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Enduring effects of an unhealthy diet during adolescence on systemic but not neurobehavioural measures in adult rats.

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Enduring effects of adolescent cafeteria diet on systemic but not neurobehavioural measures in adult rats.

The adolescent period is an important stage of maturation for various brain structures. It is during this time therefore that the brain may be more vulnerable to environmental factors such as diet that may influence mood and memory. Diets high in fat and sugar (termed a cafeteria diet) during adolescence have been shown to negatively impact upon cognitive performance, which may be reversed by switching to a standard diet during adulthood. Consumption of a cafeteria diet increases both peripheral and central levels of interleukin-1β (IL-1β), a pro-inflammatory cytokine which is also implicated in cognitive impairment during the ageing process. It is unknown whether adolescent exposure to a cafeteria diet potentiates the negative effects of IL-1β on cognitive function during adulthood. To address this, rats were fed with a cafeteria diet during the adolescent period after which time they received a lentivirus injection in the hippocampus to induce chronic low-grade overexpression of IL-1β. After viral integration, metabolic parameters, circulating and central pro-inflammatory cytokine levels, and cognitive behaviours were assessed. Our data demonstrate that rats fed the cafeteria diet exhibit metabolic dysregulations in adulthood, which were concomitant with low-grade peripheral and central inflammation. Overexpression of hippocampal IL-1β in adulthood impaired spatial working memory. However, adolescent exposure to a cafeteria diet, combined with or without hippocampal IL-1β in adulthood does not induce any lasting cognitive deficits in spatial working memory, recognition memory or locomotor activity when the diet was replaced with a standard diet in adulthood. Taken together, these data demonstrate that cafeteria diet consumption during adolescence induces metabolic and inflammatory changes, but not behavioural changes in adulthood.

Keywords: adolescence; diet; inflammation; metabolism; IL-1β; hippocampus; cognition

1 **1. Introduction**

2 Adolescence is a vulnerable period of neurobehavioural modelling essential for
3 life-long cognitive processing. Despite no fixed markers for adolescence, in rodents this
4 period is considered to be between post-natal day (PND)21 and approximately PND60
5 and in humans from ages 12 to 20[1]. Adolescence is a period of physiological, sexual and
6 neurological changes that are necessary for health in the longer term, but this period is
7 sensitive to environmental challenges such as stress or diet[2]. An increasing body of
8 evidence demonstrates a significant role of dietary habits during adolescence in overall
9 well-being, including brain health[3]. However, the changes in human dietary habits over
10 the last 50 years are potentially negatively impacting adolescent brain development.

11 In developed countries in particular, over consumption of processed foods rich in
12 saturated fat and sugar are becoming a major health problem[4]. The emergence of this
13 'cafeteria diet' (rich in fat and sugar) has led to an increase in the prevalence of obesity
14 and metabolic disturbances, as well as cognitive and emotional disorders[5]. The
15 deleterious effects of a high fat/high sugar diet have been largely documented in rodents.
16 For example, adult rats fed a cafeteria diet for 15 weeks develop obesity with glucose
17 intolerance and inflammation[6], and in adolescent rats it induces hyperinsulinemia with
18 insulin resistance[7].

19 The hippocampus, which is involved in learning, memory and the regulation of
20 emotional responses is particularly affected by an increase of fat and sugar intake. It has
21 been shown that a high fat/high sugar diet reduces hippocampal neurogenesis[8], decreases
22 expression of BDNF and synapsin[9], and induces neuroinflammation in rodents[10]. These
23 alterations are correlated with behavioural impairments in hippocampal dependent
24 processes, mainly when the diet is consumed during adolescence. For example,
25 adolescent rats fed a diet supplemented with fat or sugar display impairments in

26 hippocampal-associated pattern separation[11], and hippocampal-dependent place
27 recognition memory[12]. Additionally, adolescent male rats fed a diet supplemented with
28 sugar display increased anxiety-like behaviours[13] and impairments in reward-related
29 behaviours in adulthood[14]. Similar observations have been made in humans in that
30 adolescents consuming high fat and high sugar diets display impaired visuospatial
31 learning and memory[15]. Recent evidence has suggested that deleterious effects of a high
32 fat diet on hippocampal-associated cognition during adolescence can be reversed in
33 adulthood by a simple switch back to a standard diet[16]. Thus, it could be that side effects
34 related to poor dietary habits are transient, and that the adolescent brain is resilient.
35 Nevertheless, it is still unknown whether exposure to a high fat/high sugar diet during
36 adolescence could act as an aversive stimulus, which could pre-condition the brain's
37 reaction to an insult or a disease in later years.

38 Hippocampal function is impaired in response to various stimuli and in particular
39 to neuroinflammation. Interleukin-1β (IL-1β) is a pro-inflammatory cytokine
40 predominantly produced by microglia that orchestrates the neuroinflammatory
41 response[17]. The hippocampus is very sensitive to levels of IL-1β locally because its
42 cognate receptor IL-1R1 is expressed at high levels within this structure[18]. At
43 physiological levels, IL-1β is necessary for memory formation[19], but a transgenic
44 overexpression of IL-1β induces impairments in both spatial and contextual memory[20] as
45 well as pattern separation[21]. It is now well established that chronically heightened levels
46 of hippocampal IL-1β are implicated in development and progression of
47 neurodegenerative disease[22] and psychiatric disorders[23]. After adolescence and during the
48 ageing process, the hippocampus may be vulnerable to stimuli that induce and maintain
49 elevated levels of IL-1β. We propose that a transient exposure to negative regulator of
50 systemic and hippocampal function during adolescence such as a cafeteria diet, may

51 negatively prime the brain to such a neuroinflammatory state at a later time point. Thus,
52 this study examined whether transient consumption of a cafeteria diet during adolescence
53 followed by a chronic hippocampal IL-1β overexpression in early adulthood impacted
54 adult systemic and brain functions.
55
56 **2. Methods**
57 ***2.1: Animals***
58 Male Sprague-Dawley rats were bred in-house (Biological Services Unit, UCC) and were
59 weaned at postnatal day (PND) 21. Weaned rats were pair housed in a colony maintained
60 at 22 ± 1°C with a 12-hour light-dark cycle (lights on at 7:30am). All animal procedures
61 were performed under licenses issued by the Health Products Regulatory Authority
62 (HPRA, Ireland), in accordance with the European Communities Council Directive
63 (2010/63/EU) and approved by the Animal Experimentation Ethics Committee of
64 University College Cork.
65
66 ***2.2: Experimental design and diet***
67 Rats were randomly divided into four experimental groups; standard chow fed animals
68 injected with an mCherry expressing lentivirus (Ctrl; n=8), cafeteria diet fed animals
69 injected with an mCherry expressing lentivirus (Caf. Diet; n=10), standard chow fed
70 animals injected with an IL-1β expressing lentivirus (Ctrl - IL-1β; n=10), and cafeteria
71 diet fed animals injected with an IL-1β expressing lentivirus (Caf. diet - IL-1β; n=10)
72 (Figure 1A). The cafeteria diet consisted of high fat and high sugar components and was
73 given in addition to standard chow to diet fed rats. Foods were weighed prior to being
74 placed in the cages and were given in excess. The leftover amount of each component
75 was weighed after 24 hours to determine the amount consumed by rats in each cage.

