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Dual role of striatal astrocytes in behavioral flexibility and metabolism in the context of obesity

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35 **ABSTRACT**

36

37 Brain circuits involved in metabolic control and reward-associated behaviors are potent
38 drivers of feeding behavior and are both dramatically altered in obesity, a multifactorial
39 disease resulting from genetic and environmental factors. In both mice and human, exposure
40 to calorie-dense food has been associated with increased astrocyte reactivity and pro-
41 inflammatory response in the brain. Although our understanding of how astrocytes regulate
42 brain circuits has recently flourish, whether and how striatal astrocytes contribute in
43 regulating food-related behaviors and whole-body metabolism is still unknown. In this study,
44 we show that exposure to enriched food leads to profound changes in neuronal activity and
45 synchrony. Chemogenetic manipulation of astrocytes activity in the dorsal striatum was
46 sufficient to restore the cognitive defect in flexible behaviors induced by obesity, while
47 manipulation of astrocyte in the nucleus accumbens led to acute change in whole-body
48 substrate utilization and energy expenditure. Altogether, this work reveals a yet
49 unappreciated role for striatal astrocyte as a direct operator of reward-driven behavior and
50 metabolic control.

51

52

53 **KEYWORDS**

54 Astrocytes, behavior, obesity, cognitive flexibility, dorsal striatum, nucleus accumbens,
55 synchrony, metabolism, fatty acid oxidation

56

57 **Introduction**

58 Obesity is a major public health problem, which increases the relative risk of a set of
59 pathological conditions (e.g. heart disease, hypertension, type 2 diabetes, steatosis and
60 some form of cancers) (Must et al., 1999; GBD 2015 Obesity Collaborators et al., 2017).
61 Although both genetic and lifestyle factors thoroughly participate in the development of
62 obesity, the contribution of each factor widely varies from individual to individual. Over
63 consumption of highly rewarding high fat, high sugar diet (HFHS) is definitively an identified
64 culprit. While homeostatic circuits located in the hypothalamic-brainstem axis are potent
65 contributors of feeding behaviors, the rewarding nature of food is another powerful drive of
66 feeding (Berthoud et al., 2017). The rewarding aspect of food involves the release of
67 dopamine (DA) within the cortico-mesolimbic system (Berridge, 1996; Alcaro et al., 2007;
68 Björklund and Dunnett, 2007). Consumption of HFHS enhances DA release within the
69 Nucleus accumbens (NAc) and the dorsal striatum (DS) (Lenoir et al., 2007), which in turn
70 influences the striato-hypothalamic circuits promoting food intake (Kenny, 2011; Kempadoo
71 et al., 2013; O'Connor et al., 2015). Alterations in the DA transmission have been shown to
72 be implicated in addictive/compulsive-like ingestive behaviors as well as altered cognitive
73 flexibility (Yang et al., 2018) and reward processing (Koob and Volkow, 2010), two well-
74 established endophenotypes of overweight individuals, which largely depend on striatal
75 processing. It is therefore suggested that, by hijacking the reward system, exposure to
76 palatable hypercaloric diets can switch feeding from a goal-directed and flexible behavior, to
77 an impulsive (Babbs et al., 2013; Adams et al., 2015), inflexible, and ultimately compulsive-
78 like behavior [see (Wang et al., 2001; Johnson and Kenny, 2010; Kenny, 2011; Michaelides
79 et al., 2012)]. In line with this, increasing evidence support that the development of obesity
80 and obesity-related disorders not only results from metabolic dysregulation, but also from
81 dysfunctions of the fronto-striatal circuit, a main substrate for inhibitory behaviors and
82 cognitive control, which can be altered in response to food and associated cues (Stice et al.,
83 2008; Seabrook et al., 2023). However, the cellular and molecular events that underlie the
84 mal adaptive response of the reward system to obesogenic environment remain elusive.

85 Increasing evidence point to an alteration of astrocytes, the most abundant type of glial cells
86 (García-Cáceres et al., 2019), as a pathophysiological feature of obesity. Astrocytes
87 reactivity, reflected by both morphological and functional remodeling has already been
88 described in the hypothalamus, in response to days or weeks of HFHS exposure, well before
89 fat accumulation and systemic inflammation (Thaler et al., 2012; Clyburn and Browning,
90 2019). Consumption of enriched diet and the excess of adipose tissue further favor
91 inflammatory cascades associated with secretion of pro-inflammatory signals (Thaler et al.,

92 2012), triggering vascular hyper permeability and maladaptation in both microglia and
93 astrocytes (García-Cáceres et al., 2019).

94 Despite the physiological evidence that astrocyte are primary target of caloric dense food, it
95 is yet unclear if they play a dominant role in the cognitive and metabolic defect associated
96 with obesity. In the current study, we show that long-term exposure to HFHS leads to
97 profound changes in striatal astrocytes states and activity, associated with loss of synchrony
98 in neuronal activity and impairs mice reversal learning. Second, we show that selective
99 manipulation of striatal astrocyte through chemogenetic approaches helps reinstate neural
100 network coordination. Third, we identified a neuroanatomical distinction by which activation of
101 astrocytes in the dorsal striatum can directly rescue HFHS diet-induced cognitive dysfunction
102 while manipulating astrocytes activity in the Nucleus accumbens exert a dominant control
103 onto whole-body substrate utilization and energy expenditure.

104 **Results**

105 **High-fat diet-induced obesity leads to reactive astrocytes in both the Nucleus** 106 **Accumbens and the Dorsal Striatum.**

107 Previous studies have demonstrated that HFHS exposure results in reactive astrocytes
108 (Douglass et al., 2017) and alters astrocytic calcium signals in the hypothalamus (Herrera
109 Moro Chao et al., 2022). Anatomical and functional studies have suggested a functional
110 heterogeneity within the striatum, with the ventral striatal regions more likely to be involved in
111 goal directed behaviors, and the dorsal subdivisions rather related to motor control and
112 habits development (Kravitz and Kreitzer, 2012; Lee et al., 2012). Therefore, we explored the
113 distinctive astrocytic adaptations in both the DS and the NAc. Mice were exposed to HFHS
114 for a minimum of 3 months (**Fig-1A**), leading to a significant increase in fat mass compared
115 to chow fed littermates (**Fig-1A**). In both NAc and DS, exposure to HFHS diet enhanced
116 immunoreactivity of the structural protein glial fibrillary acidic protein (GFAP) (**Fig-1B,C,F**), a
117 proxy of increased astrocyte reactivity (Escartin et al., 2021). In HFHS fed mice, the increase
118 in GFAP signal intensity was also accompanied by a decrease in the sphericity of the
119 segmented GFAP positive regions in both the DS and the NAc (**Fig-1D,G**), indicating an
120 effect of HFHS diet on astrocytes morphology. In HFHS-fed groups, the total surface of
121 GFAP staining was significantly increased in the NAc indicating an increase of astrocytic
122 coverage, (**Fig-1E**) while unchanged in the DS (**Fig-1H**). Altogether, these data indicate a
123 functional heterogeneity in the striatal astrocyte response to HFHS diet exposure.

