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# CHEMOGENETIC SILENCING OF HIPPOCAMPUS AND AMYGDALA REVEALS A DOUBLE DISSOCIATION IN PERIADOLESCENT OBESOGENIC DIET-INDUCED MEMORY ALTERATIONS

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## 1 ABSTRACT

In addition to numerous metabolic comorbidities, obesity is associated with several adverse neurobiological outcomes, especially learning and memory alterations. Obesity prevalence is rising dramatically in youth and is persisting in adulthood. This is especially worrying since adolescence is a crucial period for the maturation of certain brain regions playing a central role in memory processes such as the hippocampus and the amygdala. We previously showed that periadolescent, **but not adult**, exposure to obesogenic high-fat diet (HFD) had opposite effects on hippocampus- and amygdala-dependent memory, impairing the former and enhancing the latter. However, the causal role of these two brain regions in periadolescent HFD-induced memory alterations remains unclear. Here, we first showed that periadolescent HFD induced long-term, but not short-term, object recognition memory deficits, specifically when rats were exposed to a novel context. Using chemogenetic approaches to inhibit targeted brain regions, we then demonstrated that recognition memory deficits are dependent on the activity of the ventral hippocampus, but not the basolateral amygdala. On the contrary, the HFD- induced enhancement of conditioned odor aversion specifically requires amygdala activity. Taken together, these findings suggest that HFD consumption throughout adolescence impairs long-term object recognition memory through **alterations of ventral hippocampal activity** during memory acquisition. Moreover, these results further highlight the bidirectional effects of adolescent HFD on hippocampal and amygdala functions.

## 2 KEYWORDS (6)

obesity, adolescence, memory, DREADD, hippocampus, amygdala

## 1 1 INTRODUCTION

2 Obesity is one of the most important public health challenges and is linked to the overconsumption  
3 of energy-dense food combined with a sedentary lifestyle. In addition to being associated with  
4 several peripheral comorbidities including cardiovascular and metabolic disorders (Head, 2015;  
5 Malnick & Knobler, 2006; Walls et al., 2012), obesity is also associated with cognitive and  
6 neurobiological dysfunctions (Francis & Stevenson, 2013; Wang et al., 2016). Previous studies have  
7 demonstrated that obesity is associated with deficits in episodic and spatial **memory** (for reviews see  
8 Francis & Stevenson, 2013; Martin & Davidson, 2014; Sellbom & Gunstad, 2012; Yeomans, 2017) but  
9 also with increased emotional, **mood** and affective disorders (Mansur et al., 2015).

10 The prevalence of obesity has also risen in young people (Ogden et al., 2016; Sahoo et al., 2015).  
11 Recent studies indicate indeed that obese adolescents display blunted performance in  
12 geometric/visuospatial problems or relational memory (Khan et al., 2015; Nyaradi et al., 2014;  
13 Øverby et al., 2013). Given that childhood and adolescence are crucial periods for cognitive and brain  
14 development (Spear, 2000), they represent a window of vulnerability to external insults such as the  
15 deleterious impact of various diets (for reviews see Andersen, 2003; Noble & Kanoski, 2016; Reichelt  
16 & Rank, 2017). In rodents, we recently showed that high-fat diet (HFD) **exposure from weaning to**  
17 **adulthood, defined as periadolescent exposure (see Labouesse et al., 2017)** induced complex  
18 motivational (Naneix et al., 2017; Tantot et al., 2017) and memory deficits (Boitard et al., 2012, 2014,  
19 2015, 2016; Khazen et al., 2019). Importantly, we found that **similar duration of HFD exposure at**  
20 **adulthood does not have any effect on memory (Boitard et al., 2012, 2014, 2015; Khazen et al., 2019)**  
21 **and that periadolescent HFD alters relational and spatial memory but enhances emotional memory**  
22 (for reviews see Del Olmo & Ruiz-Gayo, 2018; Morin et al., 2017; Murray & Chen, 2019). However,  
23 how these memory alterations are supported by specific neurobiological changes is still unclear.

24 Relational **memory** and emotional **memory** are **respectively** dependent on the hippocampus (Bunsey  
25 & Eichenbaum, 1996; Hartley et al., 2014) and the amygdala (LeDoux, 2003; McGaugh, 2004; Paré,  
26 2003). In humans, clinical studies have shown that obese patients present hippocampal (Mestre et  
27 al., 2017; Mueller et al., 2012) and amygdala **alterations** (Connolly et al., 2013; Pasquali et al., 2006;  
28 Widya et al., 2011). It is noticeable that both **the** hippocampus and **the** amygdala complete their  
29 development during adolescence (for review see Casey et al., 2010; McCormick & Mathews, 2010;  
30 Saygin et al., 2015; Spear, 2000). Interestingly, overweight/obese children present reduced  
31 hippocampal volumes (Bauer et al., 2015) and increased amygdala activation (Boutelle et al., 2015).  
32 Similar patterns have been reported in HFD animal models (Abbott et al., 2019; Bose et al., 2009).  
33 Therefore, hippocampus and amygdala may then be highly vulnerable to the long-term deleterious

34 effects of periadolescent HFD. However, the causal role of these two brain areas in periadolescent  
35 HFD-related memory changes remain to be demonstrated.