76 Standard chow was also weighed daily to determine consumption by rats in all cages (See
77 supplementary table 1 for a list of all foods and associated nutritional information).
78 Cafeteria diet began at PND28 and ended at PND56 when the animals underwent
79 stereotaxic surgery. Standard chow was given for the remainder of the study (Figure 1A).
80

81 ***2.3: Stereotaxic surgery***

82 Rats were anaesthetised with isoflurane/O_2 (1.5–2.5%) and placed on a stereotaxic
83 apparatus (Kopf) on a heating mat. Lentiviruses purchased from Genecopoeia (MD,
84 USA) to overexpress mCherry (Lenti-mCherry: Cat# LP-NEG-Lv80-0205-cs; 2.42 x 10^8
85 copies/ml) or mCherry-IL-1β (Lenti-IL-1β-mCherry: Cat# LP-Mm03282-Lv80-0205-cs;
86 1.93 x 10^7 copies/ml) were injected (3μL of each) into the dorsal hippocampus using the
87 coordinates AP: -3.5 mm, ML: ± 2.4 mm, DV: -3.8 mm relative to Bregma[21] at a rate of
88 1μL/min followed by a 5 min diffusion. Following lentiviral injection, incisions were
89 sutured, the wound was treated with antibacterial ointment (Fucithalmic® 10 mg/g), and
90 rats were administered the analgesic carprofen (Rimadyl® 5 mg/kg, s.c., Zoetis Ireland
91 Ltd) and 5% glucose solution. All rats were allowed to recover for 3 weeks with *ad libitum*
92 access to standard chow and water to allow viral uptake prior to the beginning of
93 behavioural testing.
94

95 ***2.4 Behavioural testing***

96 Behavioural testing started 3 weeks after the stereotaxic surgery (PND78). All
97 behavioural tests were conducted during the light phase.
98

99 *2.4.1: Open field*

100 The openfield was used to measure locomotor activity and anxiety like behaviour. Rats
101 were placed in an open field arena (90 cm diameter) under bright lighting conditions
102 (approx. 1000lux) for 10 min. Distance travelled in the arena as well as time spent and
103 the number of visits into a virtual center zone (defined as the inner 30% core of the arena)
104 was recorded using the EthoVision software (XT 8.5 version, Noldus, USA). The arena
105 was cleaned with a 50% ethanol solution between each animal to remove odour cues.
106
107 *2.4.2: Spontaneous Alternation in the Y maze*
108 The spontaneous alternation in the Y-maze test was used to measure spatial working
109 memory. The Y maze consisted of three arms (120° from each other, 50 cm x 10 cm x
110 30 cm) which were connected to form a triangular space in the centre (10 cm x 10 cm x
111 10 cm). Briefly, each animal underwent one trial and the Y-maze was cleaned with 50%
112 ethanol between animal trials. The rat was placed into the outward-extending end of one
113 arm (always the same) facing the wall and was then allowed free exploration of the maze
114 for 5 min. Arms were numbered (1–3) and the sequence of arm entries was recorded
115 manually during the test, where an arm entry was defined by the four-paw criterion. A
116 spontaneous alternation is defined as the consecutive entry in all three arms. The
117 percentage spontaneous alternations $\left(\% \, alternations = \left(\frac{\text{n alternations}}{\text{n visits}}\right) * 100\right)$ within
118 5 min was calculated.
119
120 *2.4.3 Novel Object Recognition*
121 Novel object recognition was assessed as described[24]. On day 1, rats were habituated to
122 the testing arena (L40.5 cm × W36.5 cm × H28.0 cm) under dim light (20 lux) for 10 min.
123 On day 2, rats were placed in the testing arena with 2 identical objects (i.e. ceramic mugs
124 or glass bottles) for 10 min. After a 3-hour inter-trial interval, one familiar object was

125 replaced with a novel object, and each animal was introduced in the testing arena for a 5
126 min exploration period. All behaviours were recorded, and videos were scored to
127 determine the amount of time the rats spent exploring the novel vs. familiar objects. Time
128 spent with the objects was recorded, and a discrimination ratio (DR) of object recognition
129 was calculated as DR= $\frac{Time\ exploring\ the\ novel\ object}{(Time\ exploring\ novel\ object + familiar\ object)}$.

130

131 ***2.5 Blood measurements***

132 Tail blood samples were taken before the start of the diet (PND28), and immediately at
133 the end of the diet (PND56). Trunk blood was taken at cull (PND98).

134

135 *2.5.1 Metabolic hormones*

136 Concentrations of metabolic hormones were measured in duplicate by ELISA (Millipore
137 for leptin and insulin; R&D systems for adiponectin) in plasma collected from cafeteria
138 diet fed animals only at PND28, PND56 and PND98 according to the manufacturers'
139 guidelines.

140

141 *2.5.2 Cytokines*

142 The concentrations of inflammatory cytokines (interleukin-1β (IL-1β), IL-4, IL-6, IL-10,
143 tumor necrosis factor-α (TNFα) and interferon-γ (IFNγ)) was measured in plasma
144 collected at PND28, PND56 and PND98, using an electrochemoluminescence (ECL)-
145 based assay (V-plex, MesoScale Discovery, Rockville, MD, USA) following the
146 manufacturer's instructions.

147

148

149

150 ***2.6 Tissue collection***

151 At PND98 rats were sacrificed by rapid decapitation. Freshly dissected brain region
152 (hippocampus, prefrontal cortex and hypothalamus) were processed for molecular
153 analysis as described below.

154

155 *2.6.1 Quantitative Reverse-Transcription PCR (RT-qPCR)*

156 Total RNA was extracted from hippocampal, prefrontal cortex and hypothalamus tissue
157 using the mirVanaTM total RNA extraction kit (Ambion/Life Technologies) and treated
158 using a Turbo DNA-free kit (Ambion/life technologies) as per the manufacturer's
159 instructions. Total RNA yield and purity were determined using the Nanodrop System
160 (Thermo Scientific). Synthesis of cDNA was performed using the high capacity cDNA
161 reverse transcription kit (Applied Biosystems) using the SureCycler® 8800 (Agilent
162 Technologies) and diluted to a final concentration of 10ng/µl. All qPCR was performed
163 in 3 technical replicates for each biological sample on a LightCycler® 480 Instrument II
164 (Roche). Each reaction consisted of 1µl of sample (5ng/µl), 5µl of Sybr MasterMix
165 (KiCqStart® SYBR® Green qPCR ReadyMix™ with ROX™ for ABI instruments,
166 Sigma-Aldrich), 0.1µl of both forward and reverse primers, and 3.8µl of RNase free H_2O.
167 Relative gene expression was adjusted to β-Actin and quantified using the $2^{-\Delta\Delta CT}$
168 method [28].

169

170 *2.6.2 Western Blot*

171 Total protein was extracted from hippocampal tissue using commercially available RIPA
172 Lysis and Extraction Buffer (Thermo Fisher Scientific, 89901) in combination with
173 CompleteTM Mini EDTA-Free Protease Inhibitors (Roche, 04693159001) in a 1:1 ratio
174 with dH_2O. Equal amounts of total proteins (30 µg), as determined by the Bradford

175 method (BioRad), were separated by 12% SDS-PAGE, then transferred to a nitrocellulose
176 membrane. Blots were incubated in PBS 0.1% Tween 20, 2% BSA with an antibody
177 directed against IL-1β (R&D systems, goat anti-mouse IL-1β polyclonal, 1:500) and β-
178 actin (Sigma, mouse polyclonal, 1:1000) and subsequently incubated with the appropriate
179 HRP-tagged secondary antibodies. Proteins were then visualised on exposure film using
180 ECL detection kit (GE healthcare). Densitometry analysis of the developed immunoblot
181 bands was carried out using ImageJ software. Intensity readings for each band were
182 expressed as arbitrary units relative to controls (standard chow fed non-IL-1β injected
183 rats).

184

185 *2.6.3 Histology*

186 Epididymal white adipose tissue (eWAT) was excised and weighed before freezing. Then
187 eWAT was fixed in PBS containing 4% paraformaldehyde for 24 h at 4°C, dehydrated
188 and embedded in paraffin. Following paraffin embedding, eWAT was sectioned at 8 μm
189 using a microtome (Leica), and tissue was stained with haematoxylin and eosin. The
190 numbers and size of cells from five random fields containing ~100 cells/field were
191 evaluated from four sections per rat per group using the Adiposoft plugin on ImageJ
192 software.