124 **Chemogenetic manipulation of DS astrocytes affects spiny projections neurons**
125 **activity**

126 The finding that HFHS exposure triggers structural and functional changes in striatal
127 astrocytes led us to assess metabolic and behavioral consequences of astrocytic
128 manipulation in the striatum of lean and obese mice. We first probed the consequence of
129 chemogenetic (Designed Receptors Exclusively activated by Designer Drug)-mediated
130 manipulation of DS astrocytes on DA signaling mediated by pharmacological intervention
131 onto dopamine 1 receptor (D1R) and dopamine 2 receptor (D2R). Lean and obese mice
132 expressing the CRE recombinase under the control of the astrocytes-specific promoter
133 Aldehyde dehydrogenase family 1, member L1 (Aldh1l1-Cre) (Cahoy et al., 2008) were
134 stereotactically injected with Cre-dependent pAAV-EF1 α -DIO-hM3Dq-mCherry in the DS
135 allowing for the astrocyte-specific expression of the Gq-coupled receptor (DS^{hM3Dq}) (**Supp.**
136 **Fig-1**). Astrocytic-specific targeting was confirmed by co-immunolocalization of the mCherry
137 signal in striatal astrocytes with the astrocyte's marker GFAP (**Supp. Fig-1A**). Next, to
138 validate the DREADD-induced Ca²⁺ signaling in astrocytes, we co-expressed the Ca²⁺
139 indicator GCaMP6f using Cre-dependent AAV vector. Intraperitoneal injection (IP) of the
140 DREADD ligand Clozapine N-Oxide (CNO, 0.6 mg/kg) led to significant increase of astrocytic
141 Ca²⁺ activity as assessed *in vivo* through fiber photometry recording of DS GCaMP6-based
142 fluorescence (**Supp. Fig-1B, C**). As a functional readout, we observed that Gq-DREADD-
143 mediated manipulation of astrocytes in the in DS astrocyte did not alter hyper locomotion
144 triggered by a single injection of the D1R agonist (SKF-81297) (**Supp Fig-1D**), while the
145 cataleptic effects induced by the D2R antagonist haloperidol (0.5 mg/kg) was significantly
146 decreased in response to the DREADD ligand CNO. Interestingly, this effect was further
147 enhanced in obese mice (**Supp. Fig-1E-F**).

148 **Diet-induced obesity leads to increased temporal correlation of astrocyte Ca²⁺ signals**
149 **in the DS**

150 Next, we investigated how HFHS exposure impacts onto spontaneous astrocytic Ca²⁺
151 dynamics, an important feature of astrocyte signalling (Agulhon et al., 2008; Khakh and
152 McCarthy, 2015). To do so, we used mice expressing the genetically encoded Ca²⁺ sensor
153 GCaMP6f under the astrocyte-specific promoter of the glutamate-aspartate transporter
154 (*Slc1a3*, GLAST) (Glast-GCaMP6f), (Pham et al., 2020; Herrera Moro Chao et al., 2022).
155 Wide-field imaging of striatal astrocytes in acute brain slices of lean and obese Glast-
156 GCaMP6f mice showed that exposure to HFHS does not affect Ca²⁺ spontaneous activity in
157 the DS (**Fig-2A**). However, in the DS, obesity associates with an increased temporal
158 correlation of astrocyte Ca²⁺ signals, as showed by calculating the paired Pearson's

159 coefficient, a correlation coefficient between individual Ca^{2+} signals reflecting signal
160 synchronicity. While the distribution of temporal correlation of Ca^{2+} signals from all active
161 domains appeared bimodal in lean mice, suggesting that astrocyte Ca^{2+} signals are
162 segregated into two populations of asynchronous temporal features, this distribution is right
163 shifted in obese mice indicating increased temporal correlation (**Fig-2B**). These results show
164 that beyond a change of astrocyte Ca^{2+} signaling intensity, exposure to HFHS changes the
165 temporal organization of astrocyte activation. Because astrocytes are tightly linked with
166 synaptic activity, it is likely that this shift also affects neuronal synchronization.

167 **DS astrocytes chemogenetic manipulation in obese mice rescues neuronal** 168 **synchronization**

169 Using brain slice preparation for GCaMP6f monitoring we observed that obesity had little
170 impact on the overall strength of neuronal Ca^{2+} signals (**Supp. Fig-2A**), but significantly
171 decreased temporal correlation pattern of neuronal events and reduced on average their
172 correlation level as compared to lean animals (**Fig-3B**), suggesting that obesity compromised
173 the synchrony in the DS neuronal network. To further explore the impact of obesity on
174 astrocyte-neuron communication in the DS, we examined the effect of astrocytes
175 chemogenetic manipulation on neuronal Ca^{2+} signals *ex vivo*. C57Bl6 mice received a
176 mixture of viral vectors allowing for simultaneous expression of GCaMP6f in neurons (AAV-
177 synapsin-GCaMP6f) and hM3Gq in astrocytes (AAV-GFAP-hM3Gq-mCherry) (**Fig-3A**).

178 Next, we analyzed neuronal time courses during Gq-DREADD astrocyte activation in lean
179 and obese animals. We first validated that coincident activation of neuronal populations can
180 enhance their synchrony. To do so, we used glutamate whose receptors are abundantly
181 expressed in DS neurons (Montalban et al., 2022) and applied a concentration ($30\mu\text{M}$) that
182 targets perisynaptic mGluR and/or NMDA receptors, hence mimicking the activation of
183 glutamate receptors targeted by Gq-DREADD astrocyte activation. Application of glutamate
184 did synchronize the Ca^{2+} events in GCaMP6f-expressing DS neurons, as reflected by
185 simultaneous fluorescence rises (**Supp. Fig-2B, C**) and the right shifted distribution of the
186 temporal correlations (**Supp. Fig-2D, E**).

187

188 **Activation of DS astrocytes rescues neuronal synchronization defect associated with** 189 **obesity**

190

191 We then examined whether Gq-mediated activation of DS astrocytes could modulate
192 neuronal activity profile as assessed by GCaMP6f activity. Bath application of CNO

193 significantly increased the level of temporal correlation between neuronal Ca^{2+} signals,
194 leading to a right shift of the correlation distribution (**Fig-3C-D**). Notably, the impairment of
195 neuronal Ca^{2+} signals synchrony associated with obesity was largely restored by Gq-
196 DREADD-mediated astrocytes activation (**Fig-3D**), along with overall enhancement of
197 neuronal activity (**Fig-3E**). To further confirm this effect, Aldh111-Cre mice were co-injected
198 with Gq-DREADDs or mCherry control viruses ($\text{DS}^{\text{mCherry}}$ and DS^{hM3Dq}) and AAV-synapsin-
199 GCaMP6f to target neurons. As previously observed, the strength (**Supp. Fig-3A-B**) and
200 temporal correlation (**Supp. Fig-3C**) of DS neuronal Ca^{2+} signals were increased by both
201 glutamate bath application and CNO-mediated DREADD manipulation of astrocytes.
202 Together, these results show that the signal synchronization of DS neurons is dampened in
203 obese mice, but can be restored by selective activation of striatal astrocytes.

204

205 **Obesity-associated impairment in cognitive flexibility can be rescued by selective**
206 **activation of striatal astrocyte.**

207 We next explored the functional outcome of DS astrocytes manipulation onto obesity-induced
208 cognitive alteration. Reversal learning is a form of cognitive flexibility highly dependent on to
209 the integrity of the DS and that was shown to be impaired in human and rodent obesity (Foldi
210 et al., 2021; Montalban et al., 2023). Neuroimaging studies in humans show that reversal
211 learning requires the integrity of the ventral prefrontal cortex and the DS (Jocham et al.,
212 2009). Previous studies already showed that activation of astrocytes in the DS facilitate the
213 switch from habitual to goal directed behavior in lean mice in a operant conditioning
214 paradigm (Kang et al., 2020). We first evaluated if DS-dependent flexible behavior was
215 altered in obese mice. To do so, lean and obese mice of matched age were tested in a food-
216 cued T-maze, in which mice learnt to locate the baited arm with no external cues, using an
217 egocentric strategy (Oliveira et al., 1997; Watson and Stanton, 2009; Baudonnat et al., 2013)
218 followed by a reversal learning task, in which locations of the baited and non-reinforced arms
219 are inverted (**Fig-4A**). While no differences were observed during the learning phase, obese
220 mice displayed impaired ability to relearn the new location of the baited arm during reversal
221 task (**Fig-4A**). Their performances did not reach criterion even after 3 sessions of reversal
222 test (**Supp. Fig-4**) whereas lean mice reached 80% of correct choice during the first reversal
223 session (**Fig-4A**).

224 Next, we assessed the consequence of Gq-DREADDs activation of DS astrocyte on reversal
225 learning in lean and obese Aldh111 $\text{DS}^{\text{mCherry}}$ and DS^{hM3Dq} mice. Reversal learning was
226 assessed in response to CNO injection 30 minutes before the first trial of the reversal phase

227 **(Fig-4B, C)**. Importantly, while activation of Gq-DREADD in DS astrocytes in lean mice led to
228 a small though significant increased performance **(Fig-4B)**, CNO injection in obese DS^{hM3Dq}
229 led to an almost complete restoration of reversal learning during the reversal phase **(Fig-4C)**.
230 Our results indicate that DS astrocytes activation during reversal learning was sufficient to
231 restore obesity-induced impairment in cognitive flexibility.

