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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. Introduction

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

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

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

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

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

negatively prime the brain to such a neuroinflammatory state at a later time point. Thus, this study examined whether transient consumption of a cafeteria diet during adolescence followed by a chronic hippocampal IL-1β overexpression in early adulthood impacted adult systemic and brain functions.

2. Methods

2.1: Animals

Male Sprague-Dawley rats were bred in-house (Biological Services Unit, UCC) and were weaned at postnatal day (PND) 21. Weaned rats were pair housed in a colony maintained at 22 ± 1°C with a 12-hour light-dark cycle (lights on at 7:30am). All animal procedures were performed under licenses issued by the Health Products Regulatory Authority (HPRA, Ireland), in accordance with the European Communities Council Directive (2010/63/EU) and approved by the Animal Experimentation Ethics Committee of University College Cork.

2.2: Experimental design and diet

Rats were randomly divided into four experimental groups; standard chow fed animals injected with an mCherry expressing lentivirus (Ctrl; n=8), cafeteria diet fed animals injected with an mCherry expressing lentivirus (Caf. Diet; n=10), standard chow fed animals injected with an IL-1β expressing lentivirus (Ctrl - IL-1β; n=10), and cafeteria diet fed animals injected with an IL-1β expressing lentivirus (Caf. diet - IL-1β; n=10) (Figure 1A). The cafeteria diet consisted of high fat and high sugar components and was given in addition to standard chow to diet fed rats. Foods were weighed prior to being placed in the cages and were given in excess. The leftover amount of each component was weighed after 24 hours to determine the amount consumed by rats in each cage.

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

2.3: Stereotaxic surgery

Rats were anaesthetised with isoflurane/O_2 (1.5–2.5%) and placed on a stereotaxic apparatus (Kopf) on a heating mat. Lentiviruses purchased from Genecopoeia (MD, USA) to overexpress mCherry (Lenti-mCherry: Cat# LP-NEG-Lv80-0205-cs; 2.42 x 10_8 copies/ml) or mCherry-IL-1β (Lenti-IL-1β-mCherry: Cat# LP-Mm03282-Lv80-0205-cs; 1.93 x 10_7 copies/ml) were injected (3µL of each) into the dorsal hippocampus using the coordinates AP: -3.5 mm, ML: ± 2.4 mm, DV: -3.8 mm relative to Bregma[21] at a rate of 1µL/min followed by a 5 min diffusion. Following lentiviral injection, incisions were sutured, the wound was treated with antibacterial ointment (Fucithalmic® 10 mg/g), and rats were administered the analgesic carprofen (Rimadyl® 5 mg/kg, s.c., Zoetis Ireland Ltd) and 5% glucose solution. All rats were allowed to recover for 3 weeks with *ad libitum* access to standard chow and water to allow viral uptake prior to the beginning of behavioural testing.

2.4 Behavioural testing

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

2.4.1: Open field

The openfield was used to measure locomotor activity and anxiety like behaviour. Rats were placed in an open field arena (90 cm diameter) under bright lighting conditions (approx. 1000lux) for 10 min. Distance travelled in the arena as well as time spent and the number of visits into a virtual center zone (defined as the inner 30% core of the arena) was recorded using the EthoVision software (XT 8.5 version, Noldus, USA). The arena was cleaned with a 50% ethanol solution between each animal to remove odour cues.

2.4.2: Spontaneous Alternation in the Y maze

The spontaneous alternation in the Y-maze test was used to measure spatial working memory. The Y maze consisted of three arms (120° from each other, 50 cm x 10 cm x 30 cm) which were connected to form a triangular space in the centre (10 cm x 10 cm x 10 cm). Briefly, each animal underwent one trial and the Y-maze was cleaned with 50% ethanol between animal trials. The rat was placed into the outward-extending end of one arm (always the same) facing the wall and was then allowed free exploration of the maze for 5 min. Arms were numbered (1–3) and the sequence of arm entries was recorded manually during the test, where an arm entry was defined by the four-paw criterion. A spontaneous alternation is defined as the consecutive entry in all three arms. The percentage spontaneous alternations $\left(\% \, alternations = \left(\frac{\text{n alternations}}{\text{n visits}}\right) * 100\right)$ within 5 min was calculated.