193

194 ***2.7. Statistical analysis***

195 Statistical analyses were performed using GraphPad Prism 5 software. Data was checked
196 for normality using the Shapiro-Wilk test. Two-way analysis of variance (ANOVA) tests
197 were used where appropriate to determine statistical significance followed a Bonferroni
198 post-hoc analysis. When the sample size was small ($n \leq 8$) and/or in case of non-normal
199 distribution, differences between more than two groups were analysed with Kruskal–

200 Wallis test followed by a post hoc Dunn's. Differences between two conditions were
201 assessed by the nonparametric Mann–Whitney test. Data are presented as means ±
202 standard error of the mean (SEM); statistical significance was set at *$P<0.05$, **$P<0.01$
203 and ***$P<0.001$.
204

205 **3. Results**

206 ***3.1. Cafeteria diet during adolescence increased calorie intake and metabolic hormone***
207 ***levels without affecting body weight***

208 Neither consumption of a cafeteria diet during adolescence or IL-1β overexpression in
209 the hippocampus in adulthood (in combination or alone) induced a significant change in
210 body weight for the duration of the study (Figure 1B). Animals that received the cafeteria
211 diet consumed foods with a significantly larger energy content than control animals
212 (***$p<0.001$, Figure 1C). Immediately after the end of the cafeteria diet, animals
213 consumed foodstuffs amounting to a lower energy content than control chow fed animals,
214 and this was mirrored by the amount of chow intake by animals that were previously fed
215 the cafeteria diet (***$p<0.001$, Figure 1C). This effect normalised within 2 weeks of
216 cessation of the cafeteria diet. Concentrations of the metabolic hormones insulin, leptin
217 and adiponectin were measured in the plasma from animals fed the cafeteria diet using
218 ELISA prior to the start of the diet (PND28), immediately after the end of the dietary
219 intervention (PND56), and at the end of the study (PND98). Consumption of the cafeteria
220 diet during adolescence induced a significant increase in the concentrations of both
221 insulin and leptin immediately after diet cessation (PND56) without affecting adiponectin
222 levels. Interestingly, 6 weeks after cessation of the diet (PND98), insulin, leptin and
223 adiponectin levels were still significantly higher in comparison to levels at baseline
224 (PND28) (Figure 1 D-G *$p<0.05$ **$p<0.01$ compared to PND28). The leptin/adiponectin

225 ratio is considered to be a reliable indicator of metabolic dysfunction and was increased
226 in cafeteria diet fed rats, immediately after diet cessation (at PND56). This increase was
227 persistent until the end of the study (at PND98) (Figure 1G $*p<0.05$ $**p<0.01$ compared
228 to PND28). The current data suggest that consumption of a cafeteria diet during
229 adolescence induces metabolic dysregulation. Moreover, a switch back to a standard diet
230 for 6 weeks is not sufficient to revert to baseline levels.
231

232 ***3.2. Validation of lentiviral-mediated increase in IL-1β in the hippocampus at PND98.***
233 To assess the efficacy of lentivirus-delivered IL-1β overexpression, the hippocampus was
234 dissected from rats at PND98 and protein expression of IL-1β was analysed by Western
235 blot. IL-1β protein expression in the hippocampus was significantly increased 6 weeks
236 after animals were injected with the lentivirus overexpressing mCherry-IL-1β (at PND98)
237 compared to rats injected with a lentivirus overexpressing mCherry, independent of diet
238 ($*p<0.05$, $**p<0.01$ vs ctrl, Figure 2A-B). Our previous studies using lentivirus
239 overexpressing mCherry and mCherry-IL-1β have established that there is minimal
240 damage at the injection site[21,25].
241

242 ***3.3. Cafeteria diet during adolescence induced an adipose tissue expansion associated***
243 ***with mild inflammation in the periphery, which persisted in adulthood.***
244 Because adipokine secretion is regulated by the white adipose tissue and is sensitive to
245 food intake, particularly to high fat and high sugar, we focused next on epididymal white
246 adipose tissue (WAT) physiology. The epididymal WAT mass (Figure 3A) and the
247 adipocyte surface area (Figure 3B-C) were significantly increased in cafeteria diet fed
248 rats compared to controls, independent of IL-1β hippocampal overexpression. The
249 expansion of adipose tissue relates to an enhanced secretion of systemic inflammatory

250 factors[29]. We observed that plasma levels of pro-inflammatory cytokines IL-6 and IFNγ
251 were increased by cafeteria diet consumption at PND56 and this increase persisted 6
252 weeks after cessation of the diet at PND98 (Figure 4B-C *$p<0.05$ **$p<0.01$ compared to
253 corresponding control group at PND28; $\$p<0.05$ compared to control at PND56). Levels
254 of IL-1β and TNFα in plasma remained unaffected by the diet (Figure 3D-E). The plasma
255 concentrations of IL-4 and IL-10 were below the detection threshold at the three time
256 points in all groups of animals (data not shown). The hippocampal overexpression of IL-
257 1β did not impact upon the systemic levels of cytokines.
258
259 ***3.4. Cafeteria diet during adolescence and IL-1β overexpression in the hippocampus***
260 ***during adulthood differentially affected the expression of inflammatory genes in the***
261 ***hippocampus and in the prefrontal cortex.***
262 We next investigated if consumption of a cafeteria diet during adolescence and/or IL-1β
263 overexpression in adulthood affected brain homeostasis by disrupting the
264 neuroinflammatory state. We assessed gene expression of pro-inflammatory cytokine IL-
265 6, IL-1β, TNFα and IFNγ in the hippocampus, prefrontal cortex and hypothalamus as
266 these brain regions have been shown to be affected by systemic inflammation after
267 consumption of a high fat/high sugar diet[10]. Consumption of a cafeteria diet during
268 adolescence did not impact mRNA expression of IL-1β, IL-6, TNFα and IFNγ in the
269 hippocampus compared to the hippocampus of control rats. Overexpression of IL-1β in
270 the hippocampus increased IL-1β and IFNγ mRNA expression. However, the increase in
271 IL-1β mRNA expression was abolished in the hippocampus of rats fed the cafeteria diet
272 during adolescence (Figure 5A *$p<0.05$ **$p<0.01$ compared to control group; #$p<0,05$
273 compared to IL-1β group). Consumption of the cafeteria diet during adolescence induced
274 an increase in mRNA expression of IL-1β and TNFα in the prefrontal cortex (Figure 5B

275 *$p<0.05$ compared to control group). IL-1β overexpression in the hippocampus did not
276 impact cytokine gene expression in the prefrontal cortex but when combined with pre-
277 exposure to a cafeteria diet during adolescence, it induced an increase in the mRNA
278 expression of IFNγ (Figure 5B #$p<0.05$ compared to IL-1β group). Interestingly, the
279 effect of the cafeteria diet on gene expression levels of cytokines in the hypothalamus
280 was not evident 6 weeks after the cessation of the diet (Figure S2).
281
282 ***3.5. Consumption of a cafeteria diet during adolescence and IL-1β overexpression in***
283 ***the hippocampus during adulthood did not induce anxiety-like behaviour while IL-1β***
284 ***alone impaired spatial working memory.***
285 To assess if consumption of a cafeteria diet during adolescence induced behavioural
286 changes during adulthood, rats underwent the openfield test to assess locomotor activity
287 and anxiety-like behaviour. Animals fed the cafeteria diet during adolescence with or
288 without hippocampal IL-1β overexpression in adulthood, or animals injected with IL-1β
289 did not show any changes in locomotor activity (Figure 6A) as evaluated by the distance
290 travelled in the openfield. Animals in all groups spent a similar percentage of time within
291 the centre of the openfield arena (data not shown). Spontaneous alternation behaviour, a
292 measure of hippocampal-dependent spatial working memory, was tested in the Y-Maze.
293 Consumption of a cafeteria diet has no significant effect on performance while adult rats
294 exposed to hippocampal IL-1β displayed a reduced spontaneous alternation rate
295 compared to controls (*$p<0.05$, Figure 6B). Animals in all groups exhibited a similar
296 number of arm entries (data not shown) indicating comparable activity and motivation
297 states. Finally, performance in the novel object recognition task was similar in animals
298 across all four groups (Figure 6C). All behavioural data in control animals are within
299 comparable ranges to what we have previously observed in non-injected control rats[26].

