

Food craving-like episodes during pregnancy are mediated by accumbal dopaminergic circuits

Roberta Haddad-Tóvolli, Sara Ramírez, Emma Muñoz-Moreno, Maria Milà-Guasch, Lluis Miquel-Rio, Macarena Pozo, Iñigo Chivite, Jordi Altirriba, Arnaud Obri, Alicia Gómez-Valadés, et al.

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1	Food craving-like episodes during pregnancy are mediated by accumbal
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34 Preparation for motherhood requires a myriad of physiological and behavioural 35 adjustments throughout gestation in order to provide an adequate environment for proper 36 embryonic development¹. Cravings for high-palatable foods are highly prevalent during 37 pregnancy² and contribute to the maintenance and development of gestational overweight or 38 obesity³. However, the neurobiology underlying the distinct ingestive behaviours that result 39 from craving specific foods remain unknown. Here we show that mice, similar to humans, experience gestational food craving-like episodes. These episodes are associated with a brain 40 41 connectivity reorganization that affects key components of the dopaminergic mesolimbic 42 circuitry, which drives motivated appetitive behaviors and facilitates the perception of 43 rewarding stimuli. Pregnancy engages a dynamic modulation of dopaminergic signalling through neurons expressing dopaminergic receptors D2R in the Nucleus Accumbens, which 44 45 directly modulate food craving-like events. Importantly, persistent maternal food cravinglike behaviour has long-lasting effects on the offspring, particularly in males, leading to 46 glucose intolerance, increased body weight, and increased susceptibility to develop eating 47 48 disorders and anxiety-like behaviors during adulthood. Our results reveal the cognitively 49 motivated nature of pregnancy food cravings and advocates for moderating emotional eating 50 during gestation in order to prevent deterioration of the offspring's neuropsychological and 51 metabolic health.

Pregnancy demands multiple physiological and behavioural adaptations to ensure a healthy embryonic development and preparation for maternal care¹. Among them, metabolic and eating behaviour changes have been reported in diverse species^{4–6}. A distinct example are food cravings, which are common features of human pregnancy, resulting in notable variations in maternal ingestive patterns towards consumption of high-palatable food (HPF)². Problematically, recurrent food cravings contribute to the development and maintenance of gestational overweight/obesity³, 58 59

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with potential adverse health consequences for the offspring^{7–9}. However, despite the widespread occurrence of gestational food cravings and having been documented for centuries¹⁰, its underlying neurobiology remains unknown.

61 To verify the suitability of the mouse as a model to investigate pregnancy-characteristic 62 food craving-like behaviour, we determined their drive to overconsume sweet-tasting items which are predominant cravings during human gestation². We exposed C57BL/6 virgin and pregnant 63 mice to a 2-bottle choice paradigm (Fig. 1a). To ensure a dynamic window of detectable preference 64 65 changes, we identified the lowest sucralose and sucrose concentration that did not shift tastant 66 selection in virgin females (Extended Data Fig. 1a, b, Fig. 1b, d). Remarkably, pregnant mice 67 exhibited a higher inclination for both sweet compounds, suggesting increased sweetness sensitivity (Fig. 1b-e). Gestation did not disrupt the perception of the nutritional value of sugar 68 since overall food intake was compensated (Extended Data Fig. 1c). To mimic human food 69 70 cravings, we examined the motivation of pregnant mice to consume pleasant diet in a "limited 71 access" paradigm¹¹ (Fig. 1f, Extended Data Fig. 1d). Pregnant females increased craving-like 72 behaviour and daily food intake from the second week of pregnancy (Fig. 1g, Extended Data Fig. 73 1e). These results recapitulate the accentuated ingestion of palatable food observed during human 74 gestation, thus validating the mouse as an appropriate model.

A prevalent hypothesis posits that gestational food-cravings are necessary to support embryonic growth¹². To test this, we generated pseudopregnant females thus mimicking gestational features without actual embryonic development. Pseudopregnancy was validated by the presence of vaginal plugs and increased progesterone levels (Extended Data Fig. 1f). Pseudopregnant mice exhibited enhanced sweet sensitivity (Fig. 1b-e) and exacerbated compulsive eating similarly as genuinely-pregnant counterparts (Fig. 1g), without changes in daily food intake 81 (Extended Data Fig. 1e). These results suggest that pregnancy food craving-like behaviour does
82 not arise to support embryonic growth.

Anxiety-like states can also promote compulsive eating¹³. Therefore, we questioned if gestation was associated with anxiety-like phenotypes that could alter eating patterns. Pregnant females showed unchanged exploratory time in the anxiety-related central area and total distance in the open field test, as well as equivalent performance in the dark-light box test when compared to virgin females (Extended Data Fig. 1g-j). Although not exhaustive, our results suggest that the distinct ingestive habits during gestation are not caused by major anxiety-related states.

89 Appetite and dietary patterns are controlled by a coordinated network of multiple and distributed neuronal circuits^{14,15}. Thus, we reasoned that deviations in food consumption patterns 90 91 during pregnancy may have a neural basis. To assess the female mouse brain resting-state 92 architecture and uncover temporal features of the pregnant functional connectivity, we conducted 93 functional Magnetic Resonance Imaging (fMRI) before, during and after pregnancy (Fig. 2a). The 94 resting-state network was obtained by independent component analysis set to examine 20 95 independent components. Amongst them, 13 functional networks were identified¹⁶ (Extended Data 96 Fig. 2). Two of these networks (i.e., cortico-striatal and the salience networks) showed enhanced 97 transient activity (based on amplitude analysis) during pregnancy (Fig. 2b-e). Spatial maps 98 identified robust functional connectivity changes in gustatory, sensorimotor and reward centres 99 during pregnancy (Fig. 2c, e). It is noteworthy that gestation was associated with changes in key 100 components of the dopaminergic mesolimbic circuitry (dorsal and ventral striatum (Stri), ventral 101 tegmental area (VTA), substantia nigra (SN)) that couple homeostatically-relevant stimuli with the 102 incentive salience of palatable foods and motivated appetitive behaviours.

103Based on our connectivity analysis, we explored whether gestation-associated functional104mesolimbic system changes were associated with the remodelling of key molecular determinants

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105 of dopamine circuits. Dopaminergic neurons in the VTA project and release this neurotransmitter 106 into the ventral striatum, where it binds to its receptors (D1R and D2R) located mainly on medium-107 sized spiny neurons (MSNs). Tyrosine hydroxylase (Th; rate-limiting enzyme in dopamine 108 synthesis) and dopamine transporter (Slc6a3) gene expression, as well as the number of VTA-109 TH+/Fos+ neurons and TH-producing neurons were unchanged in the VTA of pregnant mice 110 (Extended Data Fig. 3a-d). Drd1 and Drd2 mRNA expression (encoding D1R and D2R 111 respectively), as well as Fos-dependent activity, was unchanged in the dorsal striatum (dStri) 112 during pregnancy (Extended Data Fig. 4a-e). However, in the nucleus accumbens (NAc; part of 113 the ventral striatum), pregnant females displayed increased Drd2 expression and augmented 114 proportion of Drd2⁺/Fos⁺ neurons without changes in Drd1 (Fig. 3a-d, Extended Data Fig. 4f). 115 Enhanced accumbal Drd2 expression returned to non-pregnant status after weaning of the 116 offspring (Fig. 3c, Extended Data Fig. 4f). These results evidenced that gestation specifically 117 upregulates NAc Drd2 expression and activity of D2R neurons, suggesting that this receptor is the 118 main dopaminergic effector involved in food craving-like behaviour.

