Parvocellular
861 neurons were targeted for patch clamp using morphological properties such small soma size
862 relative to their magnocellular counterparts, as well as their proximity to as many GCaMP-
863 expressing astrocytes as possible. Whole cell current clamp recordings were obtained from PVN
864 parvocellular neurons using pipettes (2.5-4 MΩ) pulled from borosilicate glass (o.d. 1.5 mm) using
865 a P-97 flaming/brown horizontal micropipette puller (Sutter Instruments, Novato, CA). The pipette
866 internal solution consisted of (in mM): 135 KMeSO₄, 8 NaCl, 10 HEPES, 2 Mg-ATP, 0.3 Na-GTP,
867 6 phosphocreatine, 0.2 EGTA with pH 7.2-7.3 and 285-295 mOsmol (kg H₂O)⁻¹. The liquid junction

868 potential for the KMeSO_4 internal was approximately -10 mV and was not corrected. Occasionally,
869 Alexa 555 (50 μM , Invitrogen, MA, USA) was included to visualize the neuron. For current clamp
870 recordings, traces were obtained with an Axopatch 200B amplifier (Axon Instruments, Foster City,
871 CA) and digitized using an Axon 1440B Digitizer (Axon Instruments, Foster City, CA) at 10 kHz
872 on a desktop computer running Clampex 10 software (Molecular Devices). In the experiments
873 where DL-threo-beta-Benzyloxyaspartate (TBOA, 1 μM , Tocris, Bristol, UK) alone was bath
874 applied, cells were recorded in voltage clamp, held at -70 mV, and filtered at 2 kHz. Data were
875 discarded if series resistance exceeded a 20% change over the course of the recording. To
876 positively confirm a patched cell was indeed a parvocellular neuron, a series of increasingly
877 negative current injections was applied to the cell from a resting membrane potential of -60mV.
878 The activation of a low threshold depolarization in response to the cessation of current injection
879 confirms parvocellular identity ([Tasker and Dudek, 1991](#)). Basal recordings of parvocellular and
880 astrocytic Ca^{2+} activity were firstly performed for a minimum of 250 seconds before bath
881 application of either CNO (10 μM) or SalB (100 μM) for 250 seconds. Additional recordings adding
882 CNO or SalB combined with kynurenic Acid (KYN, 1 mM, Abcam, Cambridge, UK) or TBOA (1
883 μM , Tocris, Bristol, UK), were performed after washout for a minimum of 5 minutes after initial
884 application of CNO/SalB. Data were analyzed using either Clampfit or Igor Pro 8 (Wavemetrics
885 Inc.).

886 Simultaneously, GCaMP6f expression in astrocytes was visualized using the Dragonfly 200 laser
887 spinning disk confocal imaging system and an iXon 888 EMCCD camera (Andor Technology,
888 Belfast, UK). Using Andor's FUSION software, we captured time series images with z-stacking to
889 maximize acquisition of astrocytic Ca^{2+} events near the patched neuron. To quantify network-wide
890 Ca^{2+} signals within the confocal plane, we employed the Astrocyte Quantification and Analysis
891 (AQuA) method that extracts event-based information from optical sectioning image series ([Wang
892 et al., 2019](#)). Image background was subtracted, and images were converted from FUSION's
893 native file format to TIFFs in ImageJ (NIH). Imaging videos of astrocytic GCaMP were then

894 processed using the AQuA script run through Matlab (v2019b) (MathWorks, Natick, MA, USA).
895 Full details of the script functionality and code can be found at: <https://github.com/yu-lab-vt/AQuA>.
896 The parameters used for the analysis are as follows: intensity threshold scaling factor 1,
897 smoothing (sigma) 1.5, minimum Size (pixels) 24, temporal cut threshold 2, growing z threshold
898 1, rising time uncertainty 2, slowest delay in propagation 2, propagation smoothness 1, z score
899 threshold 2, maximum distance 0, minimum correlation 0, maximum time difference 2, temporally
900 extended events disabled, ignore delay tau enabled. We filtered out events lasting longer than 60
901 seconds and whose amplitude exceeded a 10 dF/F₀ change in amplitude to minimize inclusion of
902 artifacts in the analysis. Event data were imported into Prism 8 for analysis (GraphPad, San
903 Diego, CA, USA). Some waveform data and figures were generated in Igor Pro (Wavemetrics,
904 Portland OR, USA).

905

906 **Brain tissue Immunofluorescence**

907 Viral injected male Aldh1-L1-Cre^{+/-} and C57BL/6J mice received Vehicle, CNO or SalB ip injection.
908 Sixty minutes later, the mice were euthanized with pentobarbital (500 mg/kg, Dolethal, Vetoquinol,
909 France). An additional group of GCaMP6f/Glast-CreER^{T2} mice was also euthanized. Mice were
910 transcardially perfused with 0.1 M sodium phosphate buffer (PBS, pH 7.5) followed by 4%
911 paraformaldehyde in phosphate buffer (0.1 M, pH 7.2). Brains were removed and post-fixed
912 overnight in 4% paraformaldehyde. Afterwards, the brains were transferred to 30% sucrose in
913 PBS for 2 days for cryoprotection. Next, 25 μm brain sections were cut in a freezing cryostat
914 (Leica, Wetzlar, Germany) and further processed for immunofluorescence following the
915 procedure previously described (Berland et al., 2020). Sections were processed as free-floating
916 and incubated at 4°C overnight with chicken anti-Green fluorescent protein (GFP, 1:500, Aves
917 Labs, Davis, California, USA), rabbit anti-s100β (1:500, Abcam, Cambridge, UK), rabbit Ds Red
918 (1:1000, Living colors, Takara Bio, St-Germain-en-Laye, France), mouse anti-Glial fibrillary acidic