36 Here we investigated the causal role of ventral hippocampus (vHPC) and the basolateral amygdala  
37 (BLA) in memory deficits induced by HFD consumption, from weaning to adulthood (covering  
38 adolescence). We previously assessed spatial and relational memory using aversive (Boitard et al.,  
39 2014, 2016) or rewarded (Boitard et al., 2012) learning tasks. Here we used different variations of  
40 non-aversive, non-rewarded, spontaneous learning tasks using objects, i.e. object recognition  
41 memory (ORM). By manipulating the delay between training and test, as well as the arousal levels  
42 during training (through habituation or not to the training context), we used different situations that  
43 could differentially recruit the hippocampus (for review see Cohen & Stackman, 2015) and the BLA  
44 (Maroun & Akirav, 2008; Okuda et al., 2004; Roozendaal et al., 2006), respectively. Regarding  
45 hippocampus, if most of the ORM studies have focused on the role of the dorsal hippocampus  
46 (dHPC), manipulations of both dHPC and vHPC have stronger effect on ORM performance than  
47 similar manipulations restricted to the dHPC suggesting a complementary role of vHPC in ORM  
48 (Broadbent et al., 2004; Hales et al., 2015). Here, we first show that periadolescent HFD decreased  
49 specifically hippocampal-dependent form of long-term ORM in non-habituated rats (high arousal  
50 conditions). Using a chemogenetic DREADD approach (Armbruster et al., 2007; Rogan & Roth, 2011),  
51 we then demonstrate that this ORM deficit is abolished by the inhibition of the vHPC, but not the BLA  
52 projecting neurons during the acquisition. Interestingly, we additionally observed that the HFD-  
53 induced enhancement of aversive odor memory is dependent on BLA, but not vHPC, activity.

54

## 55 2 METHODS

### 56 2.1 Animals and diets

57 Naïve male Wistar rats (Janvier), aged 3 weeks when they arrived, were housed in groups of two to  
58 four individuals in polycarbonate cages (48 x 26 x 21 cm) in an acclimatized (22 ± 1°C) housing room  
59 maintained under a 12 h light/dark cycle (lights on at 8:00 am, lights off at 8:00 pm). They had *ad*  
60 *libitum* access to food and water from their arrival until euthanasia day. At their arrival, rats were  
61 maintained either on a control diet (CD; 2.9 kcal/g; 8% lipids, 19% proteins, 73% carbohydrates; A04,  
62 SAFE) or on a high fat diet (HFD; 4.7 kcal/g; 45% lipids, 20% proteins, 35% carbohydrates; D12451,  
63 Research Diet). Animals' body weight was recorded weekly. Rats were exposed to CD or HFD for 12  
64 weeks (from weaning to adulthood) before the start of the behavioral experiments (Figure 1A). After  
65 the completion of the ORM task, rats were housed individually in identical cages (48 x 26 x 21 cm) to

66 **measure individual drinking behavior during all phases** of the conditioned odor aversion (COA)  
67 procedure.

68 All procedures were performed in agreement with the French (Directive 2013-118, 1 February 2013)  
69 and international (directive 2010-63, 22 September 2010, European Community) legislations and  
70 received approval from the local Ethics Committee (5012047-A).

71

## 72 2.2 *Viral vector and drugs*

73 An adeno-associated viral vector (AAV) carrying the inhibitory hM4D(Gi) DREADD driven by the  
74 CaMKII promoter (to limit expression to **projecting** neurons) was obtained from University of North  
75 Carolina Vector core (Chapel Hill, NC, USA). The vector used was an AAV8-CaMKII-hM4D(Gi)-mCherry  
76 ( $3-4 \times 10^{12}$  vp/ml).

77 The exogenous ligand Clozapine-N-Oxyde (CNO; Enzo Life Sciences) was dissolved in 0.9% saline  
78 containing 0.5% of dimethyl sulfoxide (DMSO; Sigma) at a final concentration of 1 mg/ml. Saline  
79 solution (0.9%) with 0.5% DMSO was used for vehicle injections. Both CNO and vehicle were  
80 prepared fresh for every injection day and injected (i.p.) 45 min before behavioral testing.

81

## 82 2.3 *Surgery*

83 After 7-8 weeks under CD or HFD, rats were anaesthetized under isoflurane (5% induction; 1-2 %  
84 maintenance), injected with the antalgic buprenorphine (Buprecare; 0.05 mg/kg, s.c.) and mounted  
85 on a stereotaxic apparatus (David Kopf Instruments). The scalp was shaved, cleaned and locally  
86 anaesthetized with and local application of xylocaine. The viral vector was infused using repeated  
87 pressure pulses delivered via a glass micropipette connected to a Picospritzer III (Parker, NH, USA).  
88 For the vHPC, 1  $\mu$ l of the AAV was injected over 5 min (200 nl/min) at 2 sites in each hemisphere (i.e.  
89 2  $\mu$ l per hemisphere). The vHPC coordinates were AP -5.5 mm, ML  $\pm$ 5.5 mm from Bregma, DV -4 and -  
90 6 from the skull surface (Paxinos & Watson, 2007). For the BLA, 1  $\mu$ l of the AAV was injected over 5  
91 min at 1 site in each hemisphere: AP -3.0 mm, ML  $\pm$ 5.5 mm from Bregma, DV -8 mm from the skull  
92 surface. The pipette was left in place for 5 additional minutes before being slowly removed. Rats  
93 were housed in pairs immediately after surgery and were allowed at least 4 weeks to recover before  
94 the start of behavioral testing to allow ample time for virus expression.

95

96 2.4 Object recognition memory (ORM)

97 ORM is a classical procedure to assess non-spatial memory based on the recognition of a familiar  
98 object and rodent's natural tendency to explore novel, non-threatening, object. ORM requires a  
99 single trial and does not involve any aversive or food reward component (Ennaceur, 2010; Ennaceur  
100 & Delacour, 1988).