300 Our data suggest that exposure to a cafeteria diet during adolescence does not impact
301 upon cognitive function but rather appears to prime the adult brain against the deleterious
302 effect of IL-1β overexpression in spatial working memory in adulthood.
303
304 **4. Discussion:**
305 Evidence now suggests that consumption of a high fat/high sugar diet during
306 adolescence induces detrimental effects on hippocampal associated cognition[27,14,28]. Data
307 also suggest that the effect of a high fat diet on hippocampal cognition may be reversed
308 by switching the diet to a standard diet in adulthood[16]. In the context of a cafeteria diet
309 (high fat/high sugar), our data support this later finding. We demonstrate that 4 weeks of
310 cafeteria diet consumption during the adolescent period (PND28 – PND56) does not
311 result in cognitive deficits in adulthood when the animals were switched to a standard
312 diet at adulthood for 3 weeks prior to testing. However, the cafeteria diet fed animals still
313 maintained elevated levels of insulin, leptin and adiponectin, pro-inflammatory cytokines
314 (IL-6 and IFNγ) and white adipose tissue expansion 6 weeks after the cessation of the
315 diet. This peripheral effect was concomitant with an increase in pro-inflammatory
316 cytokines (IL-1β and TNFα) expression in the prefrontal cortex. We also demonstrate that
317 hippocampal overexpression of the proinflammatory cytokine IL-1β during adulthood
318 does not induce any cognitive impairments in cafeteria diet fed animals. However,
319 overexpression of IL-1β during adulthood after standard diet intake during adolescence,
320 induced impairments in spatial working memory. This behavioural effect was
321 complemented with an increase in IL- 1β and IFNγ expression in the hippocampus.
322 In the current study, 4 weeks of cafeteria diet intervention did not induce weight
323 gain in adolescent rats compared to those fed with standard chow. This result is consistent
324 with previously published studies in which a cafeteria diet was administered from

325 adolescence. Specifically, when a cafeteria diet intervention started at adolescence
326 (PND28, as in the current study), weight gain was only observed after 5[29], 9[30] or 12
327 weeks[31] of cafeteria diet consumption. One possible reason for a lack of weight gain
328 regardless of increased caloric consumption is that the adolescent period is characterized
329 by active growth associated with high energy consumption[32]. Interestingly, it has been
330 shown that cafeteria diet did not induce a significant increase in weight even after 9 weeks
331 of intervention, but induced metabolic dysregulation similar to what we observed in the
332 current study[7]. Indeed, we demonstrate that rats fed a cafeteria diet for 4 weeks during
333 adolescence exhibit elevated levels of insulin, leptin and adiponectin in the plasma 6
334 weeks after the diet was replaced with standard chow. The leptin/adiponectin ratio also
335 remained elevated at PND56 immediately after the diet ended, and persisted until PND98.
336 This persistent change reflects the overall metabolic state, as it has been shown to be more
337 consistent than independent plasma leptin and adiponectin concentrations and correlates
338 with many of the metabolic syndrome features[33]. It has been proposed that a high fat/high
339 sugar intake during adolescence may lead to a metabolic syndrome[34], which is a cluster
340 of disorders, including abdominal obesity, insulin resistance, and dyslipidaemia[35]. In the
341 current study, some but not all of the hallmarks of metabolic syndrome were observed at
342 PND98; Indeed, an increase of abdominal fat[34], adipocyte size[36], hyperleptinemia[33] and a
343 low grade inflammation[37] were still evident 6 weeks after cessation of the diet. However,
344 the increase of insulin level is not directly related to insulin resistance, and further *in vivo*
345 experiments are required to determine if this increase is associated with insulin resistance.
346 Another consequence of a high fat/high sugar diet consumption is the occurrence
347 of low-grade systemic inflammation[38]. Previous studies have shown that 15 weeks of
348 cafeteria diet in adult mice induce an increase in plasma IL-6[39]. Here we show that
349 adolescent cafeteria diet consumption induced a low-grade inflammation characterised

350 by increased plasma IL-6 and TNFα levels, right after the diet ended (PND56), which
351 persisted up to 6 weeks after the diet has been reverted to standard chow (PND98).
352 Vinuesa and collaborators showed that adolescent mice fed a high fat diet for 6 month
353 present increased levels of plasma IL-1β 2 months but not 5 months after the beginning
354 of the diet[40]. These data and ours point to the necessity of determining the dynamics of
355 cytokines secretion following exposure to a cafeteria diet.

356 Regarding the impact of diet on levels of pro-inflammatory cytokines in the brain,
357 it has previously been demonstrated that peripheral inflammation induced by a high fat
358 diet can trigger an increase in IL-1β, IL-6 and TNFα expression as well as an increased
359 microglial activation in mouse hippocampus, prefrontal cortex and hypothalamus[41,42].
360 However, we observed that pre-exposure to a cafeteria diet during adolescence did not
361 impact upon hippocampal IL-1β-induced changes in IL-1β or IFNγ. Indeed, we showed
362 that 6 weeks after the cessation of the cafeteria diet, IL-1β and TNFα mRNA expression
363 was increased in the prefrontal cortex, while we did not observe a neuroinflammatory
364 state in the hippocampus and the hypothalamus. The hypothalamus is known as the
365 regulation centre of feeding-behaviour[43]. Thus it is surprising that it was not affected by
366 the adolescent high fat/high sugar-diet consumption and suggests that the washout period
367 was sufficient to attenuate the potential pro-inflammatory effect of the cafeteria diet on
368 the hypothalamus. Another explanation for the absence of cafeteria diet-induced
369 inflammation in the hippocampus and hypothalamus is that it may depend on the time-
370 window or duration of its administration. Indeed, a study investigating the effect of
371 cafeteria diet on neuroinflammation showed that aged rats (18 months old) fed for 12
372 consecutive weeks with a cafeteria diet exhibited high levels of IL-1β in the hippocampus
373 and prefrontal cortex[44]. In contrast in the current study, rodents were fed for 4 weeks
374 during the adolescence period (PND21-PND56) and then switched to a standard diet for

375 6 weeks after which time the cytokine analysis was carried out when the rats were 3.5
376 months old.

377 IL-1β overexpression in the hippocampus induced by a lentivirus brought about
378 an increase in endogenous mRNA expression of IL-1β, 6 weeks after its injection,
379 confirming its validity. It also induced an increase in IFNγ, which is in accordance with
380 previous findings in a rat model of traumatic brain injury[45], and in a mouse model of
381 seizure[46]. Finally, IL-1β hippocampal overexpression did not impact peripheral cytokine
382 secretion.

383 In the current study, we investigated if adolescent exposure to a cafeteria diet
384 could increase the susceptibility of the hippocampus to inflammation and consequent
385 functional impairments during adulthood. Recent data suggest that a negative effect of a
386 high fat diet during adolescence on hippocampal function may be reversed by switching
387 the diet to a standard-diet in adulthood[16,47,48]. Our findings are in accordance with these
388 studies, albeit using a high fat/high sugar diet. Specifically, 4 weeks after switching the
389 cafeteria diet to a standard diet, rats showed no cognitive deficits. Previous studies have
390 shown that high fat/high sugar diets during adolescence induce differential effects in
391 cognition and anxiety-like behaviours. For example, Pini and collaborators showed that
392 when the cafeteria diet began at weaning (PND21) and continued throughout the
393 behavioural assessments at adulthood, rats displayed lower anxiety levels and improved
394 performance on a spatial task[49]. However, another group demonstrated that when a
395 cafeteria diet began at adulthood (2 months old-PND60) and continued throughout the
396 behavioural assessments, rats showed an impaired performance in a spatial learning task[9].
397 It has been previously shown that adults rats fed for 20 days with a high fat/high sugar
398 diet showed impairments in a location recognition task whereas no impairments were
399 observed in an object recognition task[12]. However, some studies have revealed that high

400 fat and/or high sugar diets had no effect on hippocampal dependent tasks such as spatial
401 navigation and object recognition when assessed in adulthood[11]. This is similar to what
402 we have observed in the current study. It suggests that the effects of cafeteria diet may be
403 task-dependent, diet-composition-dependant, or dependent on the age at which diet
404 intervention occurs. It is important to bear in mind that the behavioural tests used in the
405 current study do not assess all characteristics of hippocampal-associated cognitive
406 function, and thus may not fully capture the impact of dietary intervention on behaviour.
407 Previous studies have assessed cognitive performance using more challenging tasks and
408 have described a negative effect of the cafeteria diet in a spatial learning task[9] and in a
409 contextual memory paradigm[28] in adult rats. These tasks are more complex than those
410 used in the current study and engage several brain regions such as the hippocampus, the
411 perirhinal cortex and the prefrontal cortex. Indeed, the spontaneous alternation task in the
412 Y-Maze and the novel object recognition task performed in this study involve only a
413 minor learning component[50,51]. Thus, application of more challenging and complex tasks
414 warrant investigation in response to adolescent cafeteria diet intervention in future
415 studies. It is also possible that any effects of a cafeteria diet on hippocampal-associated
416 cognition were reversed during the washout period as has recently been proposed in a
417 study examining adolescent cafeteria diet intervention in mice[52]. We hypothesised that
418 adolescent exposure to cafeteria diet could increase the susceptibility of the hippocampus
419 to inflammation during adulthood even after a washout period. Increased expression of
420 IL-1β has been shown to impair performance in hippocampal-associated tasks such as
421 spatial navigation in the Morris water maze[53] and induce anxiety-like behaviour in mice[54].
422 In the current study, we showed that hippocampal overexpression of IL-1β during
423 adulthood did not unmask any behavioural phenotype induced by adolescent cafeteria
424 diet consumption. Interestingly, we showed that rats injected with IL-1β during adulthood