232 **Astrocyte-mediated restoration of flexible behavior in obese mice is associated with**
233 **changes in both neuronal activity and dopamine transmission *in vivo***

234 In order to link the behavioral output with bulk neuronal activity in the DS upon Gq-DREADD
235 astrocytes activation *in vivo*, we recorded neuronal activity using Ca²⁺ sensor coupled with
236 fiber photometry during reversal learning. Our analysis showed that astrocytes activation
237 during reversal learning was accompanied by a small decrease of neuronal activity when the
238 animal enters the baited arm in obese DS^{hM3Dq} as compared to DS^{mCherry} **(Fig-4D-G)**.

239 Several studies indicate that obesity and HFHS exposure enhances DA signaling in both
240 humans (Volkow and Wise, 2005) and rodents (Johnson and Kenny, 2010; Tellez et al.,
241 2013), and recent studies point to a role of astrocytes in regulating the level of DA release in
242 the striatum (Roberts et al., 2022). Since DA transmission regulates behavioral flexibility
243 (Izquierdo et al., 2017), we investigated the role of DS astrocytes in DA transmission during
244 the reversal learning. Obese DS^{mCherry} and DS^{hM3Dq} mice were co-injected with a viral vector
245 bearing the genetically-encoded DA sensor dLight1 in the DS (AAV-CAG-
246 dLight1.1)(Patriarchi et al., 2018). Fiber photometry recording of dLight1-mediated signal was
247 used as a proxy of DA dynamics in the DS. Mice were first recorded during a reversal
248 learning after being injected with vehicle (RV1, **Fig-4H-J**) and, next, during a second reversal
249 learning after being injected with CNO (RV2, **Fig-4H-J**). Our analysis showed that astrocytes
250 activation during reversal learning potentiated DA transmission when the animal entered the
251 baited arm **(Fig-4I, J)**.

252 Overall our data showed that in DS astrocytes activation in obese mice restores reversal
253 learning impairments in relation with i) an overall decrease in neuronal activity and ii) an
254 increase in DA transmission when entering the new-bated arm during reversal learning.

255

256 **Diet induced obesity leads to increased strength and decreased temporal correlation**
257 **of astrocyte Ca²⁺ signals.**

258 Converging evidence point to a central role of the ventral part of the striatum in the regulation
259 of food intake (Sears et al., 2010; O'Connor et al., 2015; Thoeni et al., 2020), glucose
260 metabolism (Ter Horst et al., 2018) and whole body substrate utilization (Montalban et al.,
261 2023). Hence, we next considered a possible role of NAc astrocytes in the physiology and
262 pathophysiology of energy balance in obesity.

263 First, we investigated the effect of HFHS exposure on spontaneous activity of NAc astrocytes
264 in GLAST-GCaMP6f mice. Interestingly, we found that contrarily to the DS, exposure to
265 HFHS led to a significant increase in NAc astrocytic Ca^{2+} spontaneous activity (**Fig-5A**).
266 Moreover, as for the DS, lean mice showed a bimodal Ca^{2+} distribution suggesting that
267 astrocyte Ca^{2+} signals are segregated into two populations of asynchronous temporal
268 features (**Fig-5B**). However, in contrast to the DS, exposure to HFHS diet led to a left
269 monomodal distribution in the NAc, indicating a decrease in astrocytes synchronization (**Fig-**
270 **5B**). We next examined the effect of astrocyte activation in Aldh111-cre mice that received
271 intra NAc delivery of Cre-dependent viral vectors encoding for Gq DREADD and GCaMP6f.
272 We observed a significant increase in NAc astrocyte Ca^{2+} levels following CNO bath
273 application in lean mice (CNO, 10 μ M) (**Supp. Fig-5A, B**). This observation led us to
274 hypothesize that Gq-DREADD-mediated increase of astrocytic Ca^{2+} in the NAc of obese mice
275 would have little effect as compared to stimulation of astrocytes in lean mice. We used
276 pharmacology to assess whether astrocytes manipulation would influence behavioral
277 response to agonist and antagonist of D1R and D2R. We observed that NAc astrocytes
278 activation opposed SKF-81297 (3mg/kg) induced hyperlocomotion in lean mice, while this
279 effect was dampened in obese mice (**Supp. Fig-5D, E**). In contrast to DS, astrocytes
280 activation in the NAc did not trigger any significant effects in the cataleptic response to the
281 D2R antagonist haloperidol (0.5 mg/Kg) (**Supp. Fig-5C**) further supporting segregated
282 function of NAc vs DS astrocyte.

283 **Astrocytes activation in the Nucleus accumbens impacts on peripheral substrate** 284 **utilization**

285 We then assessed changes in metabolic efficiency in lean and obese Aldh111-Cre mice co-
286 injected with Gq-DREADDs or mCherry viruses (NAc^{mCherry} and NAc^{hM3Dq}) and AAV-synapsin-
287 GCaMP6f by monitoring indirect calorimetry in response to CNO-mediated astrocytes
288 manipulation (**Fig-6A**). In lean mice, acute stimulation of NAc astrocytes only marginally
289 affected feeding (**Fig-6B**), but promoted a significant decrease in respiratory exchange ratio
290 (RER, VCO_2/VO_2) indicative of substrate being used with RER=1 for carbohydrate and
291 RER=0.7 for lipids (**Fig-6C**). Correlation studies indicated that such decrease in RER
292 significantly correlated with food consumption in Aldh111 NAc^{Gq} group (**Fig-6D**). In

293 accordance, the calculated whole body fat oxidation (Fat Ox) confirmed that acute activation
294 of astrocytes in the NAc led to a shift towards lipid-based substrate (**Fig-6E**). While whole
295 body metabolism (**Supp. Fig-6**) remained unaffected by activation of DS astrocyte in both
296 lean and obese DS^{mCherry} or DS^{hM3Dq} mice, chemogenetic manipulation of NAc astrocytes also
297 resulted in a decrease of energy expenditure (EE) (**Fig. 6F**). This effect was independent
298 from the mice lean body mass (**Fig-6G**) and locomotor activity, which are not different
299 between groups (**Fig-6H**). In obese mice however, activation of astrocytes in the NAc did not
300 alter either food intake, RER, FatOx or EE (**Fig-6I-L**), further supporting the notion that
301 obesity led to maladaptive response in astrocytic control of metabolism.

302

303 **Discussion**

304

305 In the context of the obesity pandemic, the striatum has attracted increasing attention, as
306 energy-rich diets are known to promote reward dysfunctions by altering DA transmission
307 within both NAc and DS. Such alterations can lead to maladaptive habits formation, food
308 craving, inability to cut down food intake and, ultimately to body weight gain. However, while
309 the role of striatal neurons is actively investigated, the contribution of striatal astrocytes in the
310 development of metabolic defects is still largely overlooked. Here, we tested the hypothesis
311 that i) in a diet-induced obesity paradigm striatal astrocytes could be a major target of
312 nutrient overload and that ii) manipulation of astrocytes in DS or NAc could restore
313 behavioral and metabolic alterations induced by obesity. Consistently, we show that obesity
314 induces anatomically-specific change in astrocyte reactivity characterized by substantial
315 alteration in their morphology in both NAc and DS, recalling the modifications observed in the
316 hypothalamus (Thaler et al., 2012). Further, we showed that HFHS consumption translates in
317 an anatomically restricted change in overall Ca²⁺ strength in NAc astrocytes but not in DS
318 astrocytes. In contrast, while temporal correlation in astrocytes Ca²⁺ events was similar in
319 NAc and DS in lean mice, HFHS exposure led to a shift towards a monomodal Ca²⁺ events
320 distribution in the NAc (decreased synchronization), and a significant increase in temporal
321 correlation as compared to lean mice in the DS. Those findings highlight that the functional
322 heterogeneity of astrocytes may reflect different kind of activations among or within brain
323 regions according to their interactions with different subpopulations of neurons (Khakh and
324 Sofroniew, 2015).