919 protein (GFAP, 1:1000, Sigma-Aldrich, Saint-Louis, USA) or rabbit anti-HA epitope tag (1:2000,
920 Rockland, Limerick, USA) primary antibodies. The next day, sections were rinsed in Tris-buffered
921 saline (TBS, 0.25M Tris and 0.5M NaCl, pH 7.5) and incubated for 2 hours with secondary
922 antibodies (1:1000, Thermo fisher Scientific, MA, USA) conjugated with fluorescent dyes: goat
923 anti-chicken Alexa 488, donkey anti-rabbit Alexa 594, donkey anti-mouse Alexa 488 and donkey
924 anti-rabbit Alexa 647. After rinsing, the sections were mounted and coverslipped with DAPI
925 (Vectashield, Burlingade, California, USA) and examined with a confocal laser scanning
926 microscope (Zeiss LSM 510, Oberkochen, Germany) with a color digital camera and AxioVision
927 3.0 imaging software.

928

929 **Western Blotting**

930 C57BL/6J mice were euthanized with pentobarbital (500 mg/kg, Dolethal, Vetoquinol, France)
931 and shortly after decapitated. The mouse head was then immediately immersed in liquid nitrogen
932 for 4 seconds. After removal of the brain, the anterior hypothalamus was dissected on ice-cold
933 surface and brain lysates were prepared in a solution containing 1% Sodium dodecyl sulfate
934 (SDS, Sigma-Aldrich, Saint-Louis, USA), 0.2% phosphatase inhibitors (phosphatase inhibitor
935 cocktail, Sigma-Aldrich, Saint-Louis, USA) and 0.1% protease inhibitors (protease inhibitor
936 cocktail, Roche, Boulogne-Billancourt, France). Protein content in the hypothalamic lysates was
937 measured with a BC Assay protein quantification Kit (Interchim Uptima, Montlucon, France).
938 Western blotting was performed as previously described ([Berland et al., 2020](#)). Equal amounts
939 of proteins (15 µg) for each sample were loaded onto 10-12% polyacrylamide gels. Proteins were
940 separated by SDS-PAGE and transferred to PVDF membranes (Millipore). Membranes were
941 immunoblotted with rabbit anti-EEAT2 (1:500, Novus Biologicals, Abingdon, UK). HRP-coupled
942 secondary antibody anti-rabbit (1:10000, Cell Signaling Technology, Charles Renard, France)

943 was matched with an ECL detection system. Quantification was performed using Image J
944 software (NIH).

945

946 **Mono-aminergic content determination**

947 Viral injected male Aldh1-L1-Cre^{+/-} and C57BL/6J mice were injected ip with Vehicle or CNO,
948 followed by icilin ip (5 mg/kg, Tocris, Bristol, UK) and sacrificed with pentobarbital (500 mg/kg,
949 Dolethal, Vetoquinol, France) after sixty minutes. Interscapular brown adipose tissue (BAT) was
950 dissected and immediately snap frozen in liquid nitrogen. Additional viral injected male Aldh1-L1-
951 Cre^{+/-} and C57BL/6J mice received Vehicle, CNO or SalB ip injection and were euthanized sixty
952 minutes later. Urine (removed from the bladder) was collected and immediately stored at -80°C.
953 Monoamine analyses were performed at the Bioprofiler platform of the Unit “Biologie
954 Fonctionnelle et Adaptative”, University of Paris, BFA, UMR 8251 CNRS, F75205, Paris, France.
955 Catecholamines and metabolites were analyzed by reversed phase-High-performance Liquid
956 Chromatography using a Shimadzu system connected to a Waters 2465 electrochemical detector
957 (HPLC-ED, Waters, USA) with a glassy carbon working electrode (0.7V, 10nA). BAT was
958 homogenized in an ice-cold solution, containing 0.4% ethylenediaminetetraacetic acid and 0.1M
959 perchloric acid, while urine samples were processed using MonoSpin PBA columns (Interchim,
960 Montlucon, France). After centrifugation, the supernatant was further analyzed with HLPC-ED.
961 Mobile phase (58,5 mM Sodium Acetate (Sigma, St Louis, USA), 0.7mM Octane Sulfonic Acid
962 (Sigma O 0133, St Louis, USA), pH 3,8 for mobile phase A and 100% MeOH for mobile phase B)
963 was pumped at a flow rate of 1ml/min and monoamines and metabolite concentrations were
964 detected at an oxidation potential of 750mV compared to the reference electrode. Compounds
965 were separated by an isocratic flow (90% A / 10% B) using a Kromasil® AIT column (length
966 250mm; internal diameter 4.6mm; particle size 5µm) at 30°C. Catecholamines and metabolites
967 were quantified using LabSolution software (Shimadzu, Kyoto, Japan) by integration of the peak

968 absorbance area, employing a calibration curve established with known catecholamine
969 concentrations.

970
971 **Quantification and statistical analysis**

972 **Statistical analysis**

973 Results are expressed as the mean \pm SEM. All statistical analysis was performed using GraphPad
974 Prism 8 (La Jolla, CA, USA). Data were analyzed by T-test, two-way or one-way ANOVA
975 depending on each experimental design. Pairwise comparisons were evaluated with a Tukey
976 post-hoc test. Blood glucose, plasma insulin, %SO₂ and body temperature values were analyzed
977 by ANOVA of repeated measures with 2 between subject factors: *Time* and *Substance*. An
978 adjustment of Bonferroni was performed with significant values set as $p < 0.05$. All details related
979 to statistical analyses are summarized in Data S1.

980

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