119 Dopamine signalling via D2R engages two major intracellular pathways: the canonical $G_{\alpha i/o}$ protein-dependent and the non-canonical β -arrestin-dependent pathways¹⁷. While activation 120 121 of the canonical route negatively regulates cAMP production, resulting in decreased PKA activity 122 and subsequent inactivation of DARPP-32, the alternative pathway facilitates the activation (dephosphorylation) of Gsk3 β through β -arrestin-2 and Akt (Fig. 3e)¹⁷. We examined whether 123 124 pregnancy promoted molecular adjustments of these signalling routes. Dorsal striatal analysis 125 revealed no changes in TH phosphorylation levels or markers for both downstream pathways 126 (Extended Data Fig. 5a,b). In contrast, TH activity in the dopaminergic terminals that innervate 127 the NAc was negatively modulated by gestation (decreased phosphorylation of TH-Ser40) (Fig. 3f, Extended Data Fig. 5c), likely compensating enhanced D2R signal transduction¹⁸. Furthermore, 128

the phosphorylation state of DARPP-32 and Gsk3β in the NAc of pregnant mice was attenuated
denoting engagement of both D2R-mediated dopaminergic signalling (Fig. 3g, Extended Data Fig.
5d). Collectively, these results suggest that pregnancy is associated with a dynamic modulation of
dopaminergic signalling mainly through accumbal D2R neurons at both pre-and postsynaptic
levels.

134 To gain deeper insights into the role of dopamine in food craving-like behaviour during 135 gestation, we measured this neurotransmitter and related metabolites. Dopamine content was 136 unaltered in the dStri, but it was increased in the NAc of pregnant mice (Fig. 3h, Extended Data 137 Fig. 5e). While the ratio between homovanillic acid (HVA) and dopamine was unchanged, the 138 relation between 3,4-Dihydroxyphenylacetic acid (DOPAC) and dopamine (a proxy of dopamine 139 turn-over) was decreased in both brain regions in pregnancy (Fig. 3i, Extended Data Fig. 5f). This 140 is likely the result of the augmented dopamine concentration in the NAc. Though its biological 141 meaning in the dStri is uncertain and presumably related with other pregnancy-related functions.

142 Decreased TH-Ser⁴⁰ phosphorylation in the NAc probably reflects a mechanism to 143 counteract the increased dopamine content. This mechanism allows for faster adaptations to the 144 oscillatory nature of gestation and is less energy demanding than transcriptomic changes. Our results have also shown an increase in Drd2 expression concomitant with enhanced D2R signalling 145 146 in the NAc, suggesting a higher dopaminergic response (as a consequence of augmented dopamine 147 in this brain region) that may drive non-homeostatic feeding. These results support the idea that 148 the dopamine system is plastic and that particular physiological states modulate dopaminergic 149 connectivity and function.

We next explored dopamine signalling adaptations as causal determinants of gestational food craving-like behaviour. To this end, we pharmacologically blocked dopaminergic transmission in pregnant mice (Extended Data Fig. 6a). Notably, this approach suppressed

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153 gestational food craving-like episodes, without affecting daily food intake or locomotor activity 154 (Extended Data Fig. 6a-e). This suggests that dopaminergic receptor blockage restores the balance 155 between D1R and D2R activity, preventing food-craving episodes. Our studies showed a specific 156 activation of D2R-expressing neurons and remodelling of D2R-mediated signalling in the NAc 157 during gestation (Fig. 3c, d, f, g, Extended Data Fig. 4f, 5c,d). Hence, we next aimed to define the 158 involvement of D2R-expressing neurons in this brain region upon pregnancy-characteristic food 159 craving-like episodes. To this purpose, we injected inhibitory (AAV-hM4Di) Designer-Receptors-160 Exclusively-Activated-by-Designer-Drugs (DREADDs) in the NAc (and dStri as control area) of pregnant Drd2^{+/+} and Drd2^{Cre/+} mice (Extended Data Fig. 7a-c). This permitted Cre-mediated 161 162 silencing of D2R neuron activity upon Clozapine-N-oxide (CNO) injection^{19,20}, that in turn 163 released their inhibitory tone on D1R-MSNs (Extended Data Fig. 7d-i). To exclude potential unspecific effects of CNO, both Drd2^{+/+} and Drd2^{Cre/+} mice were injected with this compound at 164 165 a relatively low concentration that does not impact on behaviour²¹. Remarkably, this strategy 166 normalized the exacerbated craving-like behaviour without changes in locomotor activity (Fig. 3j, 167 Extended Data Fig. 7j-n). This effect was region-specific, as DREADD-mediated inhibition of 168 D2R-expressing neurons in the dStri did not attenuate compulsive eating of pregnant mice (Fig. 3k, Extended Data Fig. 7o-s). CNO administration to Drd2^{+/+} mice did not modify locomotor 169 170 activity or general behaviour, as assessed by visual scrutiny. Altogether, these results demonstrate 171 that gestation-related food craving-like behaviour is mediated by accumbal D2R-dependent 172 circuits.

To examine the potential dichotomy of D2R canonical *vs.* non-canonical signalling underlying food craving-like behaviour, we injected the simultaneous D2R/β-arrestin partial agonist and D2R/G_{αi/o} antagonist UNC9994^{22,23} to virgin and pregnant mice. UNC9994 administration to pregnant females blocked craving-like behaviour without changes in locomotor activity (Extended Data Fig. 8a-f). Collectively, our chemogenetic and pharmacological studies
suggest that gestational-specific craving-like episodes depends on the engagement of both
canonical and non-canonical D2R pathways.

180 Maternal exposure to diverse dietary insults perturbs the development of neurocircuits implicated in psychological, appetitive and metabolic processes of the offspring⁷⁻⁹. Therefore, we 181 182 questioned whether persistent food cravings during pregnancy affects the progeny's 183 neuropsychological and metabolic status. To address this, we implemented the "limited access" 184 protocol throughout gestation. To characterize the metabolic consequences of this paradigm on 185 dams, and compare it with common maternal obesity protocols, we studied pregnant females 186 exposed to either ad libitum chow diet, ad libitum Western diet (WD) or the "limited access" 187 paradigm. WD-fed female mice showed a progressive body weight increase, glucose intolerance 188 (in spite of unaltered fasted blood glucose and insulin levels) and higher plasma leptin (Extended 189 Data Fig. 10a-e). These results indicate that the gestational "limited access" and maternal obesity 190 protocols represent entirely different paradigms from the metabolic perspective.