2.4.3 Novel Object Recognition

Novel object recognition was assessed as described[24]. On day 1, rats were habituated to the testing arena (L40.5 cm × W36.5 cm × H28.0 cm) under dim light (20 lux) for 10 min. On day 2, rats were placed in the testing arena with 2 identical objects (i.e. ceramic mugs or glass bottles) for 10 min. After a 3-hour inter-trial interval, one familiar object was

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

2.5 Blood measurements

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

2.5.1 Metabolic hormones

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

2.5.2 Cytokines

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

2.6 Tissue collection

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

2.6.1 Quantitative Reverse-Transcription PCR (RT-qPCR)

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

2.6.2 Western Blot

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

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

2.6.3 Histology

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

2.7. Statistical analysis

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

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

3. Results

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

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

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

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

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

3.3. Cafeteria diet during adolescence induced an adipose tissue expansion associated with mild inflammation in the periphery, which persisted in adulthood.

Because adipokine secretion is regulated by the white adipose tissue and is sensitive to food intake, particularly to high fat and high sugar, we focused next on epididymal white adipose tissue (WAT) physiology. The epididymal WAT mass (Figure 3A) and the adipocyte surface area (Figure 3B-C) were significantly increased in cafeteria diet fed rats compared to controls, independent of IL-1β hippocampal overexpression. The expansion of adipose tissue relates to an enhanced secretion of systemic inflammatory

factors[29]. We observed that plasma levels of pro-inflammatory cytokines IL-6 and IFNγ were increased by cafeteria diet consumption at PND56 and this increase persisted 6 weeks after cessation of the diet at PND98 (Figure 4B-C *p<0.05 **p<0.01 compared to corresponding control group at PND28; \$$p$<0.05 compared to control at PND56). Levels of IL-1β and TNFα in plasma remained unaffected by the diet (Figure 3D-E). The plasma concentrations of IL-4 and IL-10 were below the detection threshold at the three time points in all groups of animals (data not shown). The hippocampal overexpression of IL-1β did not impact upon the systemic levels of cytokines.

3.4. 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.

We next investigated if consumption of a cafeteria diet during adolescence and/or IL-1β overexpression in adulthood affected brain homeostasis by disrupting the neuroinflammatory state. We assessed gene expression of pro-inflammatory cytokine IL-6, IL-1β, TNFα and IFNγ in the hippocampus, prefrontal cortex and hypothalamus as these brain regions have been shown to be affected by systemic inflammation after consumption of a high fat/high sugar diet[10]. Consumption of a cafeteria diet during adolescence did not impact mRNA expression of IL-1β, IL-6, TNFα and IFNγ in the hippocampus compared to the hippocampus of control rats. Overexpression of IL-1β in the hippocampus increased IL-1β and IFNγ mRNA expression. However, the increase in IL-1β mRNA expression was abolished in the hippocampus of rats fed the cafeteria diet during adolescence (Figure 5A *p<0.05 **p<0.01 compared to control group; #p<0,05 compared to IL-1β group). Consumption of the cafeteria diet during adolescence induced an increase in mRNA expression of IL-1β and TNFα in the prefrontal cortex (Figure 5B

*p<0.05 compared to control group). IL-1β overexpression in the hippocampus did not impact cytokine gene expression in the prefrontal cortex but when combined with pre-exposure to a cafeteria diet during adolescence, it induced an increase in the mRNA expression of IFNγ (Figure 5B #p<0.05 compared to IL-1β group). Interestingly, the effect of the cafeteria diet on gene expression levels of cytokines in the hypothalamus was not evident 6 weeks after the cessation of the diet (Figure S2).

3.5. 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 impaired spatial working memory.

To assess if consumption of a cafeteria diet during adolescence induced behavioural changes during adulthood, rats underwent the openfield test to assess locomotor activity and anxiety-like behaviour. Animals fed the cafeteria diet during adolescence with or without hippocampal IL-1β overexpression in adulthood, or animals injected with IL-1β did not show any changes in locomotor activity (Figure 6A) as evaluated by the distance travelled in the openfield. Animals in all groups spent a similar percentage of time within the centre of the openfield arena (data not shown). Spontaneous alternation behaviour, a measure of hippocampal-dependent spatial working memory, was tested in the Y-Maze. Consumption of a cafeteria diet has no significant effect on performance while adult rats exposed to hippocampal IL-1β displayed a reduced spontaneous alternation rate compared to controls (*p<0.05, Figure 6B). Animals in all groups exhibited a similar number of arm entries (data not shown) indicating comparable activity and motivation states. Finally, performance in the novel object recognition task was similar in animals across all four groups (Figure 6C). All behavioural data in control animals are within comparable ranges to what we have previously observed in non-injected control rats[26].

Our data suggest that exposure to a cafeteria diet during adolescence does not impact upon cognitive function but rather appears to prime the adult brain against the deleterious effect of IL-1β overexpression in spatial working memory in adulthood.