101 ORM task was performed in an arena sized 1.0 m x 1.0 m x 0.80 m (W x L X H), between 9:00 am and  
102 1:00 pm. During the acquisition phase, rats were placed in the apparatus for 10 min and the time  
103 spent exploring two identical unfamiliar objects (either pairs of glass jars or milk cans;  
104 counterbalanced between groups) was recorded. Three or 24 h later, rats were placed back into the  
105 same apparatus containing a familiar and a novel object for 5 min at the same location than during  
106 the acquisition and the time spent exploring each object was recorded. The position of the familiar  
107 and the novel object (left or right) was counterbalanced between animals. Both objects and  
108 apparatus were cleaned with 70% of ethanol between each animal. Naïve rats usually prefer  
109 exploring the novel object, indicating memory for the familiar one, while a failure of recall is  
110 considered as a memory deficit (Cohen & Stackman, 2015; Ennaceur, 2010; Ennaceur & Delacour,  
111 1988). Videos were recorded for each individual rat. Object exploration was analyzed offline in blind  
112 conditions using a video tracking software (Videotrack; Viewpoint, France). Object exploration was  
113 considered when the rat was at a distance of at least 1.0-1.5 cm and moved its whiskers towards the  
114 object. Exploration values were excluded if the animal was not exploring during either the training or  
115 the testing phase, and if one object was moved during the test. Exploration is represented as the  
116 absolute time exploring each object in seconds. ORM was expressed as the percentage of exploration  
117 of the novel object during the testing phase, calculated as following:  $\text{time spent exploring the novel object} / (\text{time spent exploring the novel object} + \text{time spent exploring the familiar object}) \times 100$ . A  
118 value above 50% indicates a higher exploration of the new object over the familiar one. In  
119 chemogenetic experiments, rats received either vehicle or CNO (i.p.) 45 min before the acquisition  
120 session.

122 In some experiments, an initial context habituation phase was performed before the acquisition  
123 session in order to decrease arousal during training (Maroun & Akirav, 2008; Okuda et al., 2004;  
124 Roozendaal et al., 2006). Context habituation consisted of 3 x 5 min daily sessions during which rats  
125 were free to explore the arena without objects.

126

127 2.5 *Conditioned odor aversion (COA)*

128 COA results from the association of an odorized tasteless solution with a visceral malaise. In the  
129 present experiment, COA was evaluated using a previously described procedure (see Boitard et al.,  
130 2015). Rats were first acclimated to a water-deprivation regimen for 4 days. Access to water was  
131 provided in a graded bottle (with 0.5 ml accuracy) placed in the rats' home cage for 15 min each day  
132 between 9am and 11am. Baseline water consumption was obtained by averaging the intake of the  
133 last 3 days. On the fifth day, rats had access for 15 min to almond- (0.01% benzaldehyde; Sigma  
134 Aldrich) or banana-scented (0.01% isopentyl acetate; Sigma Aldrich) water, counterbalanced  
135 between rats. The percentage of odorized solution consumption with respect to water baseline was  
136 used as a measure of neophobia. Thirty minutes after, rats received an intraperitoneal injection of  
137 lithium chloride (LiCl; Sigma Aldrich; 25 mg/kg, 0.075M, 0.75 % of body weight). On days 6 and 7, rats  
138 had access to water for 15 min each day to re-establish baseline water intake. Finally, on day 8, long-  
139 term memory of the odor aversion was assessed by providing access to the almond- or banana-  
140 odorized water for 15 min, immediately followed by 15 min of water. The percentage of odorized  
141 water consumption with respect to the initial consumption of the same solution during conditioning  
142 was used as a measure of the strength of COA. In chemogenetic experiments, CD and HFD rats  
143 received either vehicle or CNO (i.p.) 45 min before the first presentation of scented water on day 5.

144

145 2.6 *Histology*

146 After the completion of behavioral testing, rats were deeply anaesthetized using a pentobarbital  
147 monosodic/lidocaine solution (20 mg/kg) before being transcardially perfused by ice-cold saline  
148 (0.9%) followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were post-fixed  
149 overnight in 4% paraformaldehyde and then switched in 0.1 M phosphate buffer saline (PBS) solution  
150 and stocked at -4 °C before slicing. Serial coronal sections (40 µm) were cut using a vibratome  
151 (VT1200S, Leica Microsystems). Free-floating sections were prepared by rinsing in 0.1 M PBS for 20  
152 min (4 x 5 min rinses), blocked for 1 h (PBS 0.1 M, 0.2% Triton-X, 4% normal goat serum) and placed  
153 in 1:1000 rabbit anti-RFP (red fluorescent protein; PM005, CliniSciences) at 4° C for 48 h. Sections  
154 were then washed in PBS for 20 min (4 x 5 min rinses), incubated in 1:200 AffiniPure rhodamine goat  
155 anti-rabbit (11-025-003; Jackson Immunoresearch) diluted in PBS for 2 h at room temperature and  
156 counterstained with 1:5000 Hoestch solution (bisBenzimide H 33258, Sigma-Aldrich). Sections were  
157 washed for 20 min in PBS (4 x 5 min rinses), mounted, and cover-slipped with Fluoromount-G  
158 (SouthernBiotech). Sections were imaged using a Nanozoomer slide scanner and analyzed with the  
159 NDP.view 2 freeware (Hamamatsu Photonics, Bordeaux Imaging Center).