191 Male and female offspring born to dams submitted to either continuous chow diet (chow-192 O) or "limited access" to HPF throughout gestation (HPF-O) were studied. At weaning (P21), HPF-O mice of both sexes were glucose intolerant when compared to chow-O counterparts without 193 194 changes in body weight (Fig. 4a, b). Metabolic perturbations in HPF-O mice were accentuated in 195 adulthood, particularly in male mice, that were heavier (Fig. 4c), exhibited increased adiposity 196 (Fig. 4d) and persistent glucose intolerance (Fig. 4e). Next, we conducted a behavioural screening 197 of anxiety-like phenotypes by testing the offspring to the open field, dark-light box and elevated 198 plus maze paradigms. While individual tests showed some disparity (Fig. 4f-j, Extended Data Fig. 199 10a), the integration of these measures via z-normalization²⁴ showed more accentuated anxiety-200 like phenotypes in male HPF-O offspring (Fig. 4k). Maternal overnutrition and obesity have been linked to cognitive dysfunction of the offspring^{7,9}. Male HPF-O mice exhibited a trend to display
cognitive impairments in the novel object recognition test (NORT) without changes in locomotion
(Fig. 41-n, Extended Data Fig. 10b). These results demonstrate that excessive food craving-like
behaviour during gestation negatively impact offspring's neuropsychological and metabolic
health.

Eating disorders often develop during adolescence²⁵. To investigate the potential 206 207 consequences of frequent food craving-like episodes during pregnancy upon progeny's disordered 208 eating, we exposed adolescent (35 days old) Chow-O and HPF-O mice to a compulsive eating 209 paradigm (Extended Data Fig. 10c). As expected¹¹, control animals did not escalate their caloric 210 intake during the limited HPF access (Fig. 4o) but progressively increased total daily food 211 consumption throughout the test (Extended Data Fig. 10d). In contrast, male HPF-O mice 212 augmented their caloric intake during late compulsive eating episodes by $\sim 35\%$ more than at the 213 start of the test (Fig. 40). Compared with controls, male HPF-O animals exhibited higher basal 214 daily food intake (chow-O: 8.48 ± 0.13 Kcal/day vs. HPF-O: 10.68 ± 0.26 Kcal/day, p<0.01; 215 Extended Data Fig. 10d). The predisposition to compulsive eating was also observed in HPF-O 216 females (Fig. 40, Extended Data Fig. 10d). These findings evidenced that excessive maternal food 217 craving-like behaviour confers offspring's susceptibility to develop eating disorders during 218 adolescence.

Given that maternal high-fat diet feeding perturbs offspring dopamine signalling^{26,27}, we sought to determine whether recurrent gestational food craving-like events have a similar impact. Indeed, HPF-O mice of both sexes displayed a downregulation of both *Th* and *Slc6a3* expression in the VTA, with modest changes in the NAc and dStri (Extended Data Fig. 10e-j). Overall, the expression of dopaminergic system determinants was moderately attenuated in HPF-O, suggesting that maternal compulsivity for HPF has long-term influences in hedonic gene expression andbehaviour of the offspring.

226 The urgent appetitive behaviours during pregnancy suggest that cravings should be conceptualized as cognitively-motivated states²⁸. Indeed, our results indicating that D2R-227 228 dependent circuits in the NAc mediate gestational food craving-like behaviour support this. The 229 NAc acts as a hub integrating sensory, emotional and cognitive inputs into reward and motivated 230 behaviours²⁹. Enhanced D2R function promotes the incentive salience of food and emotional 231 eating in mice and humans^{30,31}, and has been implicated in food addiction³². The augmented 232 expression and activity of accumbal D2R neurons during pregnancy might reflect particular 233 neurobiological adjustments associated with this physiological process, in which the connectivity 234 from cortical regions into the NAc is remodelled. This may cause a vulnerability to cope with 235 cognitively-motivated states, with subsequent intensification of food-craving episodes.

236 While our findings suggest that NAc-D2R neurons are key effectors of food craving-like 237 behaviour during pregnancy, we cannot rule out the contribution of other brain areas with afferent 238 projections into the NAc. For example, the enhanced connectivity observed in the medial 239 prefrontal cortex (prelimbic and infralimbic cortices) and in the insula (part of the cortico-striatal 240 and salience networks) during gestation could exert an interoceptive function, by integrating 241 autonomic cues with emotional and motivational states³³. Our results underline pregnancy as a 242 physiological state able to promote plasticity of the neurocircuits connecting the prefrontal cortex 243 and the basal ganglia. This could partially account for the conscious urge to food cravings, as 244 described for prevalent addictions³⁴.

We show that recurrent gestational food craving-like behaviour compromises the metabolic and neuropsychological health of the progeny, enhancing the predisposition to eating and psychological disorders during adolescence and adulthood. These perturbations seem to

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preferentially affect male offspring. Indeed, maternal obesity is generally associated with pronounced metabolic and cognitive impairments in male than in female offspring in both mice and humans⁷⁻⁹. The underlying causes of such sex dimorphism demand investigation. Additionally, the majority of programming studies to date have focused on long-term maternal insults, such as diet-induced obesity, undernutrition or permanent stress^{7,9}. Our results indicate that even acute HPF consumption during food craving-like episodes is sufficient to originate detrimental health outcomes in the offspring thus advocating for controlled nutritional care during pregnancy.

255 Multiple theories have been proposed to explain the emergence of gestational food-256 cravings, including foetus nourishment, nutrient replenishment after nausea, hormonal fluctuations 257 or cultural/psychosocial factors with inconclusive outcomes². Our studies highlight biological and 258 evolutionary-conserved attributes, that are independent of foetal growth demands, as the driving 259 forces causing food craving-like experiences. Pregnancy entails a constant fluctuation of ovarian 260 hormone levels. A concomitant increase in progesterone and oestrogen levels positively modulate 261 food intake, as well as reward sensitivity and dopaminergic behavioural responses^{35,36}. Therefore, 262 it is tempting to speculate that variations in ovarian hormone levels during gestation contribute to 263 food craving behaviours.

The purpose of food motivated behaviours during gestation remains elusive, but might have evolved to ensure the consumption of nutrient/caloric-dense food thus preventing energy deficits for periods of scarcity. In the modern lifestyle, this evolutive advantage has become detrimental and excessive food-cravings may affect mother and offspring health. In summary, our findings provide the cellular and mechanistic basis of pregnancy food cravings and evidence the benefits of moderating emotional eating during gestation.

270 Methods

271 Animal care, mouse lines and diets

Mice were maintained on a temperature controlled, 12-hour light/dark cycle with free access to water and standard chow (Teklad maintenance diet 14% protein; Envigo). For the "limited access" paradigm, Western diet (WD; 40% Kcal from fat and 43% Kcal from carbohydrates; Research Diets) was provided for 2 hours during 3 consecutive days a week¹¹. C57BL/6 mice were bred inhouse. *Drd2-Cre* mice have been previously described^{37,38}. *Cre* negative littermates were used as controls. All animal studies were performed with approval of the Universitat de Barcelona Ethics Committee, complying with local government legislation (#10637).