425 impaired spontaneous alternation in the Y-maze, but not in IL-1β-injected rats fed the
426 cafeteria diet during adolescence. Thus, it would appear that adolescent cafeteria diet
427 consumption provided a resilience to the effects of IL-1β overexpression on spatial
428 working memory. Interestingly high fat/high sugar consumption in rodents has been
429 shown to increase learning performance in the Morris water maze[55] and decrease anxiety-
430 like behaviours in the openfield[56].

431 Finally, it is possible that the effects of cafeteria diet during adolescence may
432 negatively prime the brain during another vulnerable period, such as illness or in older
433 age. Previous work using a high fat/high sugar diet intervention showed that the diet
434 potentiated cognitive decline in aged rats[57]. In the current study we showed that switching
435 to a standard diet after cafeteria diet intervention during adolescence is sufficient to
436 prevent the cognitive impairment in adulthood but not enough to alleviate the metabolic
437 and inflammatory states. It should be noted however that even if the adolescent brain in
438 our study seemed to be resilient, it does not exclude the possibility of deficits later in life,
439 due to illness, or in challenging cognitive situations.

440

441 **Conclusion:**

442 In conclusion, we demonstrated that 4 weeks of cafeteria diet consumption during
443 adolescence induced long-lasting metabolic dysregulation and systemic low-grade
444 inflammation even after reverting to a standard diet. However, the adolescent cafeteria
445 diet followed by standard diet in early adulthood did not induce impairments in locomotor
446 activity, anxiety-like behaviour, spatial working memory or recognition memory and had
447 no synergistic effect with hippocampal IL-1β overexpression. Hippocampal IL-1β
448 overexpression alone in adulthood impaired spatial working memory but had no effect on
449 the other behaviours examined thus highlighting the complex role of IL-1β in regulating

450 hippocampal-associated cognitive processes. Taken together, these data suggest that
451 consumption of a high fat/high sugar during adolescence induces long lasting systemic
452 effects without affecting certain cognitive process in adulthood.
453

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Declaration of interest statement:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure captions

Figure 1. Cafeteria-diet during adolescence increased calorie intake and metabolic hormone levels without affecting body weight

a. Experimental design; **b.** Body weight (g); **c.** Energy consumption in kilojoules (kJ) across the entire study, n=8-10. Histograms showing the plasma concentrations of **d.** Insulin, expressed in ng/ml and adipokines (**e.** Leptin, expressed in ng/ml; **f.** Adiponectin, expressed in µg/ml; **g.** ratio leptin/adiponectin) at PND28, PND56 and PND98 in cafeteria-diet fed rats injected with mCherry alone only. n=6, **p*<0.05, ***p*<0.01 compared to concentration at PND28.

Figure 2. Validation of lentiviral-mediated increase in IL-1β in the hippocampus at PND98.

a. Relative expression of IL-1β protein in the hippocampus at PND98. **b.** Representative immunoblot of rat IL-1β protein expression from hippocampal tissue, n= 6-8. All data are expressed as mean ± SEM - **p*<0.05, ***p*<0.01, ****p*<0.001 compared to controls.

Figure 3. Cafeteria-diet during adolescence induced an adipose tissue expansion in adulthood (PND98).

a. Weights of epididymal adipose tissue at PND98 expressed as grams per body weight. (n=6-7). **b.** Quantification of the surface area of adipocytes expressed in μm^2 (n=5). **c.** Representative images of eosin-hematoxylin-stained eWAT. Scale bar = 50 µm. Data are expressed as mean ± SEM, **p*<0.05 compared to control.

Figure 4. Cafeteria-diet during adolescence induced a mild inflammation in the periphery which persists in adulthood.

a. Blood sampling timeline and histograms legends. **b-e.** Histograms showing the plasma concentrations of cytokines (**b.** IL-6; **c.** IFNγ; **d.** TNFα and **e.** IL1β) expressed

as pg/ml at PND28, PND56 and PND98 n=6-7, *$p<0.05$, **$p<0.01$ compared to PND28 and \$$p<0.05$ compared to controls at PND56.

Figure 5. Cafeteria-diet during adolescence and IL-1β overexpression in the hippocampus during adulthood differentially affected the expression of inflammatory genes in the hippocampus and in the prefrontal cortex.

Relative mRNA expression of IL-1β, IL-6, TNFα and IFNγ **a.** in the hippocampus and **b.** prefrontal cortex at PND98. Data are expressed as mean ± sem. n=6 *$p<0.05$, **$p<0.01$ compared to controls, #$p<0.05$ compared to cafeteria. diet.

Figure 6. Consumption of a cafeteria diet during adolescence and IL-1β overexpression in the hippocampus during adulthood did not induce anxiety-like behaviour while IL-1β alone impairs spatial working memory.

a. Distance travelled in the openfield test. **b.** Percentage of alternation in the Y-maze test. **c.** Novel object recognition depicted as discrimination ratio (DR) between novel (N) and familiar (F) objects. All data are expressed as mean ± SEM, n=8-10, *$p<0.05$ compared to control

Supplementary material captions

Supplementary Table 1. Food list with nutritional information used for cafeteria diet and chow given to rats.

Figure S1. Food intake information.

a. Regular chow intake averaged across each cage after surgery. **b.** Proportion of each cafeteria diet component consumed across the dietary intervention and as a percentage of total food eaten for each cage. All data are expressed as mean ± SEM, n=8-10

Figure S2. Neither adolescent cafeteria-diet nor adult IL-1β overexpression impact inflammatory gene expression in the hypothalamus at adulthood

Relative gene expression of cytokines (IL-1β, IL-6, TNFα and IFNγ) in the hypothalamus. All data are expressed as mean ± SEM, n=6