4. Discussion:

Evidence now suggests that consumption of a high fat/high sugar diet during adolescence induces detrimental effects on hippocampal associated cognition[27,14,28]. Data also suggest that the effect of a high fat diet on hippocampal cognition may be reversed by switching the diet to a standard diet in adulthood[16]. In the context of a cafeteria diet (high fat/high sugar), our data support this later finding. We demonstrate that 4 weeks of cafeteria diet consumption during the adolescent period (PND28 – PND56) does not result in cognitive deficits in adulthood when the animals were switched to a standard diet at adulthood for 3 weeks prior to testing. However, the cafeteria diet fed animals still maintained elevated levels of insulin, leptin and adiponectin, pro-inflammatory cytokines (IL-6 and IFNγ) and white adipose tissue expansion 6 weeks after the cessation of the diet. This peripheral effect was concomitant with an increase in pro-inflammatory cytokines (IL-1β and TNFα) expression in the prefrontal cortex. We also demonstrate that hippocampal overexpression of the proinflammatory cytokine IL-1β during adulthood does not induce any cognitive impairments in cafeteria diet fed animals. However, overexpression of IL-1β during adulthood after standard diet intake during adolescence, induced impairments in spatial working memory. This behavioural effect was complemented with an increase in IL- 1β and IFNγ expression in the hippocampus.

In the current study, 4 weeks of cafeteria diet intervention did not induce weight gain in adolescent rats compared to those fed with standard chow. This result is consistent with previously published studies in which a cafeteria diet was administered from

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

Another consequence of a high fat/high sugar diet consumption is the occurrence of low-grade systemic inflammation[38]. Previous studies have shown that 15 weeks of cafeteria diet in adult mice induce an increase in plasma IL-6[39]. Here we show that adolescent cafeteria diet consumption induced a low-grade inflammation characterised

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

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

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

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

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

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

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

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

Conclusion:

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

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

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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

