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Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats

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ABSTRACT

In addition to metabolic and cardiovascular disorders, obesity pandemic is associated with chronic low-grade inflammation as well as adverse cognitive outcomes. However, the existence of critical periods of development that differ in terms of sensitivity to the effects of diet-induced obesity remains unexplored. Using short exposure to a high-fat diet (HFD) exerting no effects when given to adult mice, we recently found impairment of hippocampal-dependent memory and plasticity after similar HFD exposure encompassing adolescence (from weaning to adulthood) showing the vulnerability of the juvenile period (Boitard et al., 2012). Given that inflammatory processes modulate hippocampal functions, we evaluated in rats whether the detrimental effect of juvenile HFD (jHFD) on hippocampal-dependent memory is associated with over-expression of hippocampal pro-inflammatory cytokines.

jHFD exposure impaired long-term spatial reference memory in the Morris water maze without affecting acquisition or short-term memory. This suggests an effect on consolidation processes. Moreover, jHFD consumption delayed spatial reversal learning. jHFD intake did neither affect basal expression of pro-inflammatory cytokines at the periphery nor in the brain, but potentiated the enhancement of Interleukin-1-beta and Tumor Necrosis Factor-alpha expression specifically in the hippocampus after a peripheral immune challenge with lipopolysaccharide. Interestingly, whereas the same duration of HFD intake at adulthood induced similar weight gain and metabolic alterations as jHFD intake, it did neither affect spatial performance (long-term memory or reversal learning) nor lipopolysaccharide-induced cytokine expression in the hippocampus. Finally, spatial reversal learning enhanced Interleukin-1-beta in the hippocampus, but not in the frontal cortex and the hypothalamus, of jHFD-fed rats.

These results indicate that juvenile HFD intake promotes exaggerated pro-inflammatory cytokines expression in the hippocampus which is likely to contribute to spatial memory impairment.

1. Introduction

Obesity has doubled worldwide in the last thirty years, becoming pandemic (WHO, 2013). Overconsumption of energy-dense food is advanced as the major explanation for the current increase of overweight and obesity, including for children and adolescents (Ervin and Ogden, 2013). Obesity is one of the major public health challenges, since it is directly linked to various co-morbidities such as cardiovascular diseases, metabolic disorders and some cancers. In addition, studies started to demonstrate that obesity is associated with cognitive deficits in humans, especially declarative memory which depends on the hippocampus (for review, see Francis and Stevenson, 2013; Nilsson and Nilsson, 2009; Sellbom and Gunstad, 2012). In rodents, high-fat diet (HFD)-induced obesity impairs learning and memory processes, in particular those dependent on the hippocampus (for review, see Kanoski and Davidson, 2011). Obesity is increasing at an alarming rate in children and adolescents. This can be particularly problematic as these developmental periods are crucial for the maturation of the hippocampus (Spear, 2000). Using short exposure (2 months) to a HFD which exerts no effects on hippocampal function when given at adulthood

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we were able to reveal juvenile vulnerability to the effects of HFD. Indeed, exposure to this HFD from weaning to adulthood, i.e., covering adolescence, induced substantial impairment on both hippocampal plasticity and hippocampal-dependent memories indicating the juvenile period is particularly sensitive to the effect of HFD (Boitard et al., 2012).

While the mechanisms involved in the effect of HFD consumption on hippocampal-dependent memory remain poorly understood, inflammation has been proposed as a potential candidate. Indeed, there is a tight link between pro-inflammatory cytokines and hippocampal-dependent learning (for reviews: Marin and Kipnis, 2013; Yirmiya and Goshen, 2011). Whereas low hippocampal levels of pro-inflammatory cytokines can facilitate learning, high levels of cytokines, in particular interleukin-1 beta (IL-1β), specifically impairs memories relying on the hippocampal formation in adult non-obese rodents (Goshen et al., 2007; Rachal Pugh et al., 2001; Hein et al., 2010). Interestingly, obesity is considered as an inflammatory disease since both adipose tissue and gut microbiota contribute to the chronic peripheral low grade inflammation described in obese patients, as well as in rodent models (Clement et al., 2004; Cottam et al., 2004; Everard and Cani, 2013). In rodents, obesity is also associated with heightened levels of pro-inflammatory cytokines in the brain, and we and others have shown that this brain inflammation in obese animals is directly linked to the deficits of hippocampal-dependent memory (Dinel et al., 2011; Pistell et al., 2010).

However, these studies were conducted in adult or middle-aged animals. Therefore it remains to be investigated whether the higher sensitivity to the detrimental effects of juvenile HFD (jHFD) intake on hippocampal memory (Boitard et al., 2012) is associated with an exaggerated jHFD-induced hippocampal inflammation. To this end, we evaluated the effects of jHFD exposure, in comparison to adult HFD exposure, on hippocampal-dependent spatial memory and flexibility and assessed whether this could be linked to a higher cytokine production in the hippocampus. Pro-inflammatory cytokines were first measured at basal state at the periphery and in different brain structures (hippocampus, frontal cortex and hypothalamus). Then, we explored whether jHFD intake could exacerbate this cytokine production in response to a well-defined stimulatory condition, i.e., a systemic acute immune challenge. Finally, as hippocampal-dependent learning is able to increase pro-inflammatory cytokines in the hippocampus (Goshen et al., 2007; Labrousse et al., 2009), we assessed cytokine levels following our learning paradigm in control and jHFD-fed animals.