325

326 Obesity is a condition characterized by both metabolic and behavioral alterations. Among the
327 latter, non-flexible behavior is a symptomatic dimension which is well characterized in obese

328 subjects. Here we measured reversal learning, a dimension known to be particularly affected
329 in obese subjects, relying on an egocentric-based strategy, a process highly dependent on
330 the integrity of DS (van Elzelingen et al., 2022), and that requires the integrity of the ventral
331 prefrontal cortex and DS (Jocham et al., 2009). Using chemogenetics, we showed that
332 activation of DS-astrocytes in lean and obese mice facilitate flexible behavior during the
333 reversal learning phase of a T-maze task. In obese mice, astrocyte activation was sufficient
334 to restore learning flexibility during reversal. These data are in line with a key role of the DS
335 astrocytes in the switch from habitual to goal directed behavior (Kang et al., 2020), and
336 highlight a central role of astrocytes for the long-term consequences of obesity. Using both
337 chemogenetics, GCaMP6f and d-Light based imaging of neural activity and DA transmission
338 *in vivo*, we showed that in obese mice the reinstatement of a flexible behavior under
339 astrocytes activation parallels with a general decrease in neuronal activity in the DS together
340 with an increase in DA transmission during the choice phase, i.e. when mice are entering the
341 rewarded arm. Dysfunctional DA transmission is associated to several psychiatric
342 pathologies characterized by alterations in flexible behavior (Insel et al., 2010; van Elzelingen
343 et al., 2022). These data confirm that reestablishing the DA transmission within the DS
344 correlates with a gain in the ability to adapt its behavior (Leroi et al., 2013) and are in line
345 with previous reports showing that astrocytes are active players in DA signaling in the
346 striatum (Martín et al., 2015; Corkrum et al., 2020)

347 In line with this, we found that i) obesity is accompanied with a sharp decrease of Ca^{2+}
348 dynamics synchronization in spiny projection neurons SPNs of the DS, that ii) astrocytes
349 activation can restore neural Ca^{2+} event synchronicity, and that iii) in obese mice glutamate
350 application can mimic chemogenetic activation of astrocytes by restoring neural Ca^{2+} events
351 synchrony. These data extend previous works showing that astrocytes can modulate
352 neuronal networks excitability and switch dynamic states *ex vivo* and *in vivo* (Fellin et al.,
353 2004; Poskanzer and Yuste, 2011, 2016; Oliveira and Araque, 2022). Synchronized activity
354 is a defining feature of the nervous system that correlates with brain functions and behavioral
355 states. Several brain diseases are associated with abnormal neural synchronization (Uhlhaas
356 and Singer, 2006). In the striatum, rearrangement in neuronal synchronization plays a key
357 role in habitual learning (Howe et al., 2011; Thorn and Graybiel, 2014; Smith and Graybiel,
358 2016), hence it is tempting to propose that modulation of synchrony by astrocytes translates
359 into the modifications of behavior that we observed in our experiments. Gq-DREADDs
360 activations and concurrent Ca^{2+} increase can have many consequences on is the so-called
361 the tripartite synapse (Araque et al., 1999). Astrocytes release and uptake neuroactive
362 molecules that could impact both pre- and postsynaptic neuronal functions (Leybaert and
363 Sanderson, 2012; Orellana et al., 2016; Savtchouk and Volterra, 2018)- an effect that have
364 been already suggested in the NAc (D'Ascenzo et al., 2007). Astrocytes are also known to

365 shape synaptic activity and communication by precisely buffering the level of extra synaptic
366 glutamate concentration (Isaacson, 1999; Martin et al., 2012). In this study we observed that
367 in obese but not lean mice, glutamate application increased neuronal synchrony. Since
368 glutamate or CNO application, and CNO glutamate co-application resulted to comparable
369 effects in acute slices from obese mice, a possibility would be that in the DS, obesity would
370 result in a deregulation of glutamate reuptake from astrocytes, an effect that could be
371 rescued by astrocytes activation. Hence, at mechanistic level, our data suggest a central role
372 for astrocyte in controlling neural Ca^{2+} events synchrony and DA transmission. Altered
373 regulation of glutamate in obesity is a mechanism reminiscent of our recent study that
374 depicted a key role for hypothalamic astrocyte in the regulation of neurons firing ability,
375 energy expenditure and glucose metabolism through the control of ambient glutamate
376 (Herrera Moro Chao et al., 2022). We found that obesity was associated with exacerbated
377 astrocyte Ca^{2+} activity and blunted astrocyte-selective excitatory Amino-Acid Transporters
378 (EAATs)-mediated transport of glutamate (Herrera Moro Chao et al., 2022). Since a large
379 portion (~ 80%) of glutamate released is actively recaptured by astrocyte through Glutamate
380 transporter 1 (GLT-1) and EAATs transporters, it is expected that striatal astrocyte will have
381 a key role in the control of glutamate in the striatum. Indeed, it was recently demonstrated
382 that glutamate transporter in the astrocytes was in control of Hebbian plasticity expression in
383 the SPNs in the DS (Valtcheva and Venance, 2016).

384

385 In line with emerging evidence that point at the connection between the DA circuits and
386 metabolic control (Montalban et al., 2023) , we found that, activation of NAc astrocytes in
387 lean mice led to significant shift towards lipids substrate utilization. Given the connection
388 between the NAc and hypothalamic nuclei involved in metabolic control, notably the lateral
389 part of the hypothalamus (LHA) (Stratford and Kelley, 1999; Sears et al., 2010; O'Connor et
390 al., 2015; Thoeni et al., 2020) it is formally possible that NAc astrocytes activity indirectly
391 impede onto a subset of neurons projecting to the LHA, with consequences on hypothalamic
392 control of energy expenditure and lipids metabolism (Farzi et al., 2018). This hypothesis is
393 consistent with previously proposed role for a hypothalamic-thalamic-striatal axis in the
394 integration of energy balance and food reward (Kelley et al., 2005). In obese mice, activation
395 of astrocytes of the NAc failed to affect energy metabolism suggesting impaired astrocyte-
396 neural coupling induced by obesity, possibly through astrocyte over activity. Indeed, our *ex*
397 *vivo* studies showed that Gq-coupled hM3Dq activation in astrocyte leads to increases Ca^{2+}
398 signals similar to the one observed in obese conditions. Therefore, it is tempting to
399 hypothesize that activation of astrocytes in the NAc of lean mice would mimic at least in part
400 some of the obesity metabolic dimensions similarly to what has been observed in
401 hypothalamic astrocyte (Herrera Moro Chao et al., 2022).

402 Our data support the notion that the physiological outcome arising from astrocyte
403 manipulation will strongly depend on the anatomical localization and the way astrocyte
404 interacts with different subpopulations of neurons (Khakh and Sofroniew, 2015). Indeed,
405 while CNO-mediated activation of Gq-coupled DREADD astrocytes in the DS had marginal
406 effect on metabolic efficiency, chemogenetic activation of the NAc astrocyte in lean mice
407 decreased energy expenditure and sustained change in nutrient partitioning interpedently from
408 caloric intake. In the same concept, chemogenetic manipulation of DS astrocytes decreased
409 the cataleptic effects induced by the D2R antagonist haloperidol but did not affect
410 hyperlocomotion response to D1R agonist SKF-81297 suggesting a bias action of astrocyte
411 towards D2R-bearing cells. This result was mirrored in the NAc in which hyperlocomotion
412 response to D1R agonist but not cataleptic response to D2R antagonist was affected by the
413 activation of hM3Dq in the NAc astrocyte. These data directly point at a segregated action of
414 astrocyte in the dichotomic action onto specific neuronal population and DA receptor
415 signaling likely due to the selective and anatomically defined properties of astrocyte-neurons
416 communication. For instance in the DS, two distinct subpopulation of astrocytes have been
417 identified that communicate selectively with D1R or D2R-SPNs (Martín et al., 2015). In
418 addition to this intrinsic diversity in astrocyte-neurons communication, our study highlights
419 that exposure to caloric dense food differently affects astrocyte-neuron communication in
420 NAc and DS. While the consequence of NAc astrocyte activation onto metabolic efficiency
421 observed in lean mice was mitigated by obesity, the cognitive improvement associated with
422 DS astrocyte activation was magnified in obese mice. Here too, the differential impact of high
423 fat feeding might reflect the intrinsic diversity in adaptive response to metabolic signals in DS
424 vs NAc astrocyte, neurons, or both astrocyte-neurons tandem.