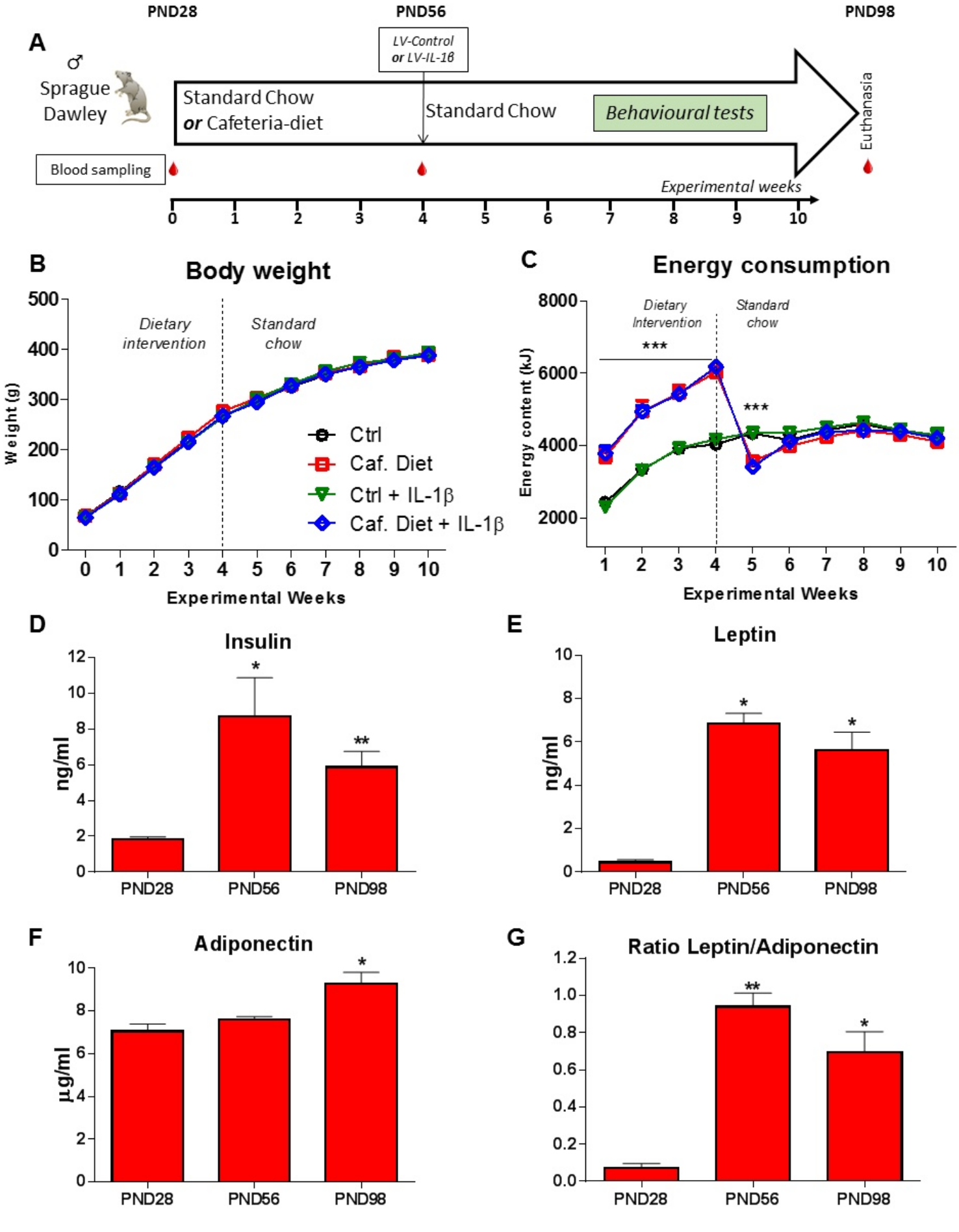


Figure 1 : Cafeteria diet during adolescence increased calorie intake and metabolic hormone levels without affecting body weight

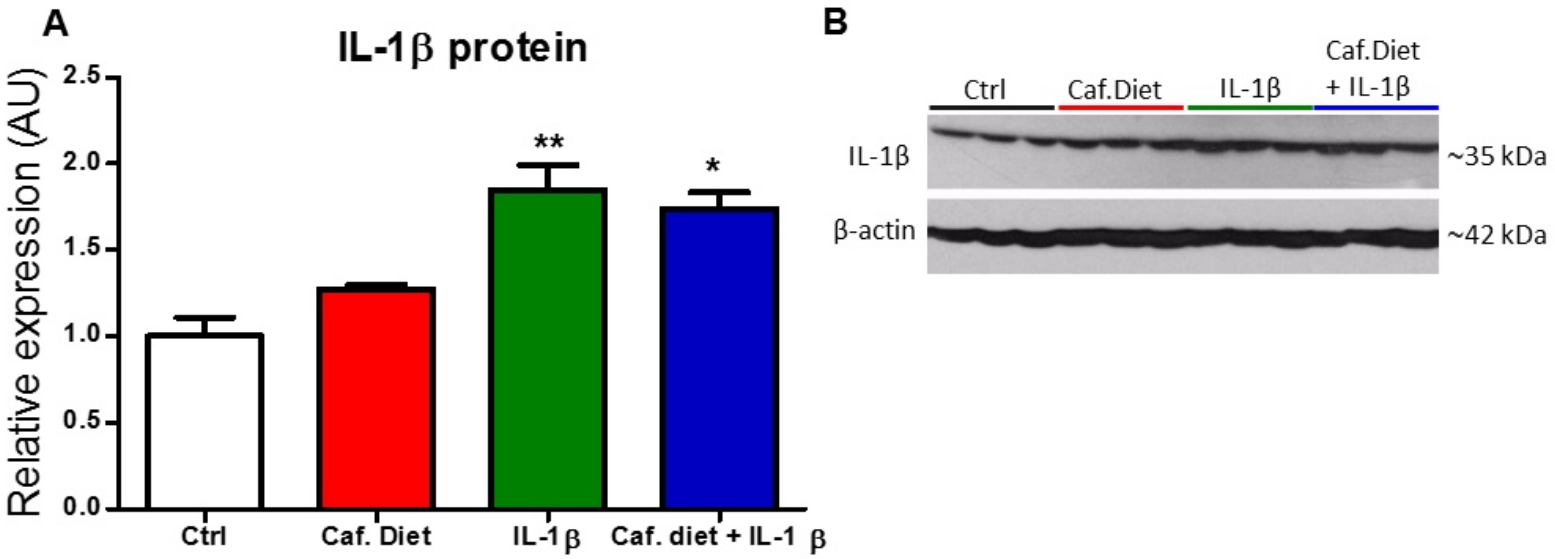


Figure 2: Validation of lentiviral-mediated increase in IL-1β in the hippocampus at PND98.