2. Materials and methods

2.1. Animals and diets

Animals were Wistar naïve male rats (Robert Janvier, Le Genest St-Isle, France) aged either 3 weeks old (juvenile groups) or 12 weeks old (adult groups) on arrival. They were housed in groups of 2–4 individuals in polycarbonate cages (48*26*21 cm) in a air-conditioned (22 ± 1 °C) animal-keeping room maintained under a 12:12 LD cycle. Animals had *ad libitum* access to food and water and were weighted once a week since arrival until sacrifice. On arrival, animals of both groups of age were divided in 2 groups with no weight differences, one receiving control diet, containing 2.5% lipids and offering 2.9 Kcal/g (CD, A04 SAFE, Augy, France) and the other receiving HFD containing 24% lipids and offering 4.7 Kcal/g (D12451, Research Diets, New Brunswick, NJ, USA). One week before the behavioral task, rats were isolated in individual cages (35*23*19 cm) and habituated to be handled by the experimenter. Rats were exposed to CD or HFD for 2 or 3 months starting either at weaning (3 weeks-old; jCD and jHFD groups), i.e., throughout adolescent development (from weaning to adulthood; Spear, 2000), or at adulthood (starting at 12 weeks-old; aCD and aHFD groups) before the beginning of behavioral tasks (Fig. 1). Some animals were exposed to CD or HFD for only one month starting at weaning in order to cover adolescence in a more restrictive manner (Fig. 1). All behavioral experiments were performed on adult animals still consuming their respective diet at the time of testing and sacrifice occurred after 4 months of diet exposure (Fig. 1).

2.2. Behavioral task: the spatial version of the Morris water maze (MWM)

2.2.1. Apparatus

A circular tank (150 cm in diameter and 50 cm high) was filled with water (22 ± 2 °C) made opaque by addition of white paint. A platform (10 cm diameter, 30 cm away from the edge of the tank) was submerged 5 cm underneath the water surface, therefore not visible for the rats. Visual cues are provided on the walls of the room to allow spatial navigation. A camera wired to an automated tracking system (SMART v2.5.20, Panlab, Barcelone, Espagne) allows recording the rat's pathway and behavior.

2.2.2. Learning schedule

During 5 consecutive days, rats were trained to localize the platform. Rats underwent 6 trials per day, with different starting locations for each trial, following a pseudo-random sequence. Before the very first trial, rats were placed on the submerged platform during 30 s. Then every trial consisted in a swim, followed by a 30 s rest on the platform. Rats that did not reach the platform within 90 s were guided to it by the experimenter. The inter trail interval was of 15 s. Latency to reach the platform, distance travelled and swimming speed were recorded.

2.2.3. Classical memory assessment

Memory was assessed through probe tests occurring 2 h (short-term memory assessment) and 4 days (long-term memory assessment) after the last learning session unless stated otherwise. The platform was removed and rats were allowed to navigate in the water maze during 90 s.

Latency to reach to target annulus, time spent in the quadrants (each representing ¼ of the maze) and the number of each annulus crossings (one in each quadrant, the target annulus being the one where the platform was localized during learning) were recorded (used as the classical measures of water maze test performance: see Maei et al., 2009). Only the annulus crossings were analyzed and presented here for two reasons. First, the number of annulus crossings reveals a more accurate search of the platform than the time spent in the quadrants (see Florian et al., 2006; Serrano et al., 2008). Second, if all variables show that CD-fed rats are able to locate the platform during the first probe trial, only annulus crossings are relevant for this control group during subsequent probe trials (above chance level).

2.2.4. Memory updating

In order to assess spatial memory updating, known to be hippocampal-dependent (Rossato et al., 2006), other animals were trained in the same learning protocol as described above after juvenile or adult diet exposure (Fig. 1). The day after learning, rats were submitted to a reversal learning protocol. The reversal learning consisted of only one session of 6 trials, with the platform in the opposite location than during the initial learning. A probe test was performed 24 h after reversal learning. However, since none of the groups exhibited preference for target quadrant or annulus during this probe test, data of this probe trial is not shown.

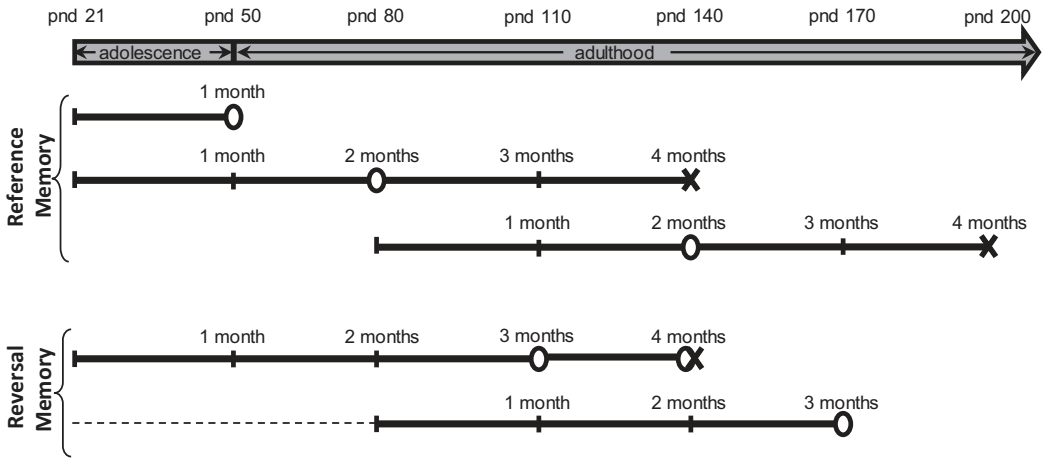

Fig. 1. Timeline representing the duration of diet treatments, memory assessment (0) and time of sacrifice for cytokines assessment (X).