160

## 161 2.7 Data analysis

162 The data never violated the homogeneity of variance measured with Levene's test or normality  
163 measured with the Shapiro–Wilk normality test. Weight was analyzed using two-way repeated  
164 measures ANOVA, followed by Bonferroni's *post hoc* tests. ORM and COA measures were analyzed  
165 using one- or two-way ANOVA followed by Bonferroni's *post hoc* tests (between groups  
166 comparisons) or Dunnett's *post hoc* tests (versus the CD-Vehicle group). ORM performance was also  
167 compared against 50% (no significant novel object exploration) using one-sample t-test. In case of  
168 missing values in the behavioral results from the ORM task (e.g. animal excluded for an absence of  
169 exploratory behavior), a Mixed-effect model was used instead for repeated measures analyses.  
170 Planned comparisons restricted to the HFD fed groups were done using multiple t-tests with  
171 Bonferroni's correction. Statistical analyses were carried out on GraphPad Prism version 7 and SPSS  
172 (IBM SPSS Statistics 25). All values were expressed as mean  $\pm$  standard error of the mean (SEM). The  
173 alpha risk of rejection of the null hypothesis was 0.05.

174

## 175 3 RESULTS

### 176 3.1 HFD intake significantly induces overweight

177 Upon arrival, animals were randomly divided to create two groups of similar body weight, and then  
178 exposed to either CD or HFD. HFD rats were significantly heavier than CD animals from 6 weeks of  
179 diet exposure until the completion of the experiments (2-way repeated measures ANOVA: Diet  $F_{1,78} =$   
180 23.9,  $p < 0.001$ ; Week  $\times$  Diet  $F_{12,546} = 11.2$ ,  $p < 0.001$ ; Bonferroni's *post hoc* tests: all  $p < 0.001$  from  
181 week 6; **Supplemental Figure 1**) as previously reported (Boitard et al., 2012, 2014, 2015, 2016).

182

### 183 3.2 HFD intake induces long-term ORM deficits when training takes place in a novel context

184 Without habituation, CD ( $n = 11$ ) and HFD-fed ( $n = 17$ ) rats were placed into a novel arena containing  
185 two novel and identical objects (**Figure 1B**). During the acquisition phase, all groups similarly  
186 explored the two objects (**Supplemental Figure 2A**; Diet  $F_{1,26} = 1.4$ ,  $p = 0.2$ ; Diet  $\times$  Retention Time  $F_{1,18}$   
187  $= 5.9$ ,  $p = 0.03$ ). Three or 24 h after training, rats were exposed to a familiar and a novel object. When  
188 tested 3 h after the acquisition phase, CD- and HFD-fed rats exhibited a similar higher exploration of  
189 the novel object (**Figure 1B**; one-sample t-test versus 50%: CD  $t_9 = 2.7$ ,  $p = 0.026$ ; HFD  $t_{16} = 2.4$ ,  $p =$   
190 0.03). However, when tested 24 h after the acquisition, only CD rats showed a significant preference

191 for the novel object (one-sample t-test versus 50%: CD  $t_7 = 2.4$ ,  $p = 0.047$ ; HFD  $t_{13} = 1.2$ ,  $p = 0.3$ ).  
192 Mixed-effect analysis confirmed a significant Diet x Retention time interaction ( $F_{1,18} = 4.9$ ,  $p = 0.04$ ;  
193 Diet  $F_{1,26} = 3.9$ ,  $p = 0.06$ ). More importantly, *post hoc* analyses confirmed a significant difference  
194 between the two diet groups when tested at 24 h ( $p < 0.05$ ) but not at 3 h ( $p > 0.9$ ; *Bonferroni's post*  
195 *hoc* tests), indicating that HFD consumption during adolescence impairs long-term, but not short-  
196 term, ORM when testing occurred without habituation to the arena.

197 We then evaluated whether previous habituation to the context would alleviate HFD-induced long-  
198 term ORM deficits. For this purpose, another batch of CD ( $n = 6$ ) and HFD-fed ( $n = 7$ ) rats was first  
199 tested in the ORM task at 24h after training without exposure to the training arena (context A). As  
200 previously observed, HFD rats tested without habituation showed a deficit in long-term ORM (one-  
201 sample t-test versus 50%: CD,  $t_5 = 6.0$ ,  $p = 0.001$ ; HFD,  $t_6 = 1.6$ ,  $p = 0.2$ ; **Figure 1C**). The same rats were  
202 then habituated during 3 days to a new training arena (context B) before being trained and tested for  
203 their ORM in this arena. Throughout context exposure, all rats maintained similar levels of locomotor  
204 activity (**Supplemental Figure 2B**; two-way repeated measures ANOVA: Diet  $F_{1,11} = 0.3$ ,  $p = 0.6$ ; Day  
205  $F_{3,33} = 0.8$ ,  $p = 0.5$ ; Diet x Day  $F_{3,33} = 0.7$ ,  $p = 0.6$ ) and increased their exploration of the center of the  
206 arena (Entries in the center: Day  $F_{3,33} = 3.2$ ,  $p = 0.04$ ; Diet  $F_{1,11} = 3.2$ ,  $p = 0.1$ ; Diet x Day  $F_{3,33} = 0.7$ ,  $p =$   
207  $0.6$ ; Time in the center: Day  $F_{3,33} = 2.5$ ,  $p = 0.07$ ; Diet  $F_{1,11} = 0.4$ ,  $p = 0.6$ ; Diet x Day  $F_{3,33} = 0.3$ ,  $p = 0.8$ )  
208 indicating efficient habituation. Context habituation decreased the time spent exploring novel  
209 objects during training in all rats (**Supplemental Figure 2C**; two-way repeated measures ANOVA:  
210 Habituation  $F_{1,11} = 5.6$ ,  $p = 0.04$ ) independently of their diet (Diet  $F_{1,11} = 0.5$ ,  $p = 0.5$ ; Diet x  
211 Habituation  $F_{1,11} = 1.1$ ,  $p = 0.3$ ). As a result of habituation, both groups significantly preferred to  
212 explore the novel object than the familiar one during the test (one-sample t-test versus 50%: CD,  $t_5 =$   
213  $5.5$ ,  $p = 0.003$ ; HFD,  $t_5 = 4.1$ ,  $p = 0.007$ ). Two-way repeated measures ANOVA on ORM test with and  
214 without habituation revealed that the time exploring the novel object was increased selectively in  
215 the HFD group after habituation (**Figure 1C**; Diet  $F_{1,11} = 8.8$ ,  $p = 0.01$ ; Diet x Habituation  $F_{1,11} = 5.8$ ,  $p =$   
216  $0.04$ ). Indeed, HFD rats tested without habituation presented a lower exploration of the new object  
217 compared to the CD group ( $p < 0.01$ ; *Bonferroni's post hoc* test), whereas, after habituation, HFD and  
218 CD animals similarly preferred to explore the novel object than the familiar one ( $p = 0.6$ ; *Bonferroni's*  
219 *post hoc* test), demonstrating in this case an intact long-term ORM.