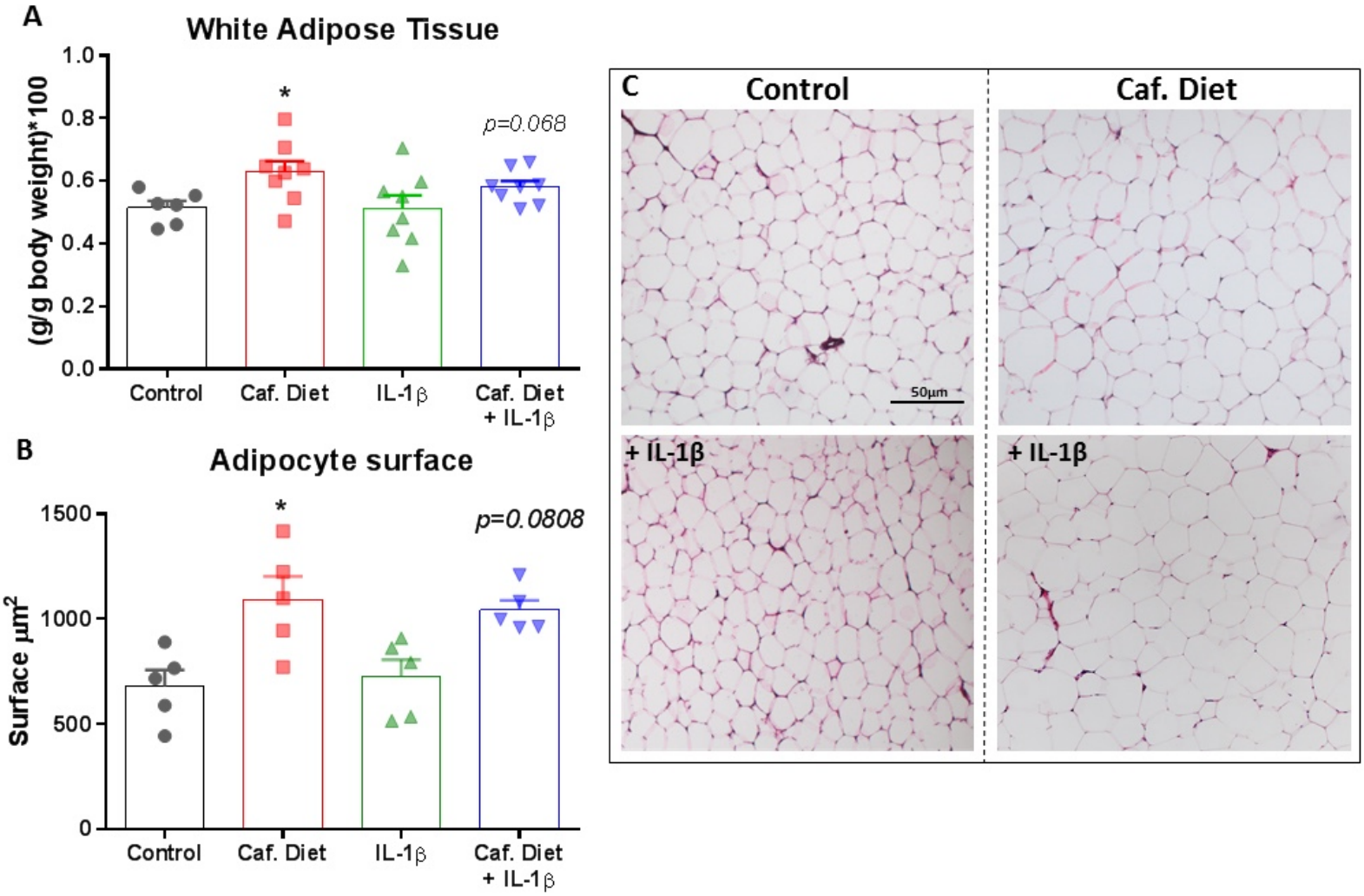


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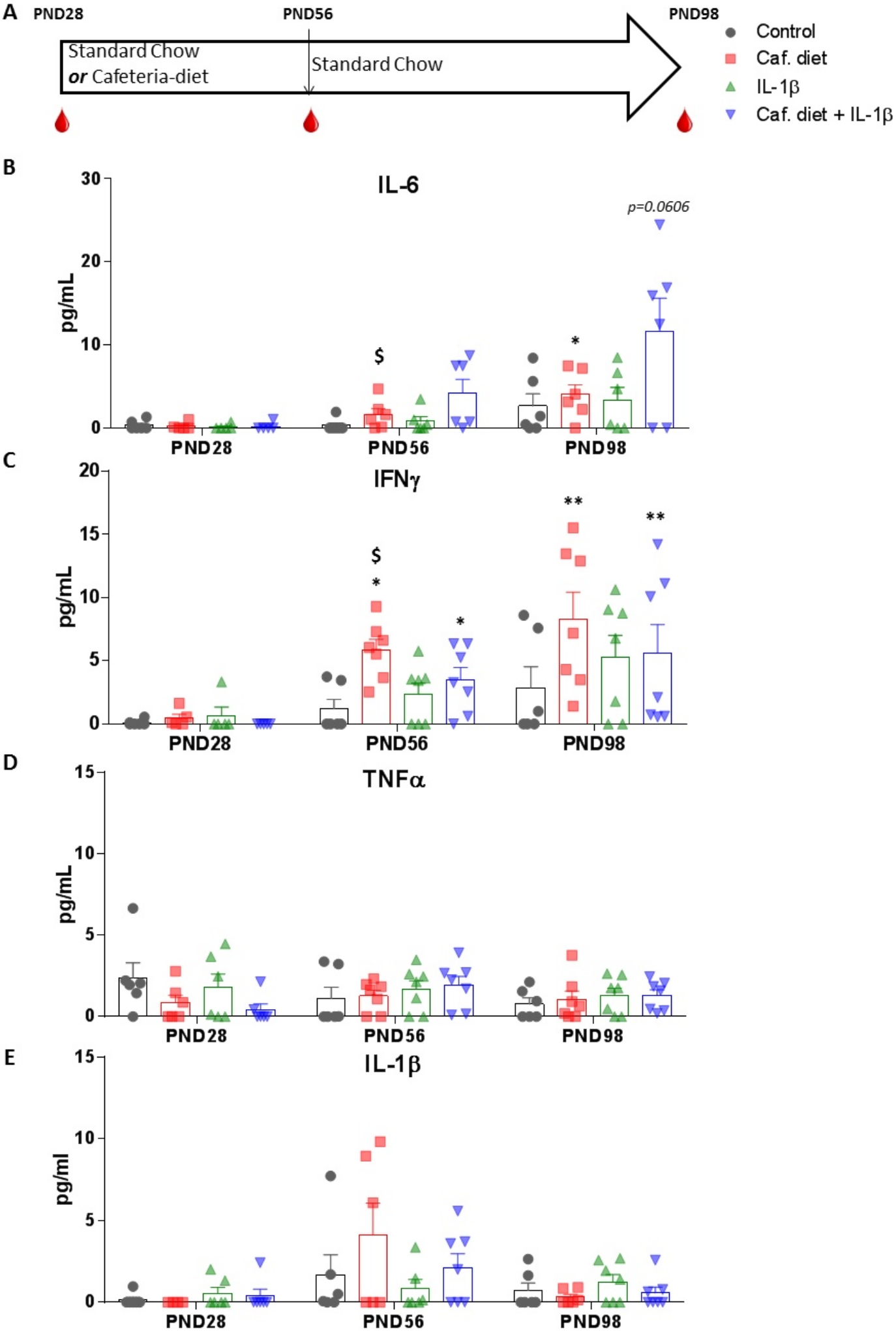


Figure 4 : Cafeteria diet during adolescence induced a mild inflammation in the periphery which persists in adulthood.