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280 Breeding strategy

C57BL/6, *Drd2*^{+/+} or *Drd2*^{Cre/+} female mice were mated at 9-12 weeks of age. Two females were housed with one male and vaginal plug was examined daily. Presence of plug denoted day 0.5 of gestation. Weight gain was measured weekly to confirm pregnancies. Dams were kept with their offspring until weaning. For pseudopregnant mice generation, eight-week-old C57BL/6 females were mated with vasectomized males. Presence of vaginal plug was considered positive pseudopregnancy.

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288 Determination of sweet compound concentration

Eight-week-old C57BL/6 female mice were trained to drink from two water bottles until they reached a 50/50 preference. They were then presented to different concentrations of either sucralose (2.5; 1.0; 0.5; 0.1; 0.05 mM) or sucrose (200; 100; 50; 25 mM) and preference of each concentration against water was tested. The concentration at which virgin females were unable to discern water from sweet compounds (50/50 intake) was determined and used in subsequent
 studies.

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296 **Two-bottle taste preference test**

297 Eight-week-old C57BL/6 female mice fed with standard diet were individualized and trained to 298 drink from two water bottles until 50/50 preference. They were then submitted to a two-bottle 299 paradigm^{39,40} to test preference for either sweet tastant (0.5 mM sucralose or 25 mM sucrose) or 300 water during 3 experimental periods: i) before pregnancy; ii) throughout pregnancy with 301 measurements every week; iii) one week after the weaning of the offspring (after pregnancy). Mice 302 were overnight water deprived. At 09:00 a.m. two bottles containing sweet compound or water 303 were provided. Mice were allowed to drink for 3 hours, without access to food. Total volume 304 intake was measured and water was provided ad libitum until 7:00 p.m. Taste preference was 305 calculated as the ratio between the sweet liquid consumption and the total volume ingested. Sweet 306 preference and total food intake were measured daily. The position of the bottles was changed 307 every day to exclude position effects. Control groups with virgin and a pseudopregnant females 308 were run in parallel.

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310 "Limited access" paradigm

The "limited access" paradigm was based on previous studies¹¹. Briefly, eight-week-old C57BL/6, $Drd2^{+/+}$ or $Drd2^{Cre/+}$ female mice were individualized and habituated to WD during 5 days. After that, females had *ad libitum* access to chow and compulsive eating was induced by limited access to WD for 2 hours a day (at the end of the light cycle) during 3 consecutive days per week during 2 experimental periods: i) before pregnancy; ii) throughout pregnancy with measurements every week. Maternal food intake was measured daily. The percentage of daily caloric intake consumed 317 during the 2 hours of intermittent WD access was considered a proxy for food craving-like318 behaviour.

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320 Maternal physiology measurements

For glucose tolerance test, E14.5-E16.5 pregnant females fed with chow diet (n=8), WD (n=8) or exposed to the "limited access" paradigm (n=8) were i.p. injected with D-glucose (2 g/kg) after 6 hours fasting. Blood glucose levels were measured using a glucometer (Nova Pro Biomedical). Plasma insulin and leptin levels were measured after fasting (6 hours) with commercial ELISA kits (Crystal Chem). Body weight was measured weekly.

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327 **Progesterone serum levels**

Plasma samples were collected from C57BL/6 female mice at 3 different time-points: i) before pregnancy; ii) throughout pregnancy with measurements every week; iii) one week after the weaning of the offspring (after pregnancy). Control groups with virgin or pseudopregnant females were run in parallel. Levels of progesterone were determined using commercial kits (Crystal Chem).

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334 MRI acquisition, processing and analysis

Each mouse was scanned at three time-points: i) before pregnancy (n=7); ii) during the second week of pregnancy (n=8); iii) one week after the weaning of the offspring (after pregnancy) (n=6). Experiments were conducted on a 7.0T BioSpec 70/30 horizontal animal scanner (Bruker BioSpin, Ettlingen, Germany), equipped with an actively shielded gradient system (400 mT/m, 12 cm inner diameter). The receiver coil is a surface coil for the mouse brain. Animals were sedated (4% isofluorane in 30% O₂ and 70% N₂), placed in supine position in a Plexiglas holder with a nose

341 cone for administering anaesthetic gases and fixed using tooth and ear bars. Eyes were protected 342 from dryness with Siccafluid ophthalmologic fluid. Once placed in the holder with constant 343 isoflurane (1.5%), a subcutaneous bolus of medetomidine (Domtor®, Orion Pharma) (0.3 mg/kg) 344 was injected. For the next 15 minutes, the percentage of isofluorane was progressively decreased 345 to 0.5%. Then, a continuous perfusion of 0.6 mg/kg/h of medetomidine was started and maintained 346 until the end of the acquisition session. After completion of the imaging session, 1µl/g of 347 atipamezol (Antisedan®, Orion Pharma) and saline were injected to reverse the sedative effect and 348 compensate fluid loss. Localizer scans were used to ensure accurate position of the head at the 349 isocenter of the magnet. Anatomical T2 RARE images were acquired in coronal orientation with 350 effective TE=33ms TR=2.3s, RARE factor=8, voxel size=0.08x0.08 mm² and slice thickness=0.7 351 mm. rs-fMRI was acquired with an EPI sequence with TR=2s, TE=19.4, voxel size 0.21x0.21 mm² 352 and slice thickness 0.5mm. 420 volumes were acquired resulting in an acquisition time of 14 353 minutes.

354 The rs-fMRI acquisition was processed to extract functional brain networks by independent 355 component analysis (ICA) and evaluate differences in connectivity between pregnant and non-356 pregnant periods. Each fMRI acquisition was pre-processed including slice timing, motion correction, pass-band frequency filtering, spatial normalization to a mice brain atlas template and 357 358 smoothing (ANTs and Python 3). All the pre-processed images were considered to extract the 359 group ICA using FSL MELODIC. The resulting independent components were compared with the 360 functional networks described¹⁶ to identify the components corresponding to functional networks 361 of interest. Afterwards, dual regression was performed to identify the network components and 362 their spatial distribution in each individual brain and estimate the network shape and amplitude 363 differences⁴¹. Network amplitude was defined as the standard variation of the time-series 364 corresponding to the independent component (IC), and shape differences were analysed based on 365 the mean value of the z-score of the subject IC within the spatial map of the component (threshold 366 at z=2.3). Kruskal-Wallis test was applied to identify differences between shape and amplitude 367 values in the pregnant period vs. non-pregnant period in the selected ICs.