425 In conclusion, this study provides a ground for a more astrocentric vision of diet and obesity
426 induced alteration in cognitive and metabolic function and open new therapeutic avenue in
427 which striatal astrocytes could represent potential target to correct behavioral and metabolic
428 diseases. However, in order to fully harvest the therapeutic potential of an astrocytic-specific
429 target strategy there is a critical need to further expand our knowledge in molecular
430 specificity and mechanism that sustain astrocyte-neuron dialogue in both physiological and
431 pathophysiological condition based on their anatomical distribution.

432

433 **Limitations of the study**

434 Due to paucity of tools readily available to characterize DS or NA astrocyte diversity, our
435 study could not provide a more detailed description of the specific features of astrocytes
436 involved in the described mechanism. Further, while changes in astrocytic or neural Ca^{2+}
437 events are indicative of cell response they most likely coexist with other intracellular changes
438 that are not accounted for in our study. Further studies are needed to establish the molecular

439 transmitters, metabolite or metabolic pathways that are engaged in astrocyte-neurons
440 connection and which of them represent the best target to leverage as future strategy to cope
441 for diet-induce metabolic and cognitive disease.

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443 --end--

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445

446 **AUTHORS CONTRIBUTION**

447 E.M. initiated, developed and supervised the project, designed and performed experiments,
448 analyzed and interpreted the data, prepared figures and wrote the original draft. CM
449 supervised and developed the research, designed and performed in vivo experiments,
450 analyzed and interpreted the data, prepared figures and participated in the writing of the
451 manuscript with the help of the co-authors. SHL provided the initial conception of the project,
452 secured and administered funding, provided guidance for experimental design and data
453 interpretation and contributed to the writing of the manuscript with the help of the co-authors.
454 DL designed and performed ex-vivo calcium imaging experiments, analyzed and interpreted
455 the data, prepared figures and participated in the writing of the manuscript. PT contributed to
456 analysis and interpretation of the data and writing of the manuscript. GG contributed to the
457 design and provided inputs to in vivo experiments and discussed the data. DHMC and CP
458 performed ex-vivo calcium imaging experiments, AC, AP, PT contributed to fiber photometry
459 experiments. DHMC, AA, JC, RH, MH, EF contributed to in vivo experiments and
460 immunohistochemistry.

461

462 **ACKNOWLEDGEMENTS**

463 We acknowledge funding supports from l'Agence Nationale de la Recherche (ANR) ANR-19-
464 CE37-0020-02, ANR-20-CE14-0020, and ANR-20-CE14-0025-01 "AstrObesity" and from the
465 Fondation pour la Recherche Médicale (FRM) FRM Project #EQU202003010155. We thank
466 the Université Paris Cité, CNRS, INRAE and Université de Bordeaux. EM was supported by
467 a post-doctoral fellowship from the FRM and awarded by the « Fondation des Treilles ». « La
468 Fondation des Treilles, créée par Anne Gruner-Schlumberger, a pour vocation de nourrir le
469 dialogue entre les sciences et les arts, afin de faire progresser la création et la recherche.
470 Elle accueille des chercheurs et des créateurs dans son domaine des Treilles (Var) www.les-treilles.com ». We thank Olja Kacanski for administrative support, Isabelle Le Parco, Aurélie
471 Djemat, Daniel Quintas, Magguy Boa Ludovic Maingault and Angélique Dauvin for animals'
472 care and Florianne Michel for genotyping. We acknowledge the technical platform Functional
473 and Physiological Exploration platform (FPE) of the Université Paris Cité, CNRS, Unité de
474

475 Biologie Fonctionnelle et Adaptative, F-75013 Paris, France, the viral production facility of
476 the UMR INSERM 1089 and the animal core facility “Buffon” of the Université Paris
477 Cité/Institut Jacques Monod. We thank the animal facility of IBPS of Sorbonne Université,
478 Paris.

479

480 **DECLARATION OF INTEREST**

481 "The authors declare no competing interests"

482

483 **FIGURES LEGENDS**

484 **Figure-1 DIO increases glial-fibrillary acidic protein (GFAP) immunoreactivity in**
485 **the DS and the NAc. A Left**-Schematic representation of the protocol. **Right**-After
486 190 days of HFHS diet obese mice showed a significant increase in fat mass as
487 compared to lean. Unpaired Mann-Whitney test **** $p < 0.0001$, $n = 24$ **B** Confocal
488 images representative of GFAP immunoreactivity in the DS and the NAc of lean and
489 DIO mice. **C-H** In the NAc and the DS, DIO increases relative expression of GFAP
490 immunoreactivity compared to lean (**C, NAc; F, DS**). DIO also results in a decrease
491 of astrocytes sphericity (**D-NAc**, unpaired t-test $p = 0.0064$ /**G-DS** unpaired t-test
492 $p < 0.0001$). Total surface of astrocytic coverage was decreased by DIO in NAc, **E-**
493 **NAc**, unpaired t-test $p < 0.0001$, while was left unchanged in the DS **H-DS** unpaired t-
494 test $p = 0.2496$). All data are expressed as mean \pm SEM. $n = 24$, 6 mice in each group.
495 **I-L** DIO increases Ca^{2+} strength and decreases the overall temporal correlation of
496 astrocyte Ca^{2+} signal intensity in Glast-GCaMP6 mice expressing GCaMP
497 selectively under the GLAST promoter (For NAc, Lean: $n = 643$ active regions, 24
498 slices, 6 mice; Obese: $n = 586$ active regions, 19 slices, 4 mice; for DS, in Lean, $n =$
499 204 active regions, 11 slices, 4 mice; Obese, $n = 178$ active regions, 8 slices, 3
500 mice).

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502
503 **Figure-2 DIO alter region specific astrocytic Ca^{2+} activity in the DS.** DIO
504 decreases the overall temporal correlation of astrocyte Ca^{2+} signal intensity in Glast-
505 GCaMP6 mice expressing GCaMP selectively under the GLAST promoter in the DS.
506 Lean, $n = 204$ active regions, 11 slices, 4 mice; Obese, $n = 178$ active regions, 8
507 slices, 3 mice. **A.** Representative pseudo-images of the GCaMP6 fluorescence
508 projection of the spontaneous Ca^{2+} activity in the NAc of lean (top) and obese
509 (middle) mice. Histogram data (bottom) are expressed as mean \pm SEM. Scale bar:
510 $20 \mu m$ **B.** Distribution of temporal correlations of Ca^{2+} responses of all paired active
511 domains (as an estimation of global synchronization) in lean (top) and obese
512 (middle). Overall Ca^{2+} strength data (bottom) are expressed as mean \pm SEM.