2.3. Sacrifice

After the behavioral task, (i.e., after 4 months of diet exposure; Fig. 1) animals were either non-injected or injected with saline or LPS (lipopolysaccharide from *Escherichia coli* 0127:B8, Sigma Aldrich, 250 μg/kg in saline) 4 h before being euthanatized with an injection of a lethal dose of pentobarbital sodium. In one experiment, 15 min before sacrifice, rats either stayed in their home cage (HC) or received 6 consecutive trials in the MWM with the platform in a new location. Blood was collected transcardially before perfusion with 0.1 M phosphate buffered saline (PBS, pH = 7.4). Brain structures (hippocampus, hypothalamus and prefrontal cortex) were collected on ice, in RNAse free conditions and stored at −80 °C. Plasma was separated from blood (centrifugation at 4000 rpm during 15 min at 4 °C) and stored at −80 °C.

2.4. Plasma measurements of metabolic parameters and cytokines levels

Plasma obtained from sacrifice was used to assess different metabolic and inflammatory markers. Levels of cholesterol and triglycerides, insulin, leptin and cytokines (IL-6, IL-1β, TNFα and MCP-1) were assessed using specific kits (Cholesterol RTU, Triglycerides enzymatique PAP 150, Biomérieux, France) or milliplex (Rat serum adipokine kit, RADPK-81K, Millipore, Billerica, USA), respectively.

2.5. PCR analysis for cytokine levels

Total RNA was extracted from hypothalamus, half hippocampus and half frontal cortex collected at sacrifice, following TRIzol reagent (Invitrogen, France) manufacturer's suggested protocol. cDNA was synthesized from 2 μg of RNA with Superscript reverse transcriptase (Invitrogen, Cergy-Pontoise, France). Real time quantitative PCR was then performed using a LightCycler system (LC480, Roche diagnostics, Germany). Briefly, to allow online real-time detection of gene amplification products, MESA GREEN qPCR MasterMix for SYBR assay (Eurogentec, Belgium) was used. The primers' oligonucleotide sequences for target genes (IL-1β, IL-6 and TNFα) and housekeeping gene (β2-microglobulin) are given in Table 1. Final quantification was performed using the comparative threshold (Ct) method fully described elsewhere (Labrousse et al., 2009). Briefly, the target gene was normalized to the housekeeping gene and relative to the control group (calibrator) was determined by $2^{-\Delta\Delta Ct}$ (relative fold change).

2.6. Data analysis

Data are expressed as the mean ± SEM. Statistical analyses were carried out on Statview software and $p < 0.05$ was the threshold chosen to consider statistically significant difference. HFD fed animals were compared to their corresponding CD-exposed rats only (same age, same exposure duration) with repeated measures ANOVAs or unpaired *t*-tests. Animal's performances were compared to chance level (for spatial memory) through one sample *t*-tests. To assess LPS effect and diet effect, we used two way's ANOVAs followed by a Fisher's PLSD post-hoc test if interaction was significant. Animals with floating behavior (staying immobile for more than 15 s) or failing to learn the location of the platform (mean escape latency of the last learning day being higher than 40 s, i.e., twice the average of the whole population) were removed from behavioral analyses. This represents a total of 14 animals (9 CD and 5 HFD) out of 143 rats tested.

3. Results

3.1. The effects of HFD exposure on bodyweight and metabolism

Whenever the diet exposure started, animals under HFD were significantly heavier than CD rats on the time of behavioral assessment, i.e., after 2 months of diet exposure (13% and 8% overweight for jHFD and aHFD groups, respectively, see Table 2), as well as on the time of sacrifice, i.e., after 4 months of diet exposure (13% and 16% overweight for jHFD and aHFD, respectively). Metabolic parameters were measured at the time of sacrifice. Both jHFD and aHFD consumption increased leptin levels and aHFD intake also slightly increased glucose levels.

3.2. The effects of juvenile and adult HFD exposure on spatial learning and memory

Spatial learning was first assessed in the MWM after 1 or 2 months of HFD exposure starting at weaning (when the animals were 3 weeks-old), i.e., covering the juvenile period (Fig. 1). jCD and jHFD groups similarly learned the location of the hidden platform during the 5 days of training (6 trials per day), as evidenced by a decreased latency to reach the platform from day to day (time effect: $F_{(4,\ 80)} = 29.3$, $p < 0.001$ after 1 month of diet exposure, Fig. 2A; $F_{(4,\ 84} = 27.1$, $p < 0.001$ after 2 months of diet exposure, Fig. 2D; diet effect and interaction: $F < 1$; similar results were obtained for the distance travelled and no group difference was found in swimming speed, data not shown). Short-term memory was

Table 1
Primers designed for real-time qPCR.

Gene of interest	Oligonucleotide sequence 5′→3′	
β2m	F - CGTGCTTGCCATTCAGAAAA	R - GAAGTTGGGCTTCCCATTCTC
IL-1β (1)	F - CTCTCCAGTCAGGCTTCCTTGT	R - CGAAAGCTGCTATTTCACAGTTGA
IL-6 (1)	F - ATATGTTCTCAGGGAGATCTTGGAA	R - GTGCATCATCGCTGTTCATACA
TNFα (1)	F - CGGGCTCAGAATTTCCAACA	R - CGCAATCCAGGCCACTACTT
IL-1β (2)	F - GACTTGGGCTGTCCAGATGAG	R - TGAGTGACACTGCCTTCCTGAA
IL-6 (2)	F - TGCCCTTCAGGAACAGCTATG	R - TGTCAACAACATCAGTCCCAAGA
TNFα (2)	F - AGGCTGTCGCTACATCACTGAA	R - TGACCCGTAGGGCGATTACA

β2m: β2-microglobulin, IL-1β: interleukin-1β, IL-6: interleukin 6, TNFα: Tumor Necrosis Factor α. Oligonucleotide (1) was used for targeting those genes in the hippocampus and oligonucleotide (2) in prefrontal cortex and hypothalamus. F: forward, R: reverse.

Table 2
Bodyweight and metabolic parameters.