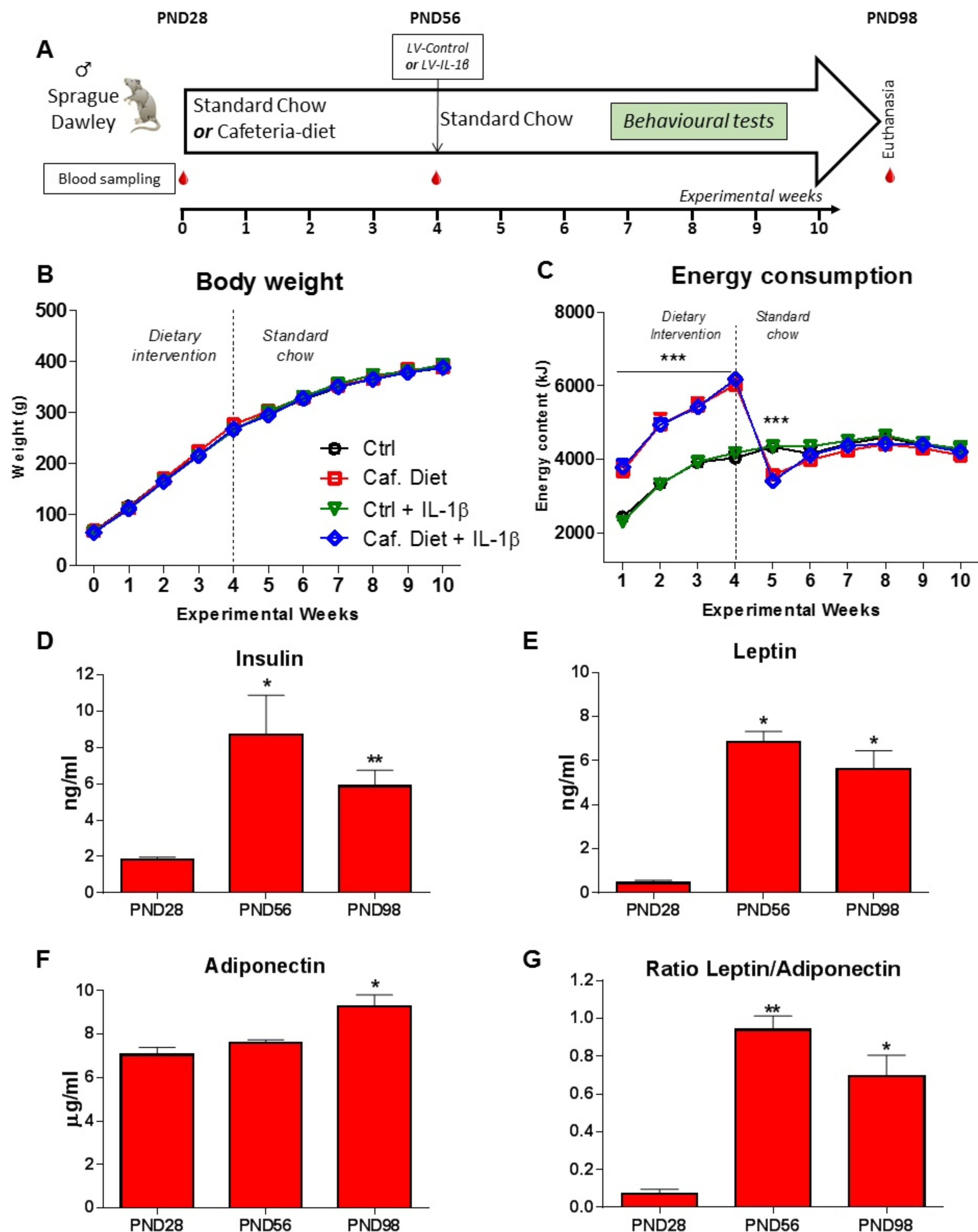


Figure 1 : Cafeteria diet during adolescence increased calorie intake and metabolic hormone levels without affecting body weight

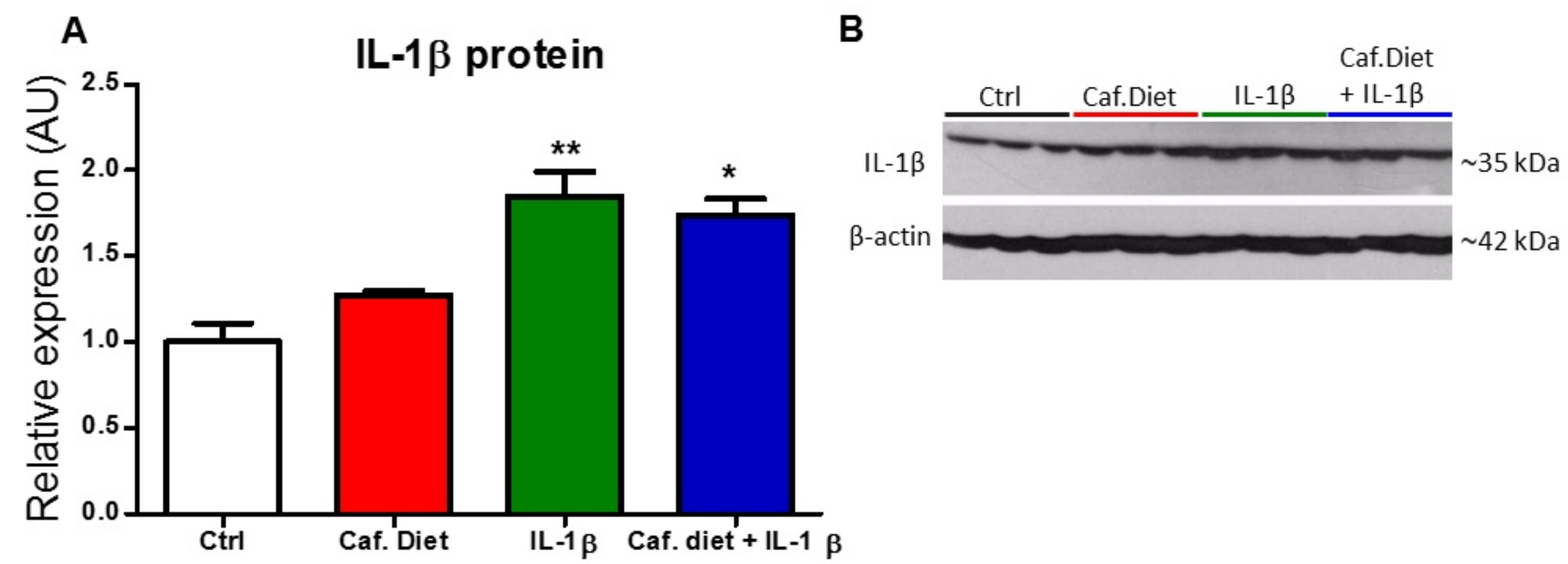
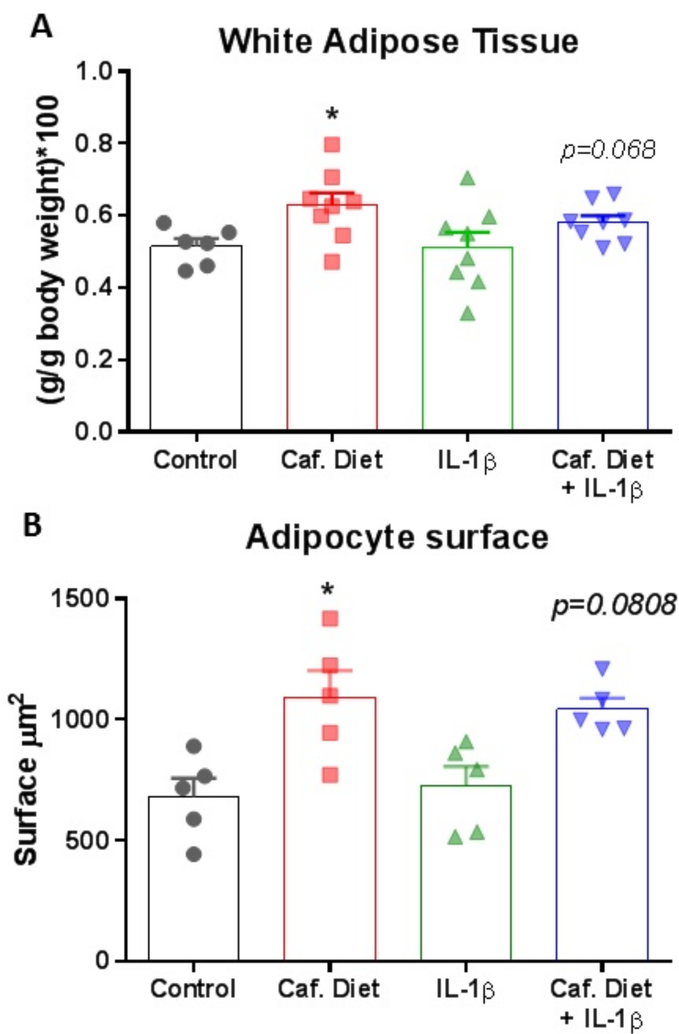


Figure 2: Validation of lentiviral-mediated increase in IL-1β in the hippocampus at PND98.