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Quantitative polymerase chain reaction (qPCR)

370 VTA, dStri and NAc samples were dissected, immediately frozen in liquid nitrogen and stored at 371 -80°C. Tissues were homogenized and total mRNA was isolated using Trizol (Invitrogen). 372 Retrotranscription was performed using reagents from Applied Biosystems. Quantitative PCR was 373 conducted using Premix Ex Taq mastermix (Takara) in an ABI Prism 7900 HT system (Applied 374 Biosystems). TaqMan Gene Expression FAM/TAMRA probes (Applied Biosystems) used were: 375 Th (Mm00447557_m1), Slc6a3 (Mm00438388_m1), Drd1 (Mm01353211_m1), Drd2 376 (Mm00438545 m1). Gene expression levels were expressed relative to Gapdh (Mm99999915_g1). 377

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379 Fluorescent in situ hybridization

380 Virgin, pregnant (during the second week of pregnancy) and after pregnancy C57BL/6 female 381 mice were transcardially perfused with 4% paraformaldehyde (PFA). Dissected brains were post-382 fixed in 4% PFA at 4°C for 24 hours and cryoprotected. Brains were cut at 20µm on a cryostat and 383 collected into 4 series (one every 4 sections) in SuperFrost Plus Gold slides (ThermoFisher) and 384 subsequently stored at -80°C.

385 Fluorescent in situ hybridization for the simultaneous detection of Drd1, Drd2 and Fos mRNA 386 was performed using RNAscope. All reagents were purchased from Advanced Cell Diagnostics 387 (ACD). All incubation steps were performed using the ACD HybEz hybridization system.

388 On assay day, one section series cut throughout the dStri and NAc was selected. From each animal, 389 one section was incubated with the negative control probe to enable calculation of background. 390 Slides were washed in PBS, baked at 60°C for 30 minutes and post-fixed with 4% PFA for 15 391 minutes. Sections were then dehydrated and baked for an additional 30 min at 60°C and submerged 392 into boiling Target Retrieval reagent for 5 minutes. The slides were dehydrated in 100% ethanol, 393 allowed to air dry for 5 minutes and placed into an RNAscope holder. Sections were treated with 394 Protease III for 30 minutes at 40°C. Probe hybridization, amplification and detection, were 395 performed according to the manufacturer's protocol. The colour module chosen labelled the Fos 396 probe with Atto 550, the Drd1 probe with Atto 647, and the Drd2 probe with Atto 488. Sections 397 were counterstained with DAPI and coverslipped with ProLong Gold Antifade Mountant 398 (ThermoFisher) and stored in the dark at 4°C.

399 Images were taken using a confocal Leica DM 2500 microscope, equipped with a 40x/1.15 oil 400 objective and using a zoom of 2x. Z-stacks of 1 µm of either the dStri or the NAc were captured 401 bilaterally from 4 rostral to caudal sections per animal (n=3 animals/group). Laser intensities were 402 kept constant throughout the entire image acquisition process. Images were imported into Fiji 403 (NIH) where maximum intensity projections were made. To acquire the minimum intensity value 404 for analysing the expression of Drd1 and Drd2, the threshold for probe recognition was calculated 405 as the mean cell intensity present in the negative control sections +3xSD. All labelling above this value was considered to be true signal⁴². Brightness and threshold were adjusted in all images. For 406 407 quantification, 8-10 Drd1+ or Drd2+ neurons per section were manually selected and Drd1 and 408 Drd2 particles were counted. After quantification, the presence of Fos expression was determined.

409

411 DStri and NAc were dissected and immediately frozen in liquid nitrogen and stored at -80°C. DStri 412 and NAc samples were sonicated in 120µl or 70µl, respectively, of RIPA buffer supplemented 413 with phosphatase and protease inhibitor cocktail (Sigma-Aldrich). Protein was quantified using 414 the BCA Protein Assay Kit (Pierce). 20µg for each sample and brain region were loaded onto a 4-415 12% SDS-polyacrylamide gel (SDS-PAGE) (Bio- Rad). Proteins were separated by SDS-PAGE, 416 electrotransferred to PVDF membranes (Millipore), blocked in 5% BSA buffer and immunoblotted 417 overnight at 4°C with the following primary antibodies: mouse anti-tubulin (1:1000, Sigma), rabbit 418 anti-phospho-DARPP-32Thr34 (1:1000, Cell Signaling Technology), rabbit anti-phospho-419 ThSer31 (1:1000, Millipore), rabbit anti-phospho-ThSer40 (1:1000, Cell Signaling Technology) 420 and rabbit anti-phospho-Gsk3ßS9 (1:1000, Cell Signaling Technology). Membranes were 421 incubated for 1 hour at room temperature with secondary antibodies: anti-mouse IgG (1:5000, GE 422 Healthcare) or anti-rabbit IgG (1:5000, GE Healthcare). Detection was performed by enhanced 423 chemiluminescence (Pierce) and images were obtained using a LAS4000 (GE Healthcare). Protein 424 expression was quantified by densitometry using ImageJ software (NIH).

425

426 **Double Th and Fos immunofluorescence**

427 Selected 20 µm-thick sections (1 out of 4 sections) throughout the VTA (bregma between -3.10mm 428 and -3.70mm) of virgin, pregnant (during the second week of pregnancy) or after pregnancy 429 C57BL/6 female mice (n=3/group) were blocked with 2% chicken serum in PBS + 0.1% Triton 430 X-100 + 3% BSA and incubated with rabbit anti-Fos antibody (1:500; Synaptic Systems) in 431 blocking solution overnight at 4°C. As secondary antibody, a chicken anti-rabbit Alexa Fluor 488 432 (1:300; Life Technologies) in PBS + 0.1% Triton X-100 + 3% BSA was used. After washing, 433 slices were blocked with 2% donkey serum in PBS + 0.1% Triton X-100 and incubated with sheep anti-Th antibody (1:600; Sigma) overnight at 4°C. As secondary antibody, a donkey anti-sheep 434

435	Alexa Fluor 594 (1:300; Life Technologies) in PBS + 0.1% Triton X-100 was used. For
436	quantification, representative images throughout the VTA of each animal were acquired using a
437	Leica DMI 6000B confocal microscope equipped with a 20x objective. The total number TH+
438	neurons and the proportion of TH+/Fos+ was counted manually using FIJI (ImageJ) software.

439

440 Neurochemical analysis

441 DStri and NAc samples of virgin (n=9) and pregnant (n=8) mice were dissected on a cold plate, 442 weighted, immediately frozen in liquid nitrogen and stored at -80°C. Dopamine, DOPAC and 443 HVA content were determined by HPLC with electrochemical detection (Waters model 2465, 444 +0.75 V) as described⁴³. Tissues were homogenized in 200 µl (dStri) or 40 µl (NAc), respectively, 445 of buffer containing 0.4 M perchloric acid with 0.1% sodium metabisulphite, 0.01% EDTA, 0.1% 446 cysteine and centrifuged at 12,000g for 30 minutes. Aliquots of supernatants were filtered through 447 0.45 µm pore size filters (Millex) and analyzed by HPLC as described. The mobile phase consisted 448 of 0.1 M KH₂PO₄, 1 mM octyl sodium sulphate, 0.1 mM EDTA (pH 2.65) and 18% methanol. 449 Dopamine and their metabolites were separated on a Kinetex Core-Shell columns (C18, 2.6 µm, 450 75×4.6 mm; Phenomenex).