	Weaning		Adulthood	
	jCD	jHFD	aCD	aHFD
Initial body weight (g)	55 ± 1	55 ± 1	496 ± 4	490 ± 5
Body weight before behavior	434 ± 12	490 ± 13*	586 ± 6	635 ± 8*
Body weight before sacrifice	566 ± 11	640 ± 16*	681 ± 12	762 ± 14*
Leptin (ng/ml)	8.0 ± 1.0	16.1 ± 2.3*	8.6 ± 1.4	20.9 ± 5.4*
Insulin (ng/ml)	4.1 ± 0.3	3.6 ± 0.3	5.6 ± 0.8	5.0 ± 0.6
Cholesterol (g/L)	119 ± 9	128 ± 7	114 ± 9	126 ± 9
Triglycerides (g/L)	103 ± 22	87 ± 11	115 ± 16	98 ± 15
Glucose (mg/dL)	108 ± 4	104 ± 3	92 ± 1	99 ± 2*

Bodyweight on arrival, before assessing behavior (2 months of diet exposure) and before sacrifice (4 months of diet exposure) and metabolic parameters at the time of sacrifice after juvenile or adult exposure to CD or HFD.
* $p < 0.05$ when compared with the corresponding CD group (unpaired *t*-test).

assessed 2 h after the last learning trial. All groups showed a preference for the target annulus compared to the other annuli, a measure of precise spatial memory (comparison to 25% chance level using one sample *t*-test: jCD: $t_{(10)} = 5.7$ and jHFD: $t_{(10)} = 5.1$, $p < 0.001$ after 1 month of diet exposure, Fig. 2B; jCD: $t_{(9)} = 4.3$ and jHFD: $t_{(12)} = 5.9$, $p < 0.001$ after 2 month of diet exposure, Fig. 2E; no diet effect: unpaired *t*-test $t < 1$; total annuli crossing: 9 ± 2 for both groups after 1 month and 10 ± 1 after 2 months). When assessing long-term memory 4 days after learning, jCD group exposed to the diets for 1 month still exhibited higher crossings of the target annulus whereas jHFD-fed rats did not (jCD: $t_{(10)} = 2.9$, $p < 0.05$; jHFD: $t_{(10)} = 1.3$, $p > 0.05$; total annuli crossing for both groups: 7 ± 1; Fig. 2C) but there was no group difference ($t_{(20)} = 1.3$, $p > 0.05$). Stronger long-term memory disturbance was obtained after 2 months of jHFD exposure: again, only jCD group showed a preference for the target annulus during long-term memory (jCD: $t_{(9)} = 2.3$, $p < 0.05$; jHFD: $t_{(12)} = 0$; total annuli crossing for both groups: 6 ± 1; Fig. 2F) with a trend towards a higher preference for the target annulus in jCD group compared to jHFD group ($t_{(21)} = 1.9$, $p = 0.068$). To rule out the possibility that long-term memory impairment may be due to an extinction effect caused by probe test repetition, long-term memory was assessed in additional groups exposed for 2 months to the diets without short-term memory test. Similar impairment in long-term memory was obtained (jCD versus jHFD: $t_{(17)} = 2.1$, $p < 0.05$; total annuli crossing for both groups: 7 ± 1; data not shown). These results indicate that jHFD consumption specifically impaired spatial long-term memory suggesting an effect on memory consolidation processes.

We then decided to evaluate the effects of jHFD exposure on cognitive flexibility in the MWM, assessed by moving the hidden platform to a new location, i.e., using reversal learning (Fig. 1). Again, spatial learning accuracy was not affected by jHFD consumption (time effect: $F_{(4,96)} = 38.7$, $p < 0.001$, with no diet effect or interaction: $F < 1$, Fig. 2G). Moreover, both jCD and jHFD groups exhibited similar preference for the target annulus during a probe trial performed 24 h after the last learning day (data not shown). Two hours after this trial, we evaluated if jHFD exposure could disrupt spatial memory updating assessed by learning a novel location of the platform in one session of 6 trials. During reversal learning, both groups decreased their latency to reach the platform from trial to trial (time effect: $F_{(5,\ 110)} = 20.2$, $p < 0.001$), but there was a clear interaction between diet and the evolution of the performance (diet × time: $F_{(5,\ 110)} = 5.1$, $p < 0.001$; Fig. 2H). This was due to the fact that jHFD group showed a higher latency to reach the platform than jCD group during the first trial (67 versus 37 s respectively; $t_{(24)} = 3.09$, $p < 0.01$), but not during the 5 subsequent trials ($t < 1$; Fig. 2H). This indicates that jHFD rats show a delayed reversal acquisition, showing less flexibility in the first trial.

We then evaluated whether 2 months of HFD exposure restricted to adulthood (i.e., starting when the animals were 12 weeks-old, aHFD), impaired spatial long-term memory as 2 months of jHFD exposure did (Fig. 1). Both aCD and aHFD groups learned to locate the hidden platform during the 5 days of acquisition (time effect: $F_{(4,\ 96)} = 34.0$, $p < 0.001$; diet effect and interaction $F < 1$; Fig. 3A; similar results were obtained for the distance travelled, and no difference in swimming speed was found between groups, data not shown). Both groups crossed preferentially the target annulus during the short-term memory test (compared to 25% chance level: aCD: $t_{(13)} = 4.4$ and aHFD: $t_{(11)} = 8.1$, $p < 0.001$; no diet effect: $t_{(24)} < 1$; total annuli crossings: 11 ± 1 for aCD, and 9 ± 1 for aHFD, no diet effect: $t_{(24)} = 1.5$, $p > 0.05$; Fig. 3B) but also during the long-term memory test ($t_{(13)} = 3.1$ and $t_{(11)} = 2.3$, $p < 0.05$ for aCD and aHFD, respectively; no diet effect, $t_{(24)} < 1$; total crossings for both groups: 8 ± 1; Fig. 3C).