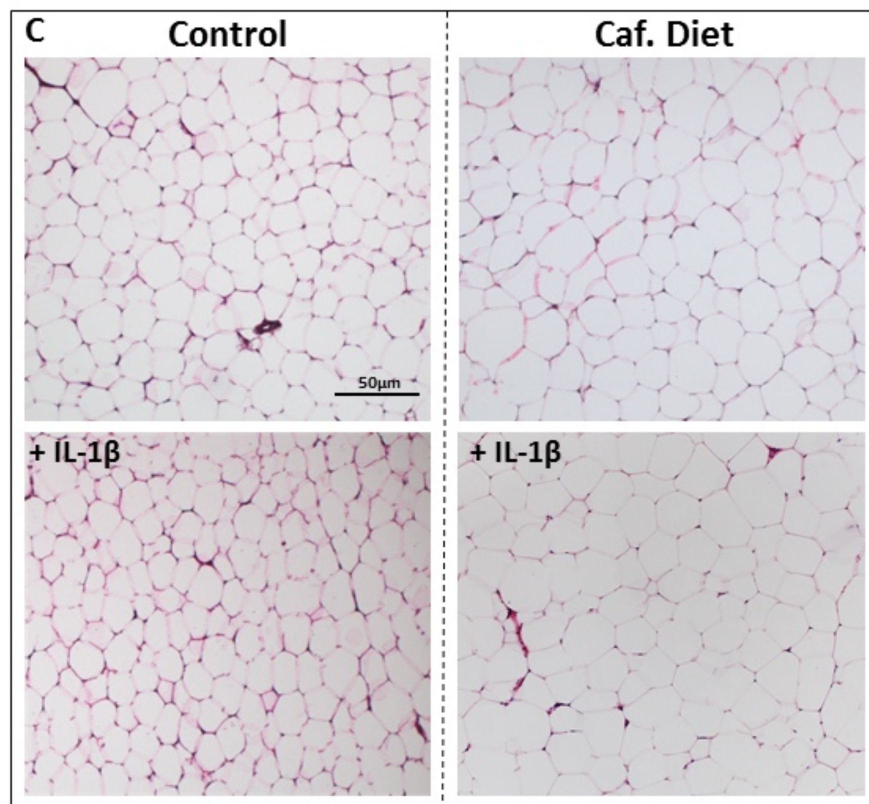


Figure 3: Cafeteria diet during adolescence induced an adipose tissue expansion in adulthood (PND98).

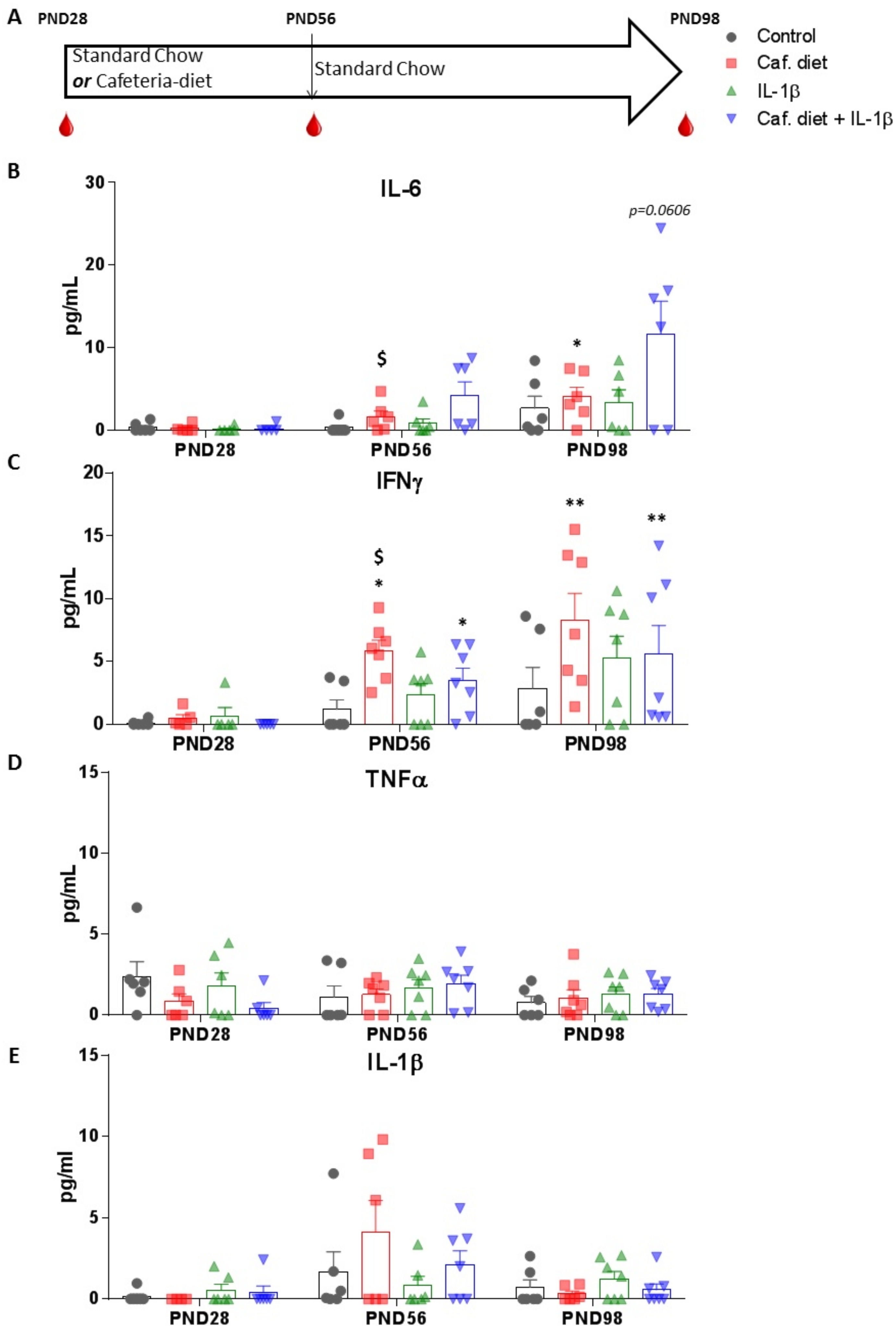


Figure 4 : Cafeteria diet during adolescence induced a mild inflammation in the periphery which persists in adulthood.