513
514
515 **Figure-3 Activation of astrocytes augments neuronal activity synchrony in the**
516 **DS in obese mice.**

517 **A.** Neuronal spontaneous activity was recorded by Ca^{2+} imaging with GCaMP6. *Left*,
518 temporal projection of GCaMP6 fluorescence, scale bar, $20 \mu m$; *middle*, identified regions
519 displaying Ca^{2+} oscillations; *right*, raster plot showing GCaMP6 fluorescence fluctuations
520 over time indicating the spontaneous Ca^{2+} signals. Scale bar, $50 \mu m$. Temporal bar, 10 s. **B.**
521 Distribution of temporal correlation of neuronal Ca^{2+} signal between lean and obese mice.
522 Temporal correlation was derived from the Pearson's correlation coefficients calculated
523 between all pairs of individual Ca^{2+} signals. 6131 signal pairs for four mice for lean, and
524 11796 pairs from three mice for obese condition. Wilcoxon rank sum (Mann-Whitney) test, p
525 $= 1.65 \times 10^{-6}$, $h = 1$, stats = [zval: 18.58, ranksum: 6.1067×10^7]. **C.** Distribution of the
526 temporal correlation of neuronal Ca^{2+} signals in response to astrocyte Gq DREADD
527 activation in lean mice (9199 signal pairs from three mice). Lean and obese as referenced
528 from **B.** **D.** In obese mice, astrocyte Gq DREADD activation by CNO enhanced the temporal
529 correlation (synchrony) of neuronal Ca^{2+} signals (20326 signal pairs from three mice; $p =$
530 6.97×10^{-125} , $h = 1$, stats = [zval: -23.7691, ranksum: 1.7042×10^8]). **E.** Activation of

531 astrocyte Gq DREADD enhances neuronal Ca^{2+} intensity in lean ($p = 1.06 \times 10^{-24}$, $h = 1$,
532 stats = [zval: -10.2608, ranksum: 4438]) and in obese (ranksum, $p = 0.032$, $h = 1$, stats =
533 [zval: -2.1403, ranksum: 82174]) mice. The Ca^{2+} strength derived from normalized temporal
534 integral was compared for control (pre CNO) and CNO application phase (Lean, 89
535 responsive regions, 3 slices, 3 mice; Obese: 294 regions, 5 slices, 3 mice).

536

537 **Figure-4 Effect of DS astrocytes activation on the reversal learning in a T-maze**
538 **paradigm in Aldh1l1-cre lean and obese mice.** A) Right behavioral paradigm. Left
539 Performances of $\text{Aldh1l1}^{\text{DS-mCherry}}$ lean and obese mice were compared for learning
540 and reversal learning skills. A significant between group difference in the reversal
541 phase indicate a decreased flexibility in obese as compare to lean mice. Reversal
542 phase, two-way ANOVA Column Factor $F(1, 14) = 33.80$ $P < 0.0001$ Data are
543 expressed as mean \pm SEM. $n = 8$. **B.** $\text{DS}^{\text{mCherry}}$ and DS^{hM3Dq} lean mice were trained in
544 a T-maze and injected with CNO before the reversal phase. CNO injections slightly
545 increases flexibility in DS^{hM3Dq} mice as compared to control. Group Factor $F(1, 27) =$
546 5.125 $P = 0.0318$, Data are expressed as mean \pm SEM. $n = 14-15$. **C** Astrocytes
547 activation before the reversal phase in obese DS^{hM3Dq} mice restore the behavioral
548 performances. Two-way ANOVA: Group Factor $F(1, 12) = 35.54$ $P < 0.0001$ Data are
549 expressed as mean \pm SEM. $n = 7$. **D-G** Neuronal Ca^{2+} activity was evaluated during
550 reversal learning by fiber photometry in the DS of $\text{DS}^{\text{mCherry}}$ and DS^{hM3Dq} mice co-
551 injected with a virus expressing GCaMP6f in DS neurons. Each mouse was injected
552 with CNO 30 minutes before the test and recorded during the T-maze session. **D,F**
553 Peri-event heat map of the single trials of $\text{DS}^{\text{mCherry}}$ and DS^{hM3Dq} mice respectively,
554 aligned to the time when mice attained the baited arm. **E,G** Plot of area under the
555 curve (AUC) during the baited arm exploration vs before turning in the baited arm (4 s
556 each, indicated by horizontal grey bars) in mice treated with CNO ($n = 16$ and 39
557 trials for $\text{DS}^{\text{mCherry}}$ and DS^{hM3Dq} mice respectively). Statistical analysis two-tailed
558 Mann-Whitney test, $p = 0.74$ for $\text{DS}^{\text{mCherry}}$ and $p = 0.0126$ for DS^{hM3Dq} mice. **H-J** DA
559 transmission was evaluated by fiber photometry during reversal learning in DS of
560 $\text{DS}^{\text{mCherry}}$ and DS^{hM3Dq} mice co-injected with a virus expressing dLight-1 in DS
561 neurons. Each mouse was recorded twice with an interval ≥ 1 day (Reversal day 1
562 and Reversal day 2), 30 min after receiving either vehicle (**Veh**) or misoprostol (**CNO**,
563 $0.06 \text{ mg} \cdot \text{kg}^{-1}$, i.p.). **H,I** Peri-event heat map of single trials of mice injected with Veh or
564 CNO aligned to the time when mice attained the baited arm. Plot of area under the
565 curve (AUC) during the baited arm exploration minus the AUC before turning in the
566 baited arm (4 s, horizontal bars) in mice treated with Veh (RV1) vs CNO (RV2),
567 Statistical analysis two-tailed Mann-Whitney test, $p = 0.0360$ ($n = 16$ and 29 trials for
568 Veh and CNO respectively).

569

570 **Figure-5 DIO increases Ca^{2+} strength and decreases the overall temporal**
571 **correlation of astrocyte Ca^{2+} signal intensity** in Glast-GCaMP6 mice expressing
572 GCaMP selectively under the GLAST promoter in the NAc. Lean: $n = 643$ active
573 regions, 24 slices, 6 mice; Obese: $n = 586$ active regions, 19 slices, 4 mice. **A**
574 Coronal brain slice showing the colocalization of GFP signal (green) and S100b (red)
575 immunostaining in GCaMP-GLAST mice. **B.** Representative pseudo-images of the
576 GCaMP6 fluorescence projection of the spontaneous Ca^{2+} activity in the NAc of lean
577 (top) and obese (middle) mice. Histogram data (bottom) are expressed as mean \pm /
578 SEM. **C.** Distribution of temporal correlations of Ca^{2+} responses of all paired active
579 domains (as an estimation of global synchronization) in lean (top) and obese
580 (middle). Overall Ca^{2+} strength data (bottom) are expressed as mean \pm SEM.

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Figure-6: Metabolic consequences of chemogenetic activation of Gq signaling in astrocytes of the NAc in lean and obese mice. **A.** Schematic representation of the DIO paradigm. Astrocytes activation in in NAc^{hM3Dq} mice fed with chow diet does not alter food intake **B** but decreases respiratory exchange ratio (RER) in NAc^{hM3Dq} mice fed with chow diet **C.** RER correlate with caloric intake for both NAc^{mCherry} and NAc^{hM3Dq} mice **D.** Astrocytes activation in lean NAc^{hM3Dq} mice increases fatty acid oxidation **E** and decreases energy expenditure (EE) **F.** EE does not correlate with LBM in neither NAc^{mCherry} mice or NAc^{hM3Dq} mice. **G.** Locomotion is not impacted by astrocytes activation **H.** Metabolic parameters are not impacted by CNO injection in obese NAc^{mCherry} and NAc^{hM3Dq} mice fed with HFHS diet. Cumulative caloric intake, FatOx, energy expenditure (EE) and respiratory exchange ratio (RER) are not significantly modified by CNO injection **I-L.** (N = 6 mice each group; VEH: vehicle).

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- 793
794

795 **METHODS**

796

797 **Experimental models and subject details**

798 **Animal studies**

799 All animal protocols were approved by the Animal Care Committee of the University of Paris
800 (APAFIS #2015062611174320), or the Institut Biologie Paris Seine of Sorbonne University
801 (C75-05-24). Twelve to fifteen-week-old male Aldh1-L1-Cre (Tg(Aldh111-cre) JD1884Htz,
802 Jackson laboratory, Bar Harbor, USA), male C57BL/6J (Janvier, Le Genest St-Isle, France)
803 or male GCaMP6f/Glast-CreERT2 (Pham et al., 2020) mice were individually housed at
804 constant temperature (23± 2°C) and submitted to a 12/12h light/dark cycle. All mice had
805 access to regular chow diet (Safe, Augy, France) and water ad libitum, unless stated
806 otherwise. Additionally, age matched C57BL/6J, GCaMP6f/Glast-CreERT2 or Aldh1-L1-Cre
807 mice groups were fed with either chow diet or high-fat high-sugar diet (HFHS, cat n. D12451,
808 Research Diets, New Brunswick, USA) for twelve to sixteen weeks. Body weight was
809 measured every week and body weight gain was estimated as the difference of body weight
810 in week one of HFHS diet consumption to twelve to sixteen weeks after HFHS diet exposure.