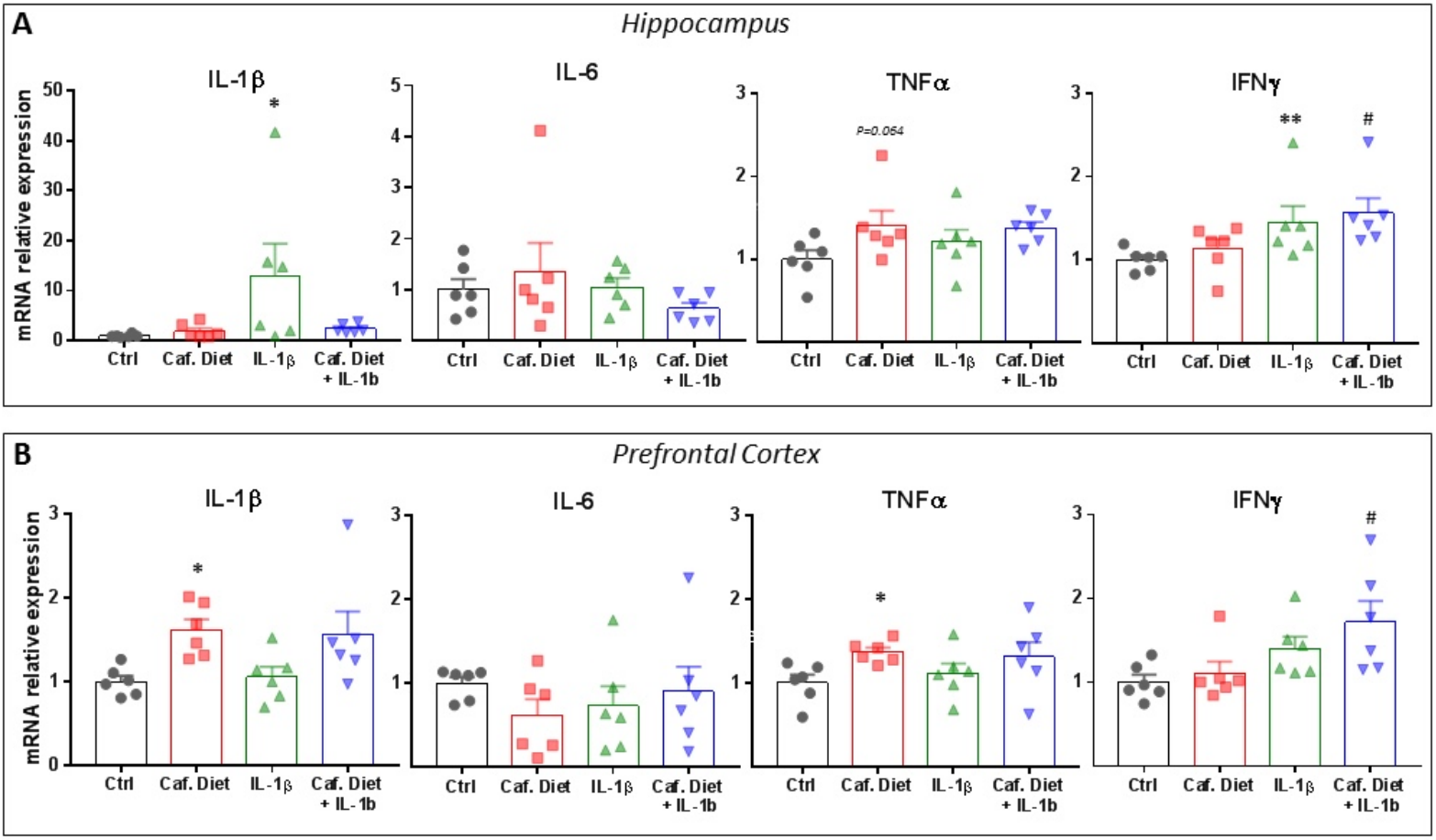


Figure 5 : Cafeteria diet during adolescence and IL-1β overexpression in the hippocampus during adulthood differentially affected the expression of inflammatory genes in the hippocampus and in the prefrontal cortex.

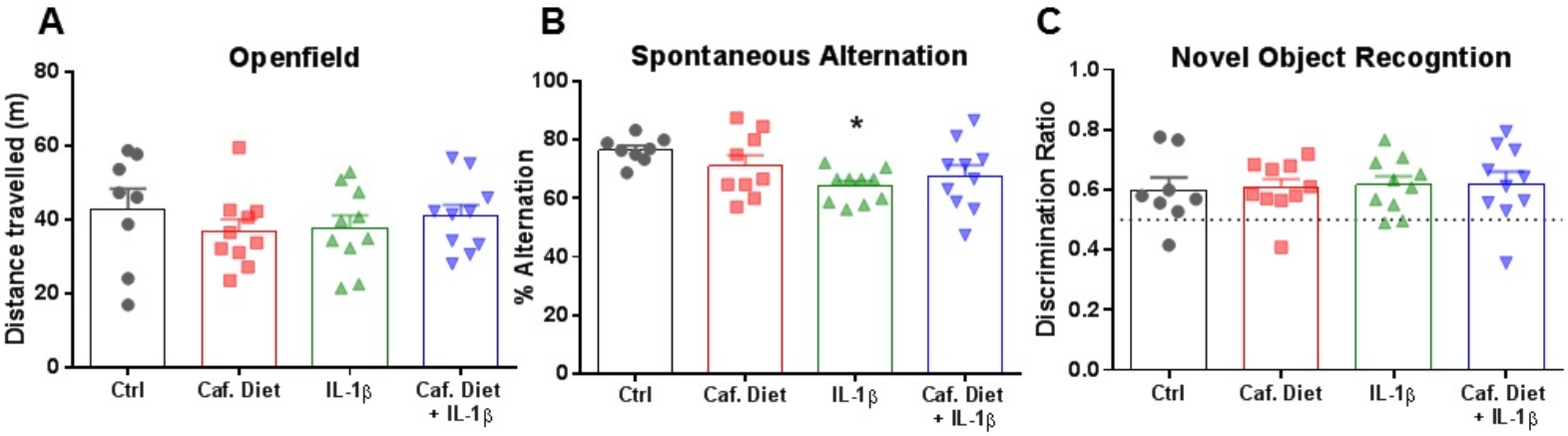
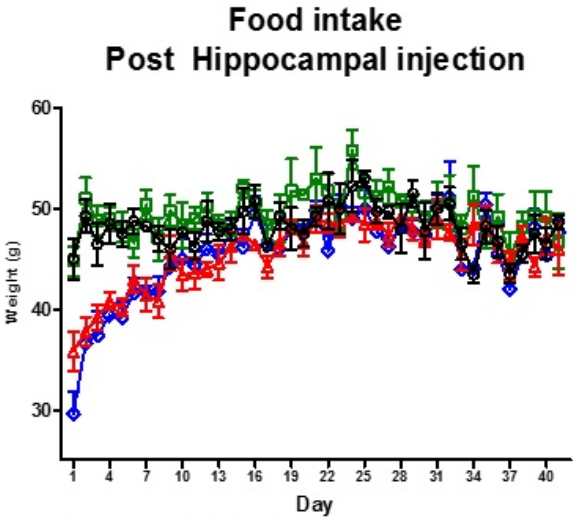