220

221 3.3 Chemogenetic inactivation of the ventral hippocampus, but not the basolateral amygdala,  
222 rescues HFD-induced recognition memory impairment

223 We then investigated the contribution of the vHPC and the BLA in **periadolescent-HFD-induced**  
224 memory alterations, using a chemogenetic approach involving the targeted expression of the  
225 inhibitory DREADD hM4Di (**Figure 2**). After histological analyses, HFD rats presenting bilateral hM4Di-  
226 mCherry expression in either the vHPC (n = 15) or in the BLA (n = 13) were kept for the statistical  
227 analyses. These rats were further divided depending if they received vehicle (vHPC, n = 8; BLA, n = 8)  
228 or CNO injection (HFD-vHPC-CNO, n = 7; HFD-BLA-CNO, n = 5). An additional group of HFD-fed rats  
229 did not receive any virus injection to control for the specificity of the CNO effects on behavioral  
230 measures (n=26). They were either injected with vehicle (n=11) or CNO (HFD-No DREADD-CNO, n =  
231 15). The HFD-fed groups injected with vehicle (with DREADD, n = 16; without DREADD, n = 11) were  
232 pooled to form **an** HFD group receiving vehicle (HFD-Vehicle, n = 27). Finally, control CD (with or  
233 without DREADD) rats which received vehicle injections were pooled to provide a single CD-Vehicle  
234 group (n = 21). To summarize, the statistical analyses were performed on the following 5 final  
235 groups: CD-Vehicle, n = 21; HFD-Vehicle, n = 27; HFD-No DREADD-CNO, n = 15; HFD-vHPC-CNO, n = 7;  
236 HFD-BLA-CNO, n = 5.

237 Four weeks after surgery, long-term ORM was tested without habituation using the exact same  
238 procedure as previously described (**Figure 3A**). All rats received an injection of either CNO or its  
239 vehicle 45 min before the acquisition phase. CNO injection did not alter the exploration of the  
240 objects during this phase (**Supplemental Figure 3A**;  $F_{4,70} = 2.2$ ,  $p = 0.08$ ). **HFD-Vehicle exhibited an**  
241 **absence of long-term ORM when tested 24 h after acquisition (one-sample t-test versus 50%:  $t_{26} =$**   
242 **1.9,  $p = 0.07$ ), whereas CD-vehicle animals showed a higher exploration of the novel object (**Figure****  
243 **3B; one-sample t-test versus 50%; CD-Vehicle  $t_{20} = 7.8$ ,  $p < 0.001$ ) as all HFD groups receiving CNO**  
244 **(HFD-No DREADD-CNO  $t_{14} = 4.6$ ,  $p < 0.001$ ; HFD-vHPC-CNO  $t_6 = 6.7$ ,  $p < 0.001$ ; HFD-BLA-CNO  $t_4 = 4.1$ ,  $p$**   
245 **= 0.02).**

246 **Group comparisons confirmed that only the HFD-Vehicle group exhibited a lower ORM performance**  
247 **than CD control rats (one-way ANOVA:  $F_{4,70} = 9.0$ ,  $p < 0.001$ ; Dunnett's *post hoc* tests versus CD-**  
248 **Vehicle: HFD-Vehicle  $p < 0.001$ , all other groups  $p > 0.3$ ). Further analyses restricted to the HFD**  
249 **groups showed that both HFD-No DREADD-CNO and HFD-vHPC-CNO groups exhibited a higher ORM**  
250 **performance than HFD-Vehicle rats ( $p < 0.05$  and  $p < 0.001$  respectively; t-test with Bonferroni's**  
251 **correction), suggesting a potential nonspecific CNO effect. However, the ORM performance of the**  
252 **HFD-No DREADD-CNO group remained lower than HFD-vHPC-CNO rats ( $p = 0.05$ ; t-test with**  
253 **Bonferroni's correction) indicating that silencing vHPC improved HFD-induced ORM deficits.**

254

255 3.4 Chemogenetic inactivation of the basolateral amygdala, but not the ventral hippocampus,  
256 prevents HFD-induced aversion memory enhancement