We then evaluated the effects of aHFD exposure on reversal learning (Fig. 1). We found a general improvement of performance after repeated trials on either reference learning (time effect: $F_{(4,76)} = 32.4$, $p < 0.001$, Fig. 3D) or reversal learning (time effect: $F_{(5,95)} = 4.9$, $p < 0.001$, Fig. 3E) but did not find any diet effect or interaction (reference learning: $F < 2.5$, Fig. 3D; reversal learning: $F < 2.5$, Fig. 3E).

Altogether, these results indicate that jHFD consumption impaired spatial memory consolidation and spatial flexibility. Similar HFD consumption at adulthood did neither affect long-term memory nor spatial flexibility indicating that juvenile period is particularly vulnerable to the effects of HFD.

3.3. The effects of juvenile and adult HFD exposure on pro-inflammatory cytokine levels

Since inflammation could be a potential mechanism explaining memory impairment due to HFD consumption, we assessed inflammation in our 4 groups of animals (jCD, jHFD, aCD and aHFD)

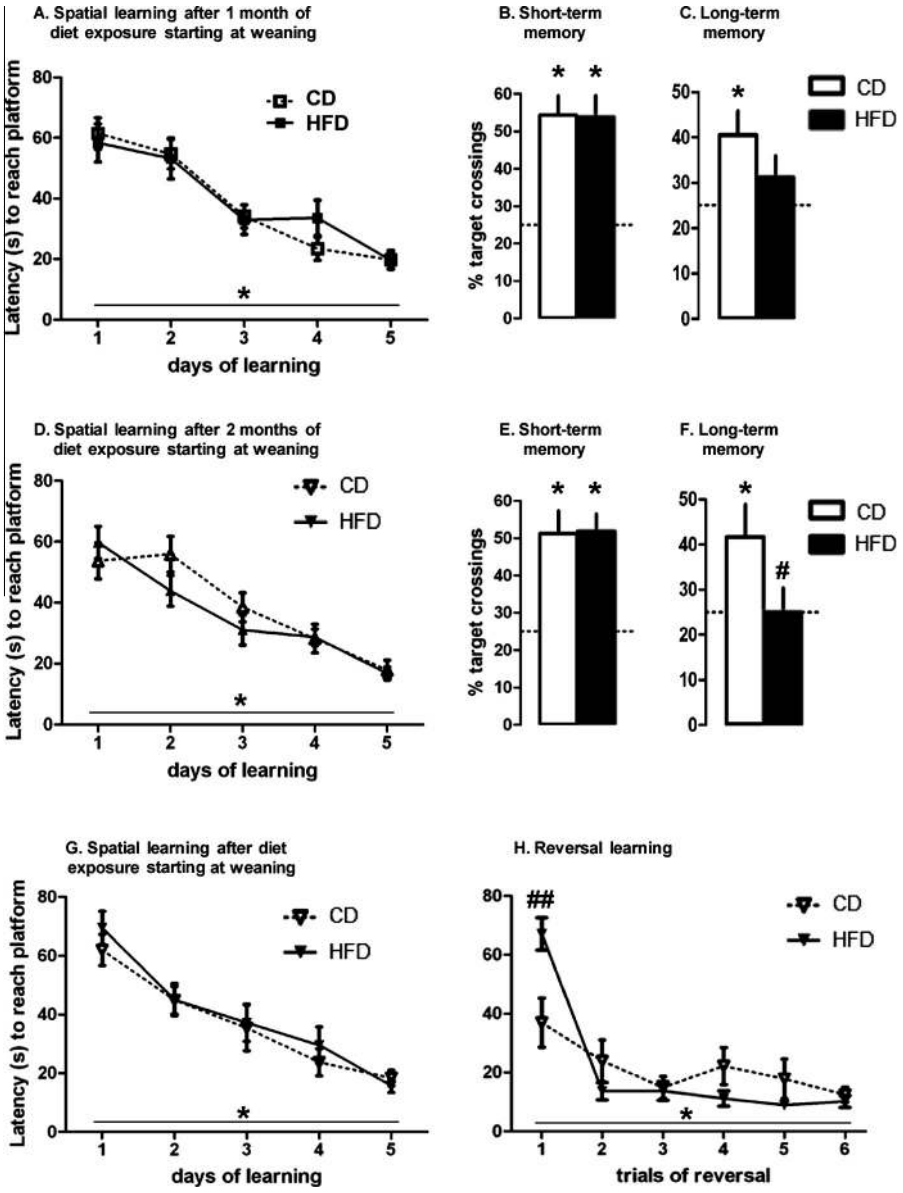

Fig. 2. Effects of juvenile HFD consumption on spatial memory. (A, D) Initial 5-days of spatial training (B, E), short-term memory (assessed 2 h after the last day of training) and (C, F) long-term memory (assessed 4 days after the last day of training) after 1 (A, B, C) or 2 months (D, E, F) of HFD or CD exposure starting at weaning (1 month: HFD: $n = 11$; CD: $n = 11$; 2 months: HFD: $n = 13$; CD: $n = 10$). (G) Initial 5-days of spatial training followed the next day by (H) the 6 trials of reversal learning after 2 months of HFD ($n = 14$) or CD ($n = 12$) exposure starting at weaning. For learning curves: $^{*}p < 0.05$ (repeated measure's ANOVA: time effect). For memory tests: $^{*}p < 0.05$ when compared to 25% (chance level) and $^{*}p < 0.07$ when compared to corresponding CD group (bilateral unpaired t-test). For reversal learning: $^{\#\#}p < 0.01$ when compared to CD group.

previously used for spatial reference memory assessment (see Fig. 1). First, we measured pro-inflammatory cytokines expression in basal conditions, and revealed no effect of either jHFD or aHFD exposure on cytokine levels in the plasma (IL-1β: jCD versus jHFD: $t_{(20)} = 1.6$, $p > 0.1$, aCD versus aHFD: $t_{(19)} = 1.4$, $p > 0.1$; IL-6 and TNFα were not detectable) or in the hippocampus (jCD versus jHFD: $t_{(14)} < 1$ for IL-1β, IL-6 and TNFα; aCD versus aHFD:, $t_{(5)} = 2.0$ for IL-1β, $t_{(6)} = 1.2$ for IL-6 and $t_{(6)} < 1$ for TNFα, $p > 0.05$; data not shown).