811

812 **Viral constructs**

813 Designer receptor exclusively activated by designer drugs (DREADD) and GCaMP6f viruses
814 were purchased from <http://www.addgene.org/>, unless stated otherwise. pAAV-EF1α-DIO-
815 hM3Dq-mCherry (2.4x10¹² vg/ml, Addgene plasmid #50460-AAV5;
816 <http://www.addgene.org/50460/>; RRID: Addgene_50460), pAAV-EF1α-DIO-mCherry
817 (3.6x10¹² vg/ml, Addgene plasmid #50462-AAV5; <http://www.addgene.org/50462/>; RRID:
818 Addgene_50462), pAAV-EF1a-DIO-hM3D(Gq)-mCherry was a gift from Bryan Roth
819 (Addgene plasmid # 50460; <http://n2t.net/addgene:50460>; RRID: Addgene_50460). pAAV-
820 CAG-Flex.GCaMP6f.WPRE (3.15x10¹³ vg/ml, working dilution 1:10, Addgene plasmid
821 #100835-AAV5; <http://www.addgene.org/100835/>; RRID:Addgene_100835) was a gift of
822 Douglas Kim and GENIE Project. pAAV-GfaACC1D.Lck-GCaMP6f.SV40 (1.53x10¹³ vg/ml,
823 working dilution 1:5, Addgene plasmid #52925-AAV5; <http://www.addgene.org/52295/>; RRID:
824 Addgene_52925) was a gift of Baljit Khak. pAAV-CAG-dLight1.1 was a gift from Lin Tian
825 (Addgene viral prep # 111067-AAV5; <http://n2t.net/addgene:111067>; RRID:
826 Addgene_111067)

827

828 **Surgical procedures**

829 For all surgical procedures, mice were first intraperitoneal (ip) injected with the analgesic
830 Buprenorphine (Buprecare, 0.3 mg/kg, Recipharm, Lancashire, UK). 30 minutes after the
831 injection mice were rapidly anesthetized with isoflurane (3%), intraperitoneal (ip) injected with

832 the analgesic Buprenorphine (Buprecare, 0.3 mg/kg, Recipharm, Lancashire, UK) and
833 Ketoprofen (Ketofen, 10 mg/kg, France) and maintained under 1.5% isoflurane anesthesia
834 throughout the surgery.

835 Stereotaxic surgery. Male *Aldh1-L1-Cre*^{+/-}, *Aldh1-L1-Cre*^{-/-} and male C57BL/6J mice were
836 placed on a stereotaxic frame (David Kopf Instruments, California, USA) and bilateral viral
837 injections were performed with 0.6ul in DS (stereotaxic coordinates: L = +/-1.75; AP = +0.6; V
838 = -3.5, and -3 in mm), or 0.3ul in NAc (L=+/- 1; AP=+1.55, V=-4.5) at a rate of 50 nl.min⁻¹.
839 The injection needle was carefully removed after 5 min waiting at the injection site and 2 min
840 waiting half way to the top. Mice recovered for at least 3 weeks after the surgery before being
841 involved in experimental procedures.

842

843 **Behavioral assays**

844 **Haloperidol-induced catalepsy.** Mice were injected with haloperidol (0.5 mg.kg⁻¹, i.p.).
845 Catalepsy was measured at several time points, 45-180 min after haloperidol injection.
846 Animals were taken out of their home cage and placed in front of a 4-cm elevated steel bar,
847 with the forelegs upon the bar and hind legs remaining on the ground surface. The time
848 during which animals remained still was measured. A behavioral threshold of 180 seconds
849 was set so the animals remaining in the cataleptic position for this duration were put back in
850 their cage until the next time point.

851 **T-maze.** Mice were tested for learning and cognitive flexibility in a gray T maze (arm 35-cm
852 length, 25-cm height, 15-cm width). All mice were mildly food deprived (85-90 % of original
853 weight) for 3 days prior to starting the experiment. The first day mice were placed in the
854 maze for 15 min for habituation. Then, mice underwent 3 days of training with one arm
855 reinforced with a highly palatable food pellet (HFHS, cat n. D12451 Research Diet). Each
856 mouse was placed at a start point and allowed to explore the maze. It was then blocked for
857 20 seconds in the explored arm and then placed again in the starting arm. This process was
858 repeated 10 times per day. At the end of the learning phase all mice showed a > 70 %
859 preference for the reinforced arm. The average number of entries in each arm over 5 trials
860 was plotted. Two days of reversal learning followed the training phase during which the
861 reinforced arm was changed and the mice were subjected to 10 trials per day with the reward
862 in the arm opposite to the previously baited one.

863 **SKF-induced locomotor activity.** Mice were placed in an automated online measurement
864 system using an infrared beam-based activity monitoring system (Phenomaster, TSE
865 Systems GmbH, Bad Homburg, Germany). After 1 day of habituation, mice were first i.p.
866 injected with CNO (0.6 mg/Kg) and 30 minutes after with SKF-81297 (3 mg/kg), and placed
867 back in the chamber for at least 80 minutes. Locomotion was recorded using an infrared

868 beam-based activity monitoring system Phenomaster, TSE Systems GmbH, Bad Homburg,
869 Germany).

870

871 **Fiber photometry**

872 Aldh1-L1-Cre mice were anaesthetized with isoflurane and received 10 mg.kg⁻¹
873 intraperitoneal injection (i.p.) of Buprécare® (buprenorphine 0.3 mg) diluted 1/100 in NaCl 9
874 g.L⁻¹ and 10 mg.kg⁻¹ of Ketofen® (ketoprofen 100 mg) diluted 1/100 in NaCl 9 g.L⁻¹, and
875 placed on a stereotactic frame (Model 940, David Kopf Instruments, California). We
876 unilaterally injected 0.6 µl of virus (pAAV.Syn.Flex.GCaMP6f.WPRE.SV40, Addgene viral
877 prep #100833-AAV9, titer ≥ 10¹³ genome copy (GC).mL⁻¹, working dilution 1:5) or d-Light1
878 (pAAV-CAG-dLight1.1, Addgene viral prep # 111067-AAV5, titer ≥ 7×10¹² vg/mL, working
879 dilution 1:1) into the DS (L = +/-1.5; AP = +0.86; V = -3.25, in mm) at a rate of 50 nl.min⁻¹.
880 The injection needle was carefully removed after 5 min waiting at the injection site and 2 min
881 waiting half way to the top. Optical fiber for calcium imaging into the striatum was implanted
882 100 µm above the viral injection site. A chronically implantable cannula (Doric Lenses,
883 Québec, Canada) composed of a bare optical fiber (400 µm core, 0.48 N.A.) and a fiber
884 ferrule was implanted 100 µm above the location of the viral injection site in the DS (L = +/-
885 1.75; AP = +0.6; V = -3.5, and -3 in mm). The fiber was fixed onto the skull using dental
886 cement (Super-Bond C&B, Sun Medical). Real time fluorescence emitted from the calcium
887 sensor GCaMP6f expressed by astrocytes with the Aldh1-L1-Cre receptor was recorded
888 using fiber photometry as described in (Berland et al., 2020). Fluorescence was collected in
889 the DS using a single optical fiber for both delivery of excitation light streams and collection
890 of emitted fluorescence. The fiber photometry setup used 2 light emitting LEDs: 405 nm LED
891 sinusoidally modulated at 330 Hz and a 465 nm LED sinusoidally modulated at 533 Hz (Doric
892 Lenses) merged in a FMC4 MiniCube (Doric Lenses) that combines the 2 wavelengths
893 excitation light streams and separate them from the emission light. The MiniCube was
894 connected to a fiber optic rotary joint (Doric Lenses) connected to the cannula. A RZ5P lock-
895 in digital processor controlled by the Synapse software (Tucker-Davis Technologies, TDT,
896 USA), commanded the voltage signal sent to the emitting LEDs via the LED driver (Doric
897 Lenses). The light power before entering the implanted cannula was measured with a power
898 meter (PM100USB, Thorlabs) before the beginning of each recording session. The light
899 intensity to capture fluorescence emitted by 465 nm excitation was between 25-40 µW, for
900 the 405 nm excitation this was between 10-20 µW at the tip of the fiber. The fluorescence
901 emitted by the GCaMP6f activation in response to light excitation was collected by a
902 femtowatt photoreceiver module (Doric Lenses) through the same fiber patch cord. The
903 signal was then received by the RZ5P processor (TDT). On-line real time demodulation of
904 the fluorescence due to the 405 nm and 465 nm excitations was performed by the Synapse