257 The results of the previous experiment raised an issue regarding the efficiency of our BLA silencing  
258 procedure. We therefore investigated in the same rats the impact of chemogenetic silencing of BLA  
259 and vHPC projecting neurons on the enhancement of aversion memory induced by periadolescent  
260 HFD exposure using a COA procedure (Boitard et al., 2015, 2016; Figure 4A; CD-Vehicle, n = 18; HFD-  
261 Vehicle, n = 23; HFD-No DREADD-CNO, n = 9; HFD-vHPC-CNO, n = 7; HFD-BLA, n = 5). Neither the diet  
262 nor the injection of CNO/vehicle affected the consumption of odorized water during its first  
263 presentation, i.e. odor-malaise association, compared to their respective water baseline  
264 consumption (Figure 4B; one-way ANOVA Group  $F_{4,57} = 0.9$ ,  $p = 0.5$ ; see also Supplemental Figure  
265 3B), and all groups presented a low level of neophobia toward the new odorized water (all  $p > 0.09$ ;  
266 one-sample t-test versus 100% water baseline). However, the long-term aversion memory was  
267 differently impacted by HFD and chemogenetic BLA or vHPC silencing during the odor-malaise pairing  
268 (Figure 4C; one-way ANOVA Group  $F_{4,57} = 3.9$ ,  $p = 0.007$ ; see also Supplemental Figure 3C). As  
269 previously observed (Boitard et al., 2015, 2016), there was a stronger aversion memory in HFD-  
270 Vehicle group as compared to the control CD group ( $p = 0.05$ , Dunnett's *post hoc* test). Further  
271 analysis restricted to the HFD groups showed that BLA silencing reduced the aversion memory as  
272 compared to HFD-Vehicle and HFD-No DREADD-CNO groups ( $p = 0.004$  and  $p = 0.03$ , respectively;  
273 Bonferroni's corrected multiple t-tests), whereas vHPC silencing had no effect ( $p > 0.9$ ). These results  
274 demonstrate that BLA activity controls HFD-induced aversive memory enhancement.

275

276 4 DISCUSSION

277 Here, we demonstrated that periadolescent HFD consumption (from weaning to adulthood) induced  
278 long term memory alterations. Specifically, we showed that HFD-fed rats presented a deficit in long-  
279 term, but not short-term, ORM when they are exposed to a novel context. Using chemogenetic  
280 silencing with high accuracy in anatomical boundaries, we found that manipulation of the vHPC, but  
281 not of the BLA, restored HFD-induced long-term ORM deficit. On the contrary, chemogenetic  
282 silencing of the BLA, but not of the vHPC, prevented the increased aversive memory observed in  
283 HFD-fed animals.

284

285 *4.1 Effects of periadolescent high-fat diet on object recognition memory*

286 The effects of HFD on object-related memory has led to contradictory results (for reviews see Abbott  
287 et al., 2019; Cordner & Tamashiro, 2015). The present study indicates that 12 weeks of exposure to  
288 HFD, starting at weaning, is sufficient to alter rats' object memory but only under certain conditions.  
289 Indeed, we observed that periadolescent HFD impaired long-term ORM tested 24h after sampling  
290 novel objects in a novel context but had no effect on short-term ORM tested 3 hours after training.  
291 These results are consistent with previous studies indicating no effect of HFD on short-term ORM  
292 (Beilharz et al., 2014, 2016; Kendig et al., 2019; Kosari et al., 2012; Lavin et al., 2011; McLean et al.,  
293 2018; Tran & Westbrook, 2015, 2017, 2018; Tucker et al., 2012), but an impairment of long-term  
294 object memory (Ayabe et al., 2018; de Andrade et al., 2017; Mucellini et al., 2019; Wang et al., 2016;  
295 Zuloaga et al., 2016). This differential impact of diet suggests a specific effect of periadolescent HFD  
296 on memory consolidation processes. Interestingly, we previously reported a similar effect on  
297 consolidation of spatial memories (Boitard et al., 2014, 2016) and emotional memories (Boitard et  
298 al., 2015), but also of object location memory (Khazen et al., 2019), which suggests that HFD  
299 consumption during early life periods could interfere with cellular substrates specifically involved in  
300 memory consolidation.

301 Importantly, we also identified that habituation to the arena prevented HFD-induced long-term ORM  
302 deficits. This could explain the absence of HFD-induced **memory deficits** reported in the literature  
303 after habituation and/or repeated tests **using ORM or object location memory (Heyward et al., 2012,**  
304 **2016; Tran & Westbrook, 2017; Tucker et al., 2012)**. Prior habituation and exploration of the arena is  
305 known to reduce the processing of contextual information during memory consolidation (Cohen &  
306 Stackman, 2015; Oliveira et al., 2010) and the arousal component of the task (Maroun & Akirav,  
307 2008; Okuda et al., 2004; Roozendaal et al., 2006). Then, the specific diet-induced deficit of long-  
308 term ORM reported here may be supported by differential neurobiological substrates involved in  
309 multiple memory systems, particularly the hippocampus and the amygdala.

310

311 *4.2 Effects of chemogenetic manipulation of vHPC and BLA on periadolescent HFD-induced object*  
312 *recognition memory deficits*

313 The hippocampus and the amygdala play a crucial role in long-term ORM (Cohen & Stackman, 2015;  
314 Roozendaal et al., 2008) and are profoundly affected by exposure to HFD during the periadolescent  
315 period (Del Olmo & Ruiz-Gayo, 2018; Morin et al., 2017; Murray & Chen, 2019; Reichelt, 2016;  
316 Reichelt & Rank, 2017). We therefore wondered whether chemogenetic inhibition of **projecting,**

317 putative excitatory, neurons in these two brain regions could alleviate the diet effects on object  
318 memory.

319 Recent studies have suggested that CNO may be metabolized *in vivo* to clozapine, an atypical  
320 antipsychotic drug, able to interact with DREADD receptors but also to induce non-DREADD related  
321 effects (Gomez et al., 2017; Ilg et al., 2018; MacLaren et al., 2016). To rule out this possibility, we  
322 included a control group which received CNO at a dose known to induce marginal behavioral effect.  
323 Our results showed a slight improvement in long-term ORM in this group which suggests that  
324 metabolism of CNO to clozapine might induce some behavioral effects as shown following clozapine  
325 administration (Addy et al., 2005; Mutlu et al., 2011). This pattern of results in the control group  
326 cannot however account for the complete restoration of ORM deficits following chemogenetic  
327 inhibition of vHPC, hence highlighting the central role of this brain region in HFD-induced long-term  
328 ORM deficits.