As basal condition did not reveal any effect of the HFD on peripheral and brain inflammation we assessed inflammatory response in other animals 4 h after an immune challenge induced by an intra-peritoneal injection of LPS (half of the animals receiving saline). In the plasma, there was a clear treatment effect: LPS induced an increase of all cytokines studied, for all groups ($F > 20$, $p < 0.001$). For IL-1β there was no diet effect or interaction. Since IL-6 and TNFα were not detectable after saline injection, we only explored the diet effect after LPS injection: an effect appears for TNFα in adult exposed rats, aHFD group showing a higher TNFα level than aCD group ($t_{(8)} = 3.2$, $p < 0.05$) whereas diet exposure did not affect IL-6 level in either juvenile or adult exposed rats (data not shown).

In the hippocampus, there was a clear LPS effect for all cytokines studied in all groups ($F > 5$, $p < 0.05$; Fig. 4A–C). A diet effect and more importantly a diet*treatment interaction appeared in juvenile groups for IL-1β and TNFα (diet effect: $F_{(1,\,24)} = 6.8$, $p < 0.05$ for Il-1β, $F_{(1,\,27)} = 6.0$, $p = 0.021$ for TNFα; interaction: $F_{(1,24)} = 6.3$, $p = 0.02$ for Il-1β, $F_{(1,27)} = 5.1$, $p = 0.033$ for TNFα, respectively), but not in adult groups ($F < 1$ for all). This was explained by an exaggerated increase of cytokine level after LPS in jHFD rats compared to jCD rats (IL-1β: $p = 0.002$, TNFα $p = 0.003$). No diet effect or interaction was revealed for IL-6 in the hippocampus ($F < 1$). In the frontal cortex

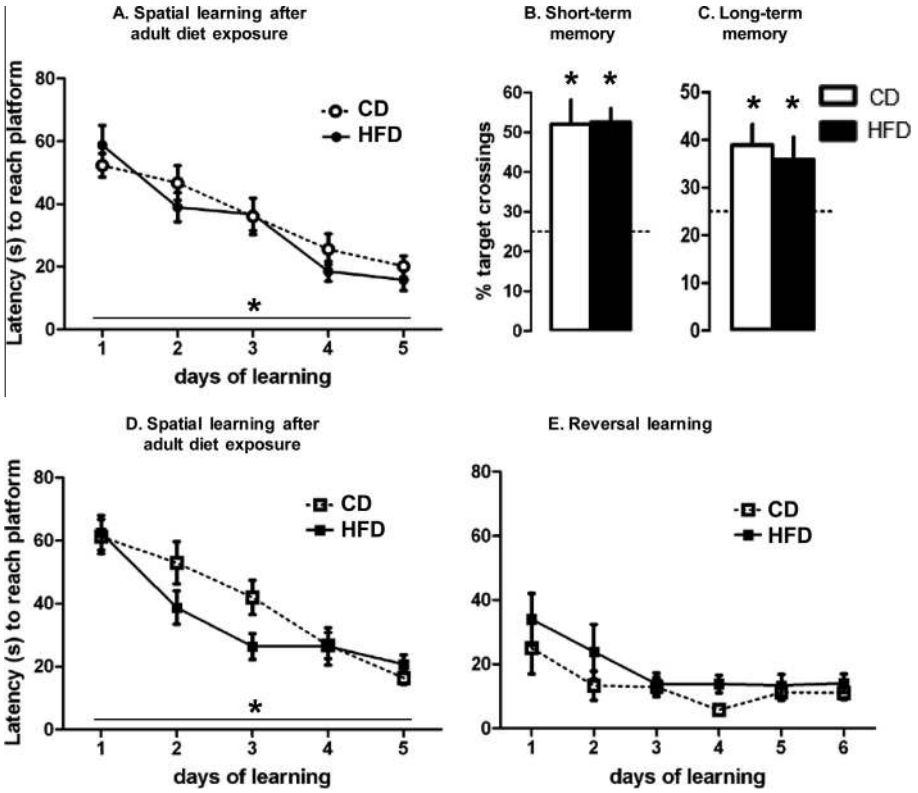

Fig. 3. Effects of adult HFD consumption on spatial memory. (A) Initial 5-days of spatial training (B), short-term memory (assessed 2 h after the last day of training) and (C) long-term memory (assessed 4 days after the last day of training) after 2 months of adult exposure to HFD ($n = 12$) or to CD ($n = 14$). (D) Initial 5-days of spatial training followed the next day by (E) the 6 trials of reversal learning after 2 months of adult exposure to HFD ($n = 11$) or to CD ($n = 10$). For learning curves: $^{*}p < 0.05$ (repeated measure's ANOVA: time effect). For memory tests: $^{*}p < 0.05$ when compared to 25% (chance level).

and the hypothalamus, there was a clear LPS effect for all cytokines studied in all groups ($F > 10$, $p < 0.05$, except for TNFα in frontal cortex of juvenile exposed rats: $F_{(1,34)} = 2.5$, $p = 0.13$) but no diet effect or interactions ($F < 1$; Fig. 4A–C). This clearly indicates that juvenile, but not adult, HFD consumption enhanced the sensitivity to LPS-induced cytokines expression specifically in the hippocampus.