905 software (TDT). A camera was synchronized with the recording using the Synapse software.
906 Signals were exported to MATLAB R2016b (Mathworks) and analyzed offline. After careful
907 visual examination of all trials, they were clean of artifacts in these time intervals. The timing
908 of events was extracted from the video. For each session, signal analysis was performed on
909 two-time intervals: one extending from -4 to 0 sec (before entering the reinforced arm) and
910 the other from 0 to +4 sec (reinforced arm). From a reference window (from -180 to -60 sec),
911 a least-squares linear fit was applied to the 405 nm signal to align it to the 465 nm signal,
912 producing a fitted 405 nm signal. This was then used to calculate the $\Delta F/F$ that was used to
913 normalize the 465 nm signal during the test window as follows: $\Delta F/F = (465 \text{ nm signal}_{\text{test}} -$
914 $\text{fitted } 405 \text{ nm signal}_{\text{ref}})/\text{fitted } 405 \text{ nm signal}_{\text{ref}}$. To compare signal variations between the two
915 conditions (before vs after entering the reinforced arm), for each mouse, the value
916 corresponding to the entry point of the animal in the reinforced arm was set at zero.

917

918 **Indirect calorimetry analysis**

919 All mice were monitored for metabolic efficiency (Labmaster, TSE Systems GmbH, Bad
920 Homburg, Germany). After an initial period of acclimation in the calorimetry cages of at least
921 two days, food and water intake, whole energy expenditure (EE), oxygen consumption and
922 carbon dioxide production, respiratory quotient ($RQ=VCO_2/VO_2$, where V is volume) and
923 locomotor activity were recorded as previously described⁸³. Additionally, fatty acid oxidation
924 was calculated as previously reported⁸³. Reported data are the results of the average of the
925 last three days of recording. Before and after indirect calorimetry assessment, body mass
926 composition was analyzed using an Echo Medical systems' EchoMRI (Whole Body
927 Composition Analyzers, EchoMRI, Houston, USA).

928

929 **Ex-vivo calcium imaging**

930 Male Aldh1-L1-Cre^{+/+} or C57BL/6J mice previously injected with GCaMP6f and DREADDs
931 viral constructs, and GCaMP6f/Glast-CreERT2 mice were terminally anaesthetized using
932 isoflurane. Brains were removed and placed in ice-cold oxygenated slicing artificial
933 cerebrospinal solution (aCSF, 30mM NaCl, 4.5mM KCl, 1.2mM NaH₂PO₄, 1mM MgCl₂,
934 26mM NaHCO₃, and 10mM D-Glucose and 194mM Sucrose) and subsequently cut into 300-
935 μm thick PVN coronal slices using a vibratome (Leica VT1200S, Nussloch, Germany). Next,
936 brain slices were recovered in aCSF (124mM NaCl, 4.5mM KCl, 1.2mM NaH₂PO₄, 1mM
937 MgCl₂, 2mM CaCl₂, 26mM NaHCO₃, and 10mM D-Glucose) at 37 °C for 60 minutes.
938 Imaging was carried out at room temperature under constant perfusion (~3 ml/min) of
939 oxygenated aCSF. The overall cellular fluorescence of astrocytes expressing GCaMP6f was

940 collected by epifluorescence illumination. A narrow-band monochromator light source
941 (Polychrome II, TILL Photonics, Germany) was directly coupled to the imaging objective via
942 an optical fiber. Fluorescence signal was collected with a 40x 0.8NA or a 63x 1.0NA water
943 immersion objective (Zeiss, Germany) and a digital electron-multiplying charge-coupled
944 device (EMCCD Cascade 512B, Photometrics, Birmingham, UK) as previously described
945 (Pham et al., 2020)(Pham, 2020). A double-band dichroic/filter set was used to reflect the
946 excitation wavelength (470 nm) to slices and filter the emitted GCaMP6 green fluorescence
947 (Di03-R488/561-t3; FF01-523/610, Semrock). The same filter was used for slices expressing
948 both GCaMP6f and DREADD-mCherry. Striatal slices were transferred to the imaging
949 chamber, where 3-minute astrocyte spontaneous activity recordings were performed in slices
950 of GCaMP6f/Glast-CreERT2 mice. In the case of striatal slices of Aldh1-L1-Cre^{+/-} and
951 C57BL/6J mice, we performed a basal epifluorescence recording (60 seconds), followed by a
952 120 second bath application of CNO (10 μ M) or Glutamate (30 μ M) and 240 seconds recording
953 over the washing of the compounds.

954 The responsive regions displaying Ca²⁺ signals were scrutinized by the three-dimensional
955 spatio-temporal correlation screening method (Pham et al., 2020). Background signal was
956 subtracted from the raw images by using the minimal intensity projection of the entire stack.
957 Ca²⁺ signals of individual responsive regions were normalized as dF/F₀, with F₀ representing
958 the baseline intensity and quantified using Matlab (The MathWorks, France) and Igor Pro
959 (Wavemetrics, USA). We gauged signal strength of Ca²⁺ traces of single responsive regions
960 by calculating their temporal integration and normalizing per minute. The global temporal
961 synchronization of detected Ca²⁺ signals was determined by the temporal Pearson's
962 correlation coefficients of all combinations between single Ca²⁺ regions (Pham et al., 2020).

963

964 **Brain tissue Immunofluorescence**

965 Mice were euthanized with pentobarbital (500 mg/kg, Dolethal, Vetoquinol, France) and
966 transcardially perfused with 0.1 M sodium phosphate buffer (PBS, pH 7.5) followed by 4%
967 paraformaldehyde in phosphate buffer (0.1 M, pH 7.2). Brains were removed and post-fixed
968 overnight in 4% paraformaldehyde. Afterwards, the brains were transferred to 30% sucrose
969 in PBS for 2 days for cryoprotection. Next, 30 μ m brain sections were cut in a freezing
970 cryostat (Leica, Wetzlar, Germany) and further processed for immunofluorescence following
971 the procedure previously described (Berland et al., 2020). Free-floating brain sections were
972 incubated at 4°C overnight with mouse anti-Glial fibrillary acidic protein (GFAP, 1:1000,
973 Sigma-Aldrich, Saint-Louis, USA) or mCherry (ab125096; 1:1000, Abcam, Cambridge, MA)
974 primary antibodies. The next day, sections were rinsed in Tris-buffered saline (TBS, 0.25M
975 Tris and 0.5M NaCl, pH 7.5) and incubated for 2 hours with secondary antibodies (1:1000,
976 Thermo fisher Scientific, MA, USA) conjugated with fluorescent dyes: goat anti-chicken Alexa

977 488, donkey anti-rabbit Alexa 594, donkey anti-mouse Alexa 488 and donkey anti-rabbit
978 Alexa 647. After rinsing, the sections were mounted and coverslipped with DAPI
979 (Vectashield, Burlingade, California, USA) and examined with a confocal laser scanning
980 microscope (Zeiss LSM 510, Oberkochen, Germany) with a color digital camera and
981 AxioVision 3.0 imaging software.

982

983 **Statistical analyses**

984 Compiled data are always reported and represented as mean \pm s.e.m., with single data
985 points plotted. Data were statistically analyzed with GraphPad Prism 9. Normal distribution
986 was tested with Shapiro-Wilk test. When n was > 7 and normality test passed, data were
987 analyzed with Student's t test, one-way ANOVA, two-way ANOVA or repeated-measures
988 ANOVA, as applicable and Holm-Sidak's post-hoc tests for two by two comparisons.
989 Otherwise non-parametric Mann-Whitney test. All tests were two-tailed. Significance was
990 considered as $p < 0.05$.