329 Such results are in agreement with previous research which has demonstrated that long-term ORM,  
330 but not short term ORM, relies on the hippocampus (for review see Cohen & Stackman, 2015).  
331 Moreover, previous studies have shown that hippocampal manipulations have a greater impact on  
332 long-term ORM when performed in an unfamiliar context (Kim et al., 2014; Oliveira et al., 2010),  
333 whereas the perirhinal cortex is crucial in both familiar and unfamiliar contexts (Kim et al., 2014).  
334 These results suggest that, in a novel context, novel objects may be encoded as part of the context  
335 thereby involving the hippocampus, whereas if the novel objects are presented in a familiar  
336 environment they are encoded under a process that probably does not involve contextual  
337 information processing and therefore does not rely on the hippocampus. We could then hypothesize  
338 that HFD-fed animals did not exhibit a memory deficit when they were previously habituated to the  
339 arena, as in that case ORM performance is not dependent on a dysfunctional hippocampus.

340 Habituation to the training context also greatly influences the impact of emotional arousal and  
341 consequently the BLA involvement in long-term ORM (Maroun & Akirav, 2008; Roozendaal et al.,  
342 2006). However, chemogenetic BLA silencing did not have a greater effect than those of CNO alone  
343 on HFD-induced ORM deficit. The amygdala is one of the major target of vHPC projection neurons  
344 (Pitkänen et al., 2000), suggesting that the vHPC-to-BLA pathway is not involved in the beneficial  
345 effect of silencing vHPC projecting neurons on long-term ORM. The vHPC involvement in memory  
346 processes also involves other projections to the nucleus accumbens or the ventromedial prefrontal  
347 cortex (Barker et al., 2019; Hsu et al., 2018; Okuyama et al., 2016; Phillips et al., 2019) and future  
348 studies are warranted to determine the role of these circuits in HFD-induced memory deficits.

349

350 4.3 *Effects of chemogenetic manipulation of BLA and vHPC on periadolescent HFD-induced aversive*  
351 *memory enhancement*

352 Few studies have examined the effects of HFD on aversive memory. Aversive cue-based memory is  
353 highly dependent on the BLA (LeDoux, 2003; McGaugh, 2004; Paré, 2003). We previously found that  
354 periadolescent HFD enhanced long-term, but not short-term, odor aversion memory as well as long-  
355 term auditory fear memory (Boitard et al., 2015, 2016). Here we replicate this finding and we provide  
356 evidence that chemogenetic silencing of the BLA, but not the vHPC, **prevented** the increased odor  
357 aversion memory observed in the HFD group. It is noticeable that, contrary to the ORM, CNO  
358 injection alone (without any DREADDs) did not have any effect by itself on HFD-induced aversive  
359 memory enhancement.

360 It is generally considered that during emotional arousal the activity of the BLA is modulated by  
361 glucocorticoids and noradrenaline, and eventually impacts aversive memory via glutamatergic  
362 projections to other structures, including the hippocampus (McEwen et al., 2016; McGaugh, 2004). In  
363 this context, we previously found that blockade of glucocorticoid receptors in the BLA is able to  
364 normalise the enhanced aversive memory of HFD group (Boitard et al., 2015). Taken together, these  
365 data suggest that periadolescent HFD consumption increases the activation of BLA through  
366 glucocorticoids in response to emotional experience, leading to an enhanced odor aversion memory.  
367 Furthermore, a recent study showed that chemogenetic inactivation of the noradrenergic pathway  
368 from the locus coeruleus to the BLA abolished aversive memory enhancement, but not ORM  
369 impairment, induced by chronic pain (Llorca-Torrallba et al., 2019). According to the differential effect  
370 of chemogenetic BLA silencing on aversive memory and ORM in HFD-fed rats, a similar impact of  
371 periadolescent HFD on the noradrenergic modulation of BLA may also be involved.

372 In contrast, chemogenetic inactivation of the vHPC did not modify odor aversion memory in HFD-fed  
373 animals. Even though the BLA is highly connected to the vHPC (Pitkänen et al., 2000) and that the  
374 BLA-to-vHPC pathway plays a central role in emotional processes (Beyeler et al., 2016; Felix-Ortiz et  
375 al., 2013; Rei et al., 2015), our results suggest that the HFD-induced enhancement of aversive  
376 memories may rather involve projections to the nucleus accumbens (Beyeler et al., 2016; Stuber et  
377 al., 2011) or to the ventromedial prefrontal cortex (Burgos-Robles et al., 2017; Felix-Ortiz et al.,  
378 2013).

379 Altogether our results demonstrate in periadolescent HFD-fed rats, that silencing vHPC, but not BLA,  
380 improves long-term ORM deficits, while silencing BLA, but not vHPC, **prevents** COA enhancement.  
381 This double dissociation suggests that vHPC and BLA, though related structures, can have distinct and  
382 independently-driven functions in HFD-induced memory changes.