3.4. The effects of reversal spatial learning and juvenile HFD exposure on brain pro-inflammatory cytokine levels

We found that jHFD exposure delayed the acquisition of the reversal task. As hippocampal-dependent memory task can raise the level of pro-inflammatory markers in the hippocampus, especially IL-1β (Goshen et al., 2007; Labrousse et al., 2009), we evaluated in our conditions whether reversal spatial learning was sufficient to enhance the level of pro-inflammatory cytokines specifically in the hippocampus and whether this increase was potentiated after jHFD exposure. Here we measured the level of IL-1β, IL-6 and TNFα expression in the hippocampus, hypothalamus and frontal cortex of jHFD and jCD rats previously trained in reversal learning (Fig. 1), 15' after a single session made of 6 consecutive trials in the MWM with the platform in a new location. jHFD and jCD animals staying in home-cage (HC) were used as controls for basal cytokine level expression.

During this reversal learning both groups decreased their latency to reach the platform from trial to trial (time effect: $F_{(5,55)} = 7.0$, $p < 0.001$) and again jHFD intake delayed reversal acquisition (diet × time: $F_{(5,55)} = 2.7$, $p = 0.032$, mean latency to reach the platform on the first trail: 25 versus 54 s, $t_{(11)} = 2.1$, $p = 0.065$ and subsequent 5 trials: 14 versus 17 s, $p > 0.1$, Fig. 5A). IL-1β expression in the hippocampus was specifically affected by the reversal spatial learning (HC versus MWM: $F_{(1,23)} = 4.4$, $p < 0.05$; with no diet effect and interaction: $F < 1$; Fig. 5B). Interestingly, when comparison was restricted to either jCD or jHFD groups, the effects of reversal training were obtained only in jHFD groups, with significantly higher IL-1β level in the group submitted to reversal learning than in HC group (MWM versus HC: $t_{(12)} = 3.1$, $p = 0.009$ in jHFD groups; $t_{(11)} < 1$ in jCD groups; Fig. 5B). IL-1β expression was not affected in the prefrontal cortex ($F < 1$; Fig. 5C) and in the hypothalamus ($F < 1$; data not shown). Moreover, IL-6 and TNFα expression was not affected in any of the structures studied ($F < 1$; data not shown). This indicates that the delayed spatial reversal acquisition of jHFD-fed rats is associated with an increase of IL-1β expression specifically in the hippocampus.

4. Discussion

Our results show that juvenile consumption of HFD by rats, from weaning to adulthood, results in a disruption of spatial long-term memory and flexibility as well as a higher inflammatory response to immune challenge, specifically in the hippocampus. The same duration of HFD consumption confined at adulthood does not yield such behavioral nor inflammatory adverse consequences. Moreover, spatial reversal learning induces a higher pro-inflammatory cytokine expression specifically in the hippocampus of jHFD. These effects are most likely due to the time of the initiation of HFD consumption and not to the age difference at the time of behavioral or inflammatory assessment. Indeed, all experiments were performed on adult animals (from 3 to 5 months of age; Fig. 1) and jCD and aCD-fed groups exhibited similar behavioral performance and similar cytokine levels. These results extend