- 451
- 452

Pharmacological blockage of dopamine receptors

453 Eight-week-old C57BL/6 female mice were i.p. injected with the D1R/D2R dopamine receptor 454 antagonist cis-(Z)-Flupenthixol dihydrochloride (flupenthixol, 0.4 mg/kg; Sigma) or saline 455 (vehicle) and exposed to the "limited access" paradigm or open field test 30 minutes later. The 456 drug was prepared 5 minutes before the tests.

- 457
- 458 Pharmacological assessment of D2R signalling dichotomy

Eight-week-old C57BL/6 female mice were i.p. injected with the D2R dopamine receptor βarrestin agonist/Gi antagonist UNC9994 (2 mg/kg; Axon MedChem) or vehicle (10% DMSO; 20%
cyclodextrin in saline) and exposed to the "limited access" paradigm or open field test 10 minutes
later.

463

464 Chemogenetic modulation of D2R neuron activity

465 Eight-week-old Drd2^{+/+} and Drd2^{Cre/+} female mice were anesthetized with Ketamine/Xylazine 466 (i.p., 100 mg/kg and 10 mg/kg) and received a Buprenorphine injection (i.p., 0.3 mg/kg) for 467 analgesia. Females were placed on a stereotaxic frame (Kopf Instruments) for subsequent injection 468 of AAV vectors encoding for inhibitory (AAV8-hSYN-DIO-hM4D(Gi)-mCherry; 1.10x10¹³ 469 gc/mL, Addgene) DREADDs. Viruses (300 nL/injection site) were bilaterally injected into the 470 dStri (AP: +1.2mm; ML: +/-1.8mm; DV: -3.5mm) or NAc (AP: +1.5mm; ML: +/-0.7mm; DV: -471 4.5mm) using a 33-gauge needle connected to a 5 µl Neuro-Syringe (Hamilton). The incision was 472 sutured by VetBondTM (3M) and mice were placed in a heated cage until they recovered from anesthesia. Experiments were conducted 3 weeks later. On experimental days, $Drd2^{+/+}$ and 473 474 Drd2^{Cre/+} female mice were i.p. injected with Clozapine-N-oxide (CNO 1 mg/kg; Tocris 475 Bioscience) and exposed to the "limited access" paradigm or open field test 30 minutes later.

476

477 Fos immunostaining on AAV8-hSYN- DIO-hM4D(Gi)-mCherry injection sites

478 Selected 20 μ m-thick sections (1 out of 4 sections), throughout the dStri and NAc, of $Drd2^{+/+}$ and 479 $Drd2^{Cre/+}$ female mice injected with AAV8-hSYN-DIO-hM4D(Gi)-mCherry were blocked with 480 2% chicken serum in PBS + 0.1% Triton X-100 + 3% BSA and incubated with rabbit anti-Fos 481 antibody (1:500; Synaptic Systems) in blocking solution overnight at 4°C. As secondary antibody, 482 a chicken anti-rabbit Alexa Fluor 488 (1:300; Life Technologies) in PBS + 0.1% Triton X-100 + 3% BSA was used. Imaging was performed using an Olympus fluorescence microscope. The
number of Fos+ cells, from 3 animals per genotype, in the dStri and NAc was counted manually
using FIJI (ImageJ) software.

486

487 Fos/DARPP32 immunostaining on AAV8-hSYN- DIO-hM4D(Gi)-mCherry injection sites

Selected 20 μ m-thick sections (1 out of 4 sections), throughout the dStri and NAc, of $Drd2^{+/+}$ and 488 489 Drd2^{Cre/+} female mice injected with AAV8-hSYN- DIO-hM4D(Gi)-mCherry were blocked with 490 2% chicken serum in PBS + 0.1% Triton X-100 + 3% BSA and incubated with rabbit anti-Fos 491 antibody (1:500; Synaptic Systems) and guinea pig anti-DARPP32 antibody (1:1000; Frontier 492 Institute) in blocking solution for 2.5 days at 4°C. As secondary antibodies, a donkey anti-rabbit 493 Alexa Fluor 647 Plus (1:300; Life Technologies) and a donkey anti-guinea pig Alexa Fluor 488 494 (1:300; Jackson ImmunoResearch) in PBS + 0.1% Triton X-100 + 3% BSA was used. Imaging 495 was performed using a Leica DMI 4000B confocal microscope. The number of Fos+ cells, from 3 496 animals per genotype, in the dStri and NAc was counted manually using FIJI (ImageJ) software.

497

498 **Offspring studies: general considerations**

Pregnant and lactating dams (n=9 chow-fed and n=11 "limited access" paradigm) underwent weekly follow-ups of body weight and food intake. Litter size was adjusted (between P1-P4) to six-to-eight pups to ensure standardized nutrition until weaning. At weaning, n=1-3 male and female offspring from each litter were randomly subdivided for subsequent studies. For physiological and behavioural tests, the offspring from n=5 chow-fed and n=5 "limited access" paradigm females were used. For compulsive eating predisposition studies, the offspring from n=4 chow-fed and 6 "limited access" paradigm females were used.

506

507 Offspring physiological measurements

508 For glucose tolerance test, mice were i.p. injected with a single bolus of D-glucose (2 g/kg) after 509 6 hours (P21 animals) (n=8-10 mice/group/sex derived from n=5 dams/group) or overnight (10-510 week-old) (n=7-8 mice/group/sex derived from n=5 dams/group) fasting. Blood glucose levels 511 were measured using a glucometer (Nova Pro Biomedical).

512

513 **Compulsive eating predisposition in the offspring**

514 Offspring of mothers continuously submitted to chow diet (n=4) or "limited access" paradigm 515 (n=6) were weaned at 21 days of age. At this time-point, male (n=5/group) and female (n=5/group) 516 pups from both experimental groups were separated and switched to standard chow diet until post-517 natal day 30. Males and females of similar body weights were submitted to a limited access WD 518 feeding to assess compulsive eating-like behaviour. Briefly, chow and HPF offspring were 519 habituated to WD during 5 days. P35 offspring were exposed to a "limited access" paradigm (2 520 hours a day, 3 times a week) during 4 weeks without food restriction¹¹. Weekly body weight and 521 daily food intake (chow and WD) were measured. The percentage of daily caloric intake consumed 522 during the two hours of intermittent WD access was considered a proxy for compulsive eating 523 behaviour.