383

#### 384 4.4 Conclusions

385 The adolescent brain is highly sensitive and prone to cognitive alterations promoted by diets rich in  
386 fat and/or sugar (for reviews see Del Olmo & Ruiz-Gayo, 2018; Morin et al., 2017; Murray & Chen,  
387 2019; Noble & Kanoski, 2016; Reichelt, 2016; Reichelt & Rank, 2017). Our study demonstrates that  
388 periadolescent HFD alters long-term memory processes, impairing recognition memory through  
389 vHPC-dependent processes while enhancing emotional memory through BLA specific effects. Such  
390 bidirectional effect on hippocampal and amygdala memory functions have also been reported in  
391 chronic stress and post-traumatic stress disorder (Elzinga & Bremner, 2002; Kaouane et al., 2012;  
392 Layton & Krikorian, 2002; Mahan & Ressler, 2012). Interestingly, obesity is linked to a higher  
393 prevalence of post-traumatic stress disorder, especially during adolescence (Pagoto et al., 2012;  
394 Perkonig et al., 2009). Future investigation is necessary to evaluate how HFD consumption during  
395 adolescence impacts preferentially the medial temporal lobe, and how the potential alterations of  
396 specific hippocampal and amygdala circuits may mediate the cognitive impact of juvenile obesity.

397

#### 398 5 AUTHORS CONTRIBUTION

399 C.B.B, G.P.L., E.C. and G.F. acquired funding; F.N., E.C. and G.F. designed research; F.N., I.B., M.S.Z.  
400 performed research; F.N., I.B., E.C. and G.F. analysed data; E.C. and G.F. supervised research; F.N.,  
401 I.B., E.C. and G.F. wrote the manuscript; C.B.B, M.S.Z. and G.P.L. edited and approved the manuscript.

402

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419

## 420 8 ANNEXE 1

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436

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778

779 CAPTIONS

780 **Figure 1. Periadolescent HFD exposure altered long-term object recognition memory.** (A) Schematic  
781 representation of the experimental design. Rats had *ad libitum* access to either CD (grey bars) or HFD  
782 (red bars) from weaning to adulthood. All behavioral testing occurred at adulthood after at least 12  
783 weeks of diet. (B) Periadolescent HFD exposure alters long-term (24h testing), but not short-term (3h  
784 testing) **object recognition memory (ORM)**. (C) Diet-induced ORM deficit at 24h is abolished when  
785 the animals were previously habituated to the arena. Data are represented as mean  $\pm$  SEM **and**  
786 **circles show individual data points**. \*  $p < 0.05$ , \*\*  $p < 0.01$  (one sample t-test versus 50%), #  $p < 0.05$ ,  
787 ##  $p < 0.01$  (Diet effect, two-way ANOVA followed by **Bonferroni's post hoc** tests).

788

789 **Figure 2. Chemogenetic targeting of the ventral hippocampus or the basolateral amygdala.** *Left,*  
790 Representative images illustrating the placement of AAV8-CaMKII-hM4Di-mCherry expression in the  
791 **ventral hippocampus (vHPC, A) and basolateral amygdala (BLA, B)**. Insets represent magnification of  
792 the area of interest (white square). *Right,* Schematics adapted from Paxinos and Watson (2013)  
793 showing the largest (light pink) and smallest (dark pink) viral infection for rats included in **behavioral**  
794 experiments.

795

796 **Figure 3. Chemogenetic inhibition of the ventral hippocampus, but not the basolateral amygdala,**  
797 **restored long-term ORM induced by periadolescent HFD exposure.** (A) Schematic representation of  
798 the experimental design. Rats had *ad libitum* access to either CD or HFD from weaning to adulthood.  
799 DREADD surgery was performed at adulthood (7-8 weeks of diet) and rats recovered 4 weeks before  
800 the start of behavioral testing. (B) Long-term ORM performance in HFD-fed rats treated with vehicle  
801 (red bars;  $\pm$  indicating with or without DREADD expression), CNO but without DREADD expression  
802 (orange bars), or CNO with DREADD expressed in the vHPC (dark blue) or the BLA (light blue).  
803 Expression of the inhibitory DREADD hM4Di is depicted by structure (vHPC or BLA), except for the  
804 vehicle group. **Data are represented as mean  $\pm$  SEM and circles show individual data points. ###  $p <$**   
805 **0.001 (one-way ANOVA followed by Dunnett's post hoc test vs. CD-Vehicle), \*  $p \leq 0.05$ , \*\*\*  $p < 0.001$**   
806 **(planned t-tests with Bonferroni's correction).**

807

808

809



810 **Figure 4. Chemogenetic inhibition of the basolateral amygdala, but not the ventral hippocampus,**  
811 **prevented enhanced aversion memory induced by periadolescent HFD exposure.** (A) Schematic  
812 representation of conditioned odor aversion (COA) protocol and chemogenetic inhibition of vHPC or  
813 BLA before COA conditioning. (B) Neither HFD or CNO injection impacted the consumption of  
814 odorized water during the conditioning phase (in percentage of water baseline consumption). (C)  
815 Long-term COA memory in HFD-fed rats treated with vehicle (red bars;  $\pm$  indicating with or without  
816 DREADD expression), CNO but without DREADD expression (orange bars), or CNO with DREADD  
817 expressed in the vHPC (dark blue) or the BLA (light blue). Expression of the inhibitory DREADD hm4Di  
818 is depicted by structure (vHPC or BLA), except for the vehicle group. **Data are represented as mean  $\pm$**   
819 **SEM and circles show individual data points. #  $p < 0.05$  (one-way ANOVA followed by Dunnett's *post***  
820 ***hoc* test vs. CD-Vehicle), \*  $p < 0.05$ , \*\*  $p < 0.01$  (planned t-tests with Bonferroni's correction).**

821

# 1. Diet exposure

Adolescence



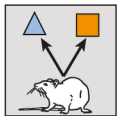
High-fat diet



Adulthood

# 2. Memory tests

Object recognition memory



Conditioned odor aversion

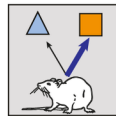


# 3. Chemogenetic (DREADD) manipulations

Chemogenetic inhibition of the **ventral hippocampus** or the **amygdala**



Object recognition memory



Conditioned odor aversion