Figure 6: Consumption of a cafeteria diet during adolescence and IL-1β overexpression in the hippocampus during adulthood did not induce anxiety-like behaviour while IL-1β alone impairs spatial working memory.



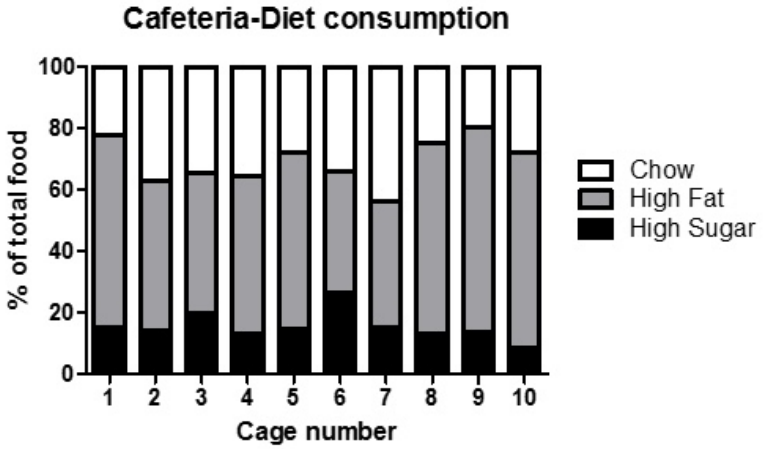


Figure S1. Food intake information

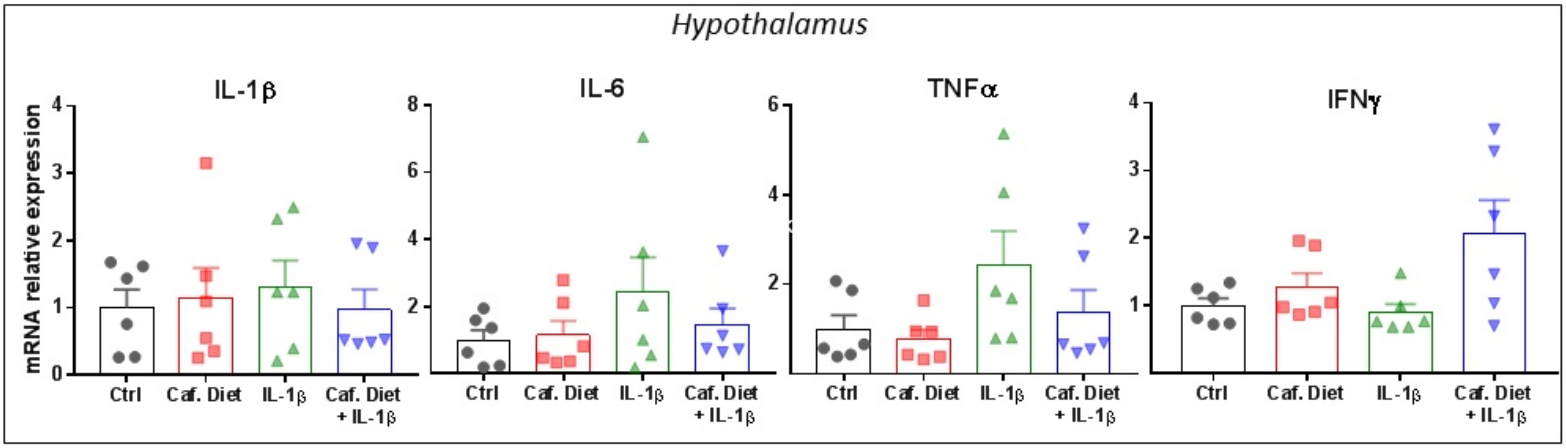


Figure S2. Neither adolescent cafeteria diet nor adult IL-1β overexpression impact inflammatory gene expression in the hypothalamus at adulthood