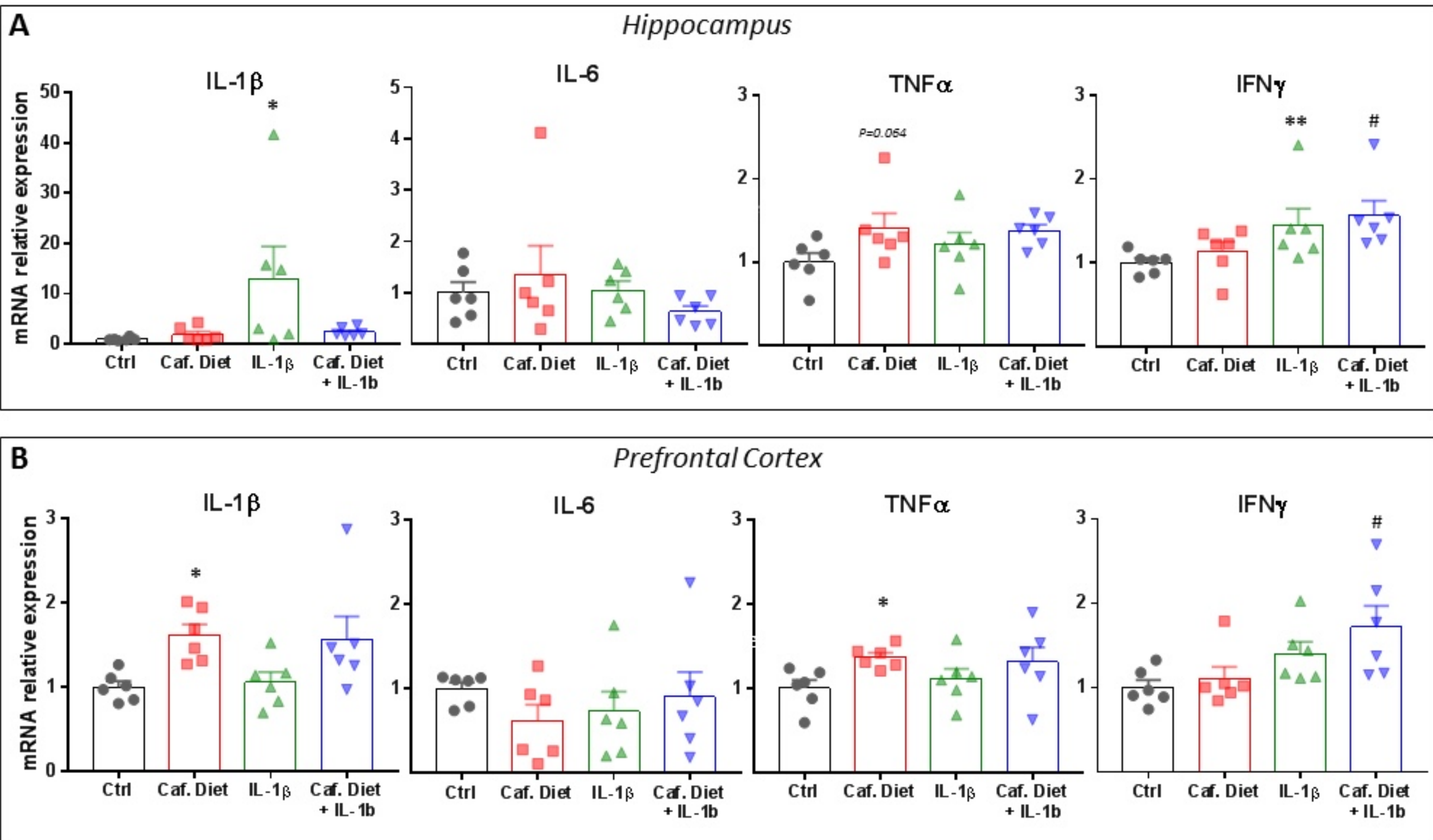


Figure 5 : Cafeteria diet during adolescence and IL-1β overexpression in the hippocampus during adulthood differentially affected the expression of inflammatory genes in the hippocampus and in the prefrontal cortex.

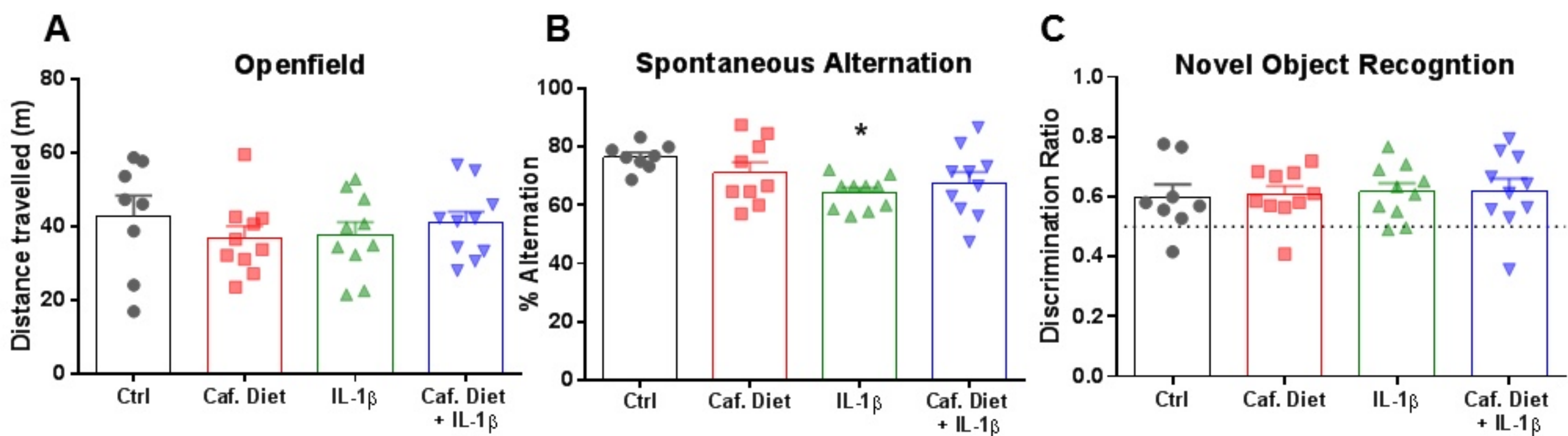
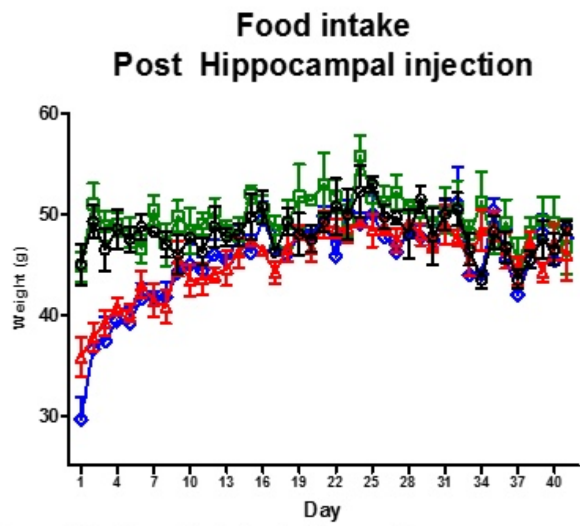


Figure 6: Consumption of a cafeteria diet during adolescence and IL-1β overexpression in the hippocampus during adulthood did not induce anxiety-like behaviour while IL-1β alone impairs spatial working memory.



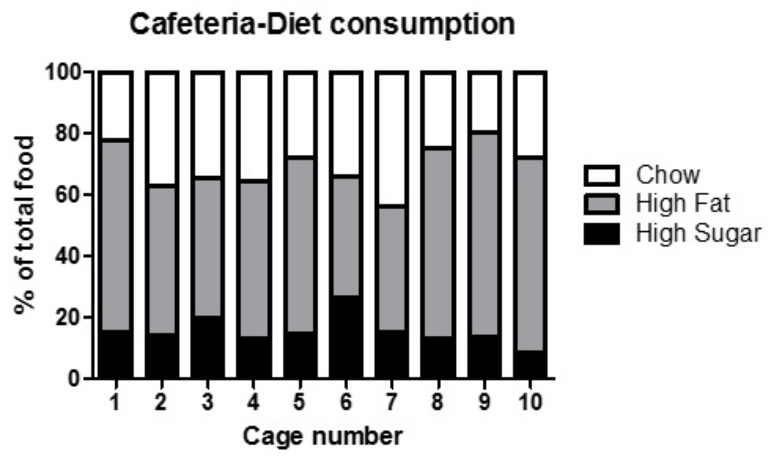


Figure S1. Food intake information

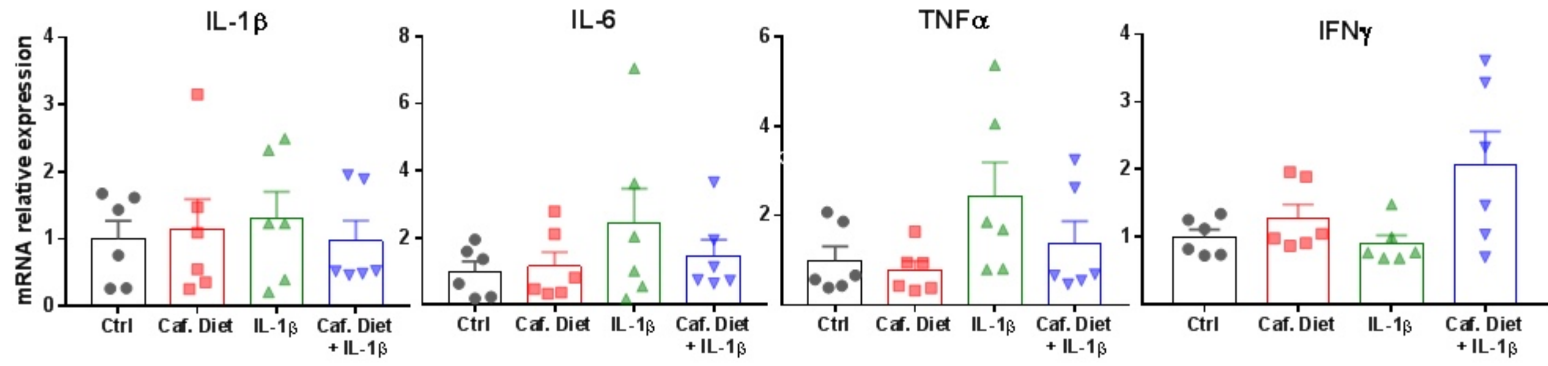


Figure S2. Neither adolescent cafeteria diet nor adult IL-1β overexpression impact inflammatory gene expression in the hypothalamus at adulthood