524

525 Behavioural procedures

526 Before each test, mice were acclimatized to the behavioural room and arena for 1 hour. The arena 527 was cleaned with 70% ethanol before and after every trial. Light intensity was adapted to each 528 task. A video camera was used to track the movement of each animal. Videos were recorded and 529 analysed with video-tracking software (SMART v3.0, Panlab). For gestational studies, the 530 experiments were conducted in virgins (n=10), pregnant (during the second week of pregnancy)

531	(n=12) and after pregnancy (n=10) females. For offspring studies, the experiments were conducted
532	in 12-14-week-old male (n=8) and female (n=7-8) offspring from chow (n=7-8 offspring/sex
533	derived from 5 dams) or "limited access" HPF (n=8 offspring/sex derived from 5 dams) mothers.
534	
535	Open field test
536	Mice were placed in the centre of a dark methacrylate arena (35x35x35 cm) and allowed to freely
537	explore it for 15 minutes. Animals were tested under a low-intensity light (< 30 lux) to avoid stress.
538	Total distance and the time spent in the corner and centre of the arena was scored.
539	
540	Dark-light box test
541	Our protocol was based on previous studies ⁴⁴ . The test apparatus consisted of a methacrylate arena
542	(35x35x35 cm) divided into a small dark (safe) compartment and a large strongly-illuminated (200
543	lux; aversive) compartment. A door connected both compartments. Mice were placed in the dark
544	compartment and allowed to freely move between the two chambers for 5 minutes. Video tracking
545	data was analysed to calculate the time spent in each compartment and the latency to enter the
546	illuminated compartment.
547	
548	Elevated plus maze test
549	Our protocol was based on previous studies ⁴⁴ . The apparatus used was a cross-shaped 4-arm maze,
550	with 2 open arms and 2 closed arms (25 x 5 cm). This structure was elevated 60 cm above the
551	floor. Mice were placed in the centre of the apparatus, facing towards a closed arm, and their
552	behaviour was video recorded for 5 minutes. Time spent in the closed arms and open arms was
553	analysed.

555 Novel object recognition test (NORT)

The novel object recognition test (NORT) was based on Leger and collaborators⁴⁵. The test was 556 557 conducted in a methacrylate (35x35x35 cm) arena under a 20 lux environment. NORT consisted 558 in three phases: habituation, training and test. During habituation (day 1), mice were allowed to 559 explore the arena for 10 minutes. In the training phase (day 2), mice were allowed to explore two 560 identical objects (equidistantly spaced) for 10 minutes. The test phase was conducted 24 hours 561 later to measure long term memory. In this phase, one of the objects was replaced by a new one 562 and the mouse was allowed to explore them for 10 minutes. The position of the 2 objects was constant across sessions. Discrimination indices were calculated as: (Timenovel - Timefamiliar) / 563 564 (Time_{novel} + Time_{familiar}). We also scored total exploratory time (Time_{novel} + Time_{familiar}) and total 565 distance travelled. Animals that showed freezing behaviour or exhibiting <5s of exploratory 566 behaviour were excluded. The trial was video-recorded and analysed off-line.

567

568 Statistical Analysis

569 Data are expressed as mean \pm SEM. Two group-one factor comparisons were performed using a 570 two-tailed unpaired Student's t test. Three groups-one factor comparisons were performed using a 571 one-way ANOVA, and two or three factors comparisons were performed using two-way or three-572 way ANOVA followed by Tukey's multiple comparison test when computing confidence intervals 573 for every comparison or the Holm-Šídák test when not. Factor results (in relation with the variables 574 Group, Time and/or the interaction Group: Time in the female studies, Fig. 1 Extended Data Fig. 1 575 and Extended Data Fig. 9) are shown next to the graph (lack of this information means that no 576 significant differences were found). For offspring studies (Fig. 4 and Extended Data Fig. 10), we 577 implemented a linear mixed-effects model analysis to account for statistical dependence among 578 individuals originating from the same dam. In the case of non-repeated measures, comparisons

579	were conducted between experimental "diet" and offspring "sex" (fixed effects), while setting the
580	variable "dam" as a random term. For physiological and compulsive-eating predisposition studies,
581	when repeated measurements were recorded over time, comparisons were made between "diet",
582	offspring "sex" and "time" of record (fixed effects). The variables "dam" and "pup ID" were
583	considered as random factors. The statistical significance of experimental treatments was assessed
584	by an analysis of deviance (ANODE). Given the substantial number of statistical comparisons
585	involved in the compulsive eating study, all p values were adjusted using the Benjamini-Yekutieli
586	test thus minimizing the false discovery rate. Results from all post-hoc analysis are depicted on
587	top of the graph bars using asterisks. Z-scores were calculated by dividing the difference between
588	the individual data determined in each experiment (x) and the mean of the control group (m), to
589	the standard deviation of the control group (s) ($z=(x-m)/s$). We defined chow diet as the control
590	group. Complementary measures (open field, dark-light box, and elevated plus maze) were
591	integrated by implementing a z-normalization across diverse behavioural tests as previously
592	described ²² . Analysis was performed with Graphpad v8 and R v4.0.2 (lme4, car and multcomp
593	packages) softwares. P<0.05 was considered significant.

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608

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performed fMRI data acquisition and analyses. R.H-T., S.R., E.M-M., E.V., G.S., L.M-R., A.B.
and M.C. contributed to method development and data interpretation. R.H-T., E.M-M., G.S. and
M.C. developed the data visualizations. R.H-T. and M.C. wrote the original draft of the paper with
editing and reviewing inputs from all authors.

616

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725	

726 Figure Legends

727 Fig. 1 | Sweet taste perception and food craving-like behaviour increase during pregnancy. 728 a, Schematic illustration of two-bottle paradigm and experimental timeline. b-c, Sucralose (0.05 729 mM) preference (b) and area under the curve (AUC) (c) throughout the study of virgin (n=5), 730 pseudopregnant (n=5) and pregnant mice (n=5). d-e, Sucrose (25 mM) preference (d) and area 731 under the curve (AUC) (e) throughout the study of virgin (n=5), pseudopregnant (n=5) and 732 pregnant mice (n=5). f, Schematic illustration of the "limited access" paradigm and experimental 733 timeline. g, Percentage of daily caloric intake, consumed during the 2-hours of HPF access 734 throughout the study, of virgin (n=8), pseudopregnant (n=22) and pregnant (n=13) mice. Dots in 735 all panels represent individual sample data. Data are expressed as mean ± SEM. Exact P values 736 are shown. One-way ANOVA with Tukey's multiple comparisons test was performed for c and e, 737 two-way ANOVA with Dunnett's multiple comparisons test for **b**, **d** and two-way ANOVA with 738 Tukey's multiple comparisons test for g. When the factors Group, Time and/or the interaction 739 Group:Time were considered significant, results are shown with the significant factor or the 740 interaction effect next to it (b, d). BP: before pregnancy; W1: first week of pregnancy (or 741 concomitant experimental week for virgins and pseudopregnants); W2: second week of pregnancy 742 (or concomitant experimental week for virgins and pseudopregnants); W3: third week of 743 pregnancy (or concomitant experimental week for virgins and pseudopregnants); AP: after 744 pregnancy.

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Fig. 2 | Brain functional networks related to reward and emotions are transiently increased
during pregnancy. a, Schematic illustration of fMRI experimental timeline. b, Shape and
amplitude analysis of the cortico-striatal network in virgins (n=7), pregnants (n=8) and after
pregnancy (n=9). c, Coronal slices of whole-brain spatial map of the cortico-striatal network,

750 including reward and motor control areas. Axial (above, left) and sagittal (below, left) 751 representative slices of the independent component spatial map are shown. **d**, Shape and amplitude 752 analysis of the salience network in virgins (n=7), pregnants (n=8) and after pregnancy (n=9). e. 753 Coronal slices of whole-brain spatial map of the salience network including gustatory, motor, 754 limbic and dopaminergic control areas. Axial (above, left) and sagittal (above, right) representative 755 slices of the independent component spatial map are shown. Dots in all panels represent individual 756 sample data. Data are expressed as mean ± SEM. Exact P values are shown. Statistical analysis 757 was performed by Kruskal-Wallis test. IL: infralimbic cortex; Ins: insula; MCx: motor cortex; 758 Pir: pirirform cortex; PrL: prelimbic cortex; SCx: sensory cortex; S: septum; Stri: dorsal and 759 ventral striatum; VCx: visual cortex; vMB: ventral midbrain.

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761 Fig. 3 | Accumbal D2R neurons underlie pregnancy-specific food craving-like behaviour. a, 762 NAc Drd1 mRNA particles per neuron (n=6 mice/group). b, Percentage of NAc Fos positive Drd1 763 neurons (n=6 mice/group). c, NAc Drd2 mRNA particles per neuron (n=6 mice/ group). d, 764 Percentage of NAc Fos positive Drd2 neurons (n=6 mice/group). e, Schematic illustration of pre-765 and postsynaptic D2R-specific signalling in the striatum. Phosphorylation status is depicted by red circle. **f**, NAc immunoblot assessing TH phosphorylation levels at residues Ser31 (p-TH^{S31}) and 766 Ser40 (p-TH^{S40}) (n=5 mice/group). g, NAc immunoblot assessing G protein-dependent and non-767 canonical β -arrestin-dependent signalling pathways showing its downstream targets, DARPP-32 768 phosphorylation levels (at residue pThr34; p-D-32^{T34}) and GSK3β phosphorylation levels (at 769 residue pSer9; p-GSK3 β^{S9}), respectively (n=5 mice/group). h-i, NAc neurotransmitter (DA, 770 771 DOPAC and HVA) levels (h), dopamine turnover (DOPAC to DA ratio) and dopamine storage 772 (HVA to DA ratio) (i) of virgins (n=9) and pregnant (n=8) females. j-k, Schematic illustration of 773 the experimental strategy (left) and percentage of daily caloric intake (right) consumed during the 774 2-hours of HPF access of female mice injected with hM4D(Gi) in the NAc (n=7 Drd2^{+/+} and 5 $Drd2^{Cre/+}$) and in the dStri (n=4 $Drd2^{+/+}$ and 3 $Drd2^{Cre/+}$) before and during the second week of 775 776 pregnancy. Dots in all panels represent individual sample data. Data are expressed as mean \pm SEM. 777 Exact P values are shown. Statistical analysis was performed by one-way ANOVA with Tukey's 778 multiple comparisons test for **a**, **b**, **c**, **d**, **f**, **g** and by two-way ANOVA with Sidak's multiple 779 comparisons test for h, i, j, k. NAc: nucleus accumbens; Drd1: dopamine receptor 1; Drd2: dopamine receptor 2; Th: tyrosine hydroxylase; DA: dopamine; PKA: protein kinase A; DARPP-780 781 32: dopamine- and cAMP-regulated phosphoprotein; $Gsk3\beta$: glycogen synthase kinase 3 beta, Mr 782 32 kDa; DOPAC: 3,4-Dihydroxyphenylacetic acid; HVA: homovanillic acid; dStri: dorsal 783 striatum; V: virgins; P: pregnants; AP: after pregnancy; BP: before pregnancy; W2: second week 784 of pregnancy.

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786 Fig. 4 | Recurrent maternal HPF craving-like behaviour deteriorates offspring health. a, 787 Body weight at P21 of male (n=9 Chow-O and 11 HPF-O) and female (n=8 Chow-O and 12 HPF-788 O) offspring born to mothers fed with chow (Chow-O) (n=5) or HPF (HPF-O) (n=5). b, GTT of 789 P21 male (n=9 Chow-O and 10 HPF-O) (left panel) and female (n=8 Chow-O and 9 HPF-O) 790 Chow-O (n=5) or HPF-O (n=5). c, Body weight of 12-week-old male (n=8 mice/group) and female 791 (n=7 Chow-O and 8 HPF-O) Chow-O (n=5) or HPF (HPF-O) (n=5). d, gWAT weight normalized 792 by total body mass in male (n=8 Chow-O and 7 HPF-O) and female (n=7 Chow-O and 8 HPF-O) 793 Chow-O (n=5) or HPF-O (n=5). e, GTT of 10-week-old male (n=8 mice/group) and female (n=7 794 Chow-O and 8 HPF-O) Chow-O (n=5) or HPF (HPF-O) (n=5). f-g, Open field performance in 12-795 week-old male (n=8 mice/group) and female (n=7 Chow-O and 8 HPF-O) Chow-O (n=5) or HPF 796 (HPF-O) (n=5), including time spent per zone (f, corner and g, center) and (h) representative traces. 797 i, Time spent in the light compartment in 12-week-old male (n=8 mice/group) and female (n=7 798 Chow-O and 8 HPF-O) Chow-O (n=5) or HPF-O (n=5). j, Time spent in the open arm in 12-week-799 old male (n=8 mice/group) and female (n=7 Chow-O and 8 HPF-O) Chow-O (n=5) or HPF-O 800 (n=5). k, Anxiety score (male n=8 mice/group and female n=7 Chow-O and 8 HPF-O) after z-801 normalization of the different behavioural anxiety paradigms shown in g, i, j. l-n, Long term 802 memory parameters in Chow-O and HPF-O 13-week-old male (n=8 mice/group) and female (n=7 803 Chow-O and 8 HPF-O) mice, including discrimination index (I), exploratory time (m), and 804 representative traces (n). Blue circle and red square depict familiar and unfamiliar objects, 805 respectively. o, Percentage of daily caloric intake, consumed during the 2-hours of HPF access 806 throughout adolescence, in Chow-O and HPF-O male and female mice (n=5 mice per group/ 807 average of 3 measurements per week). Dots in all panels represent individual sample data. Data 808 are expressed as mean ± SEM. Statistical analysis was performed by ANODE for **a**, **b**, **c**, **d**, **e**, **f**, 809 g, i, j, k, l, m or ANODE followed by Benjamini–Yekutieli adjustment for o. When the factors 810 Sex, Diet, Time and/or the interaction Sex:Diet, Sex:Time, Diet:Time, Sex:Diet:Time were 811 considered significant, results were shown with significant factor or the interaction effect with 812 Tukey's post-hoc analysis within the same sex group. Exact P values are shown.



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Pregnants

pregnants











a Cingulate/Retrosplenial Cortex



b Primary Somatosensory Area 1



c Prefrontal Cortex



d Right Thalamus



e Primary Somatosensory Area 2



f Left Hippocampus



g Cortico-striatal network







j Primary Somatosensory Area 4



k Ventral Striatum



I Temporal Association Area



m Salience network







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- **B**

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DA