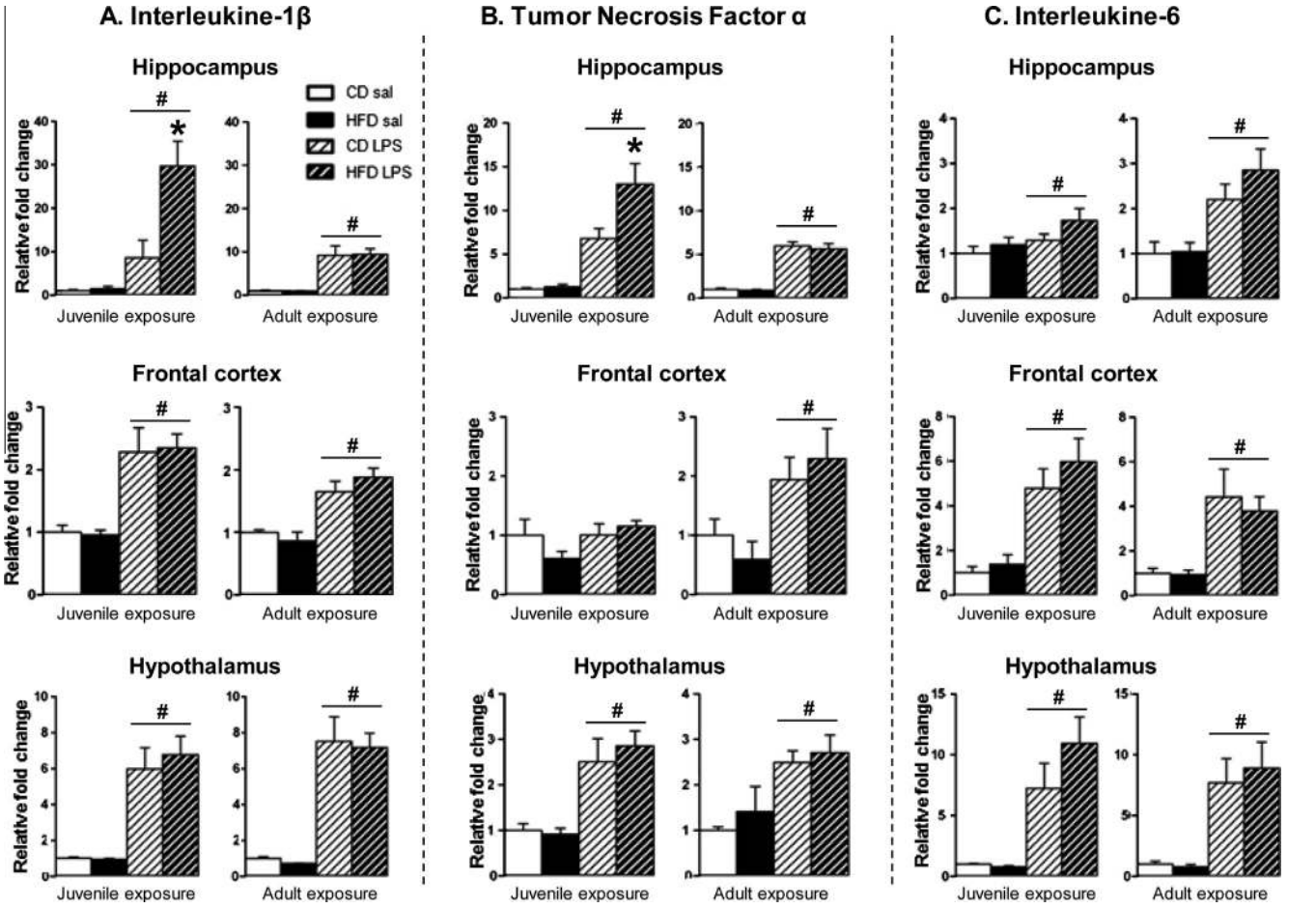

Fig. 4. Effects of juvenile or adult HFD consumption on cytokines expression in different brain structures after an immune challenge. RNA expression of (A) Interleukine-1β (B) Tumor necrosis Factor α (C) Interleukine-6 4 h after intraperitoneal injection of either saline or LPS (250 μg/kg) in juvenile or adult HFD or CD-fed rats (jHFD saline: $n = 9$; jCD saline: $n = 8$; jHFD LPS: $n = 10$; jCD LPS: $n = 8$; aHFD saline: $n = 6$; aCD saline: $n = 6$; aHFD LPS: $n = 7$; aCD LPS: $n = 6$). Relative expression levels were plotted as fold change relative to the saline-injected CD group. $^{\#}p < 0.05$ when compared to saline-injected groups (ANOVA: LPS effect); $^{*}p < 0.05$ when compared to LPS-injected CD group (ANOVA: diet*injection interaction, followed by ANOVA's post-hoc).

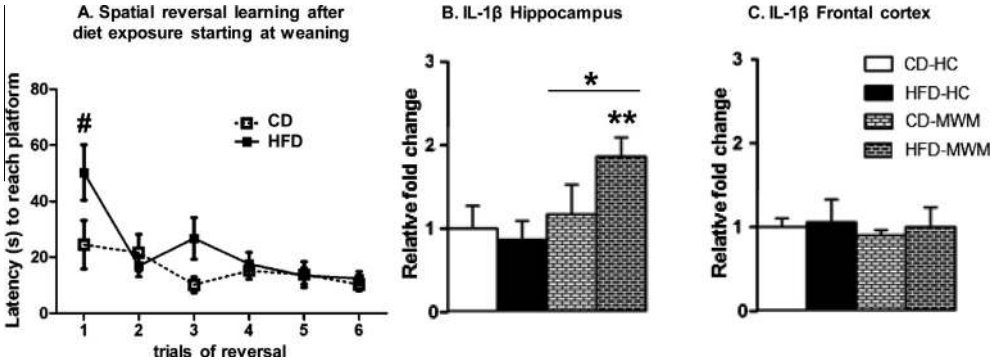

Fig. 5. Effects of juvenile HFD exposure on interleukine-1β expression in hippocampus and frontal cortex after spatial reversal learning. (A) Rats were either submitted to spatial reversal learning (MWM; jHFD: $n = 8$; jCD: $n = 7$) or stayed in their homecage prior to sacrifice (HC; jHFD: $n = 8$; jCD: $n = 6$). Relative RNA expression of Interleukine-1β in hippocampus (B) and frontal cortex (C) were plotted as fold change relative to jCD HC group. For reversal learning: $^{\#}p < 0.07$ when compared to CD group (bilateral unpaired t-test). For cytokine expression: $^{*}p < 0.05$ when compared to HC rats (ANOVA: MWM effect). $^{**}p < 0.01$ when compared to the jHFD HC group (restricted analyses to jHFD groups; unpaired t-test).

our previous work demonstrating that the juvenile period is particularly vulnerable to the adverse effects of HFD on hippocampal function in mice (Boitard et al., 2012).

In our conditions HFD exposure confined at adulthood does not impair hippocampal function. However, previous studies showed impairments of hippocampal-dependent memory after HFD exposure at adulthood to more drastic diet conditions, i.e., longer duration of HFD consumption, higher percentage of fat or a combination of HFD with high sugar (for review: Kanoski and Davidson, 2011). Our study also reveals that the juvenile vulnerability to the effect of HFD on spatial memory, first evidenced in mice (Boitard et al., 2012), can be generalized to another species, in which HFD

exposure affects metabolism in a different manner. Indeed, exposure to HFD in mice increases leptin, insulin and cholesterol, whereas only leptin levels appear to be heightened in rats. Despite such metabolic differences, both jHFD fed mice and rats exhibit hippocampal-dependent memory impairments, suggesting that these metabolic alterations do not critically contribute to the effects of jHFD on memory. Moreover aHFD mice and rats show normal spatial performances whereas they exhibit similar metabolic changes as jHFD mice and rats, respectively, suggesting an absence of metabolic contributions to the effects of jHFD.

Our study brings out new findings in the description of jHFD effects on hippocampal-dependent memory disruption. HFD exposure during the peri-adolescence period (covering late childhood, adolescence and early adulthood) impairs specifically long-term memory without affecting spatial learning or short-term memory. Interestingly, ablating hippocampal neurogenesis disrupts spatial long-term memory in the MWM (Deng et al., 2009; Snyder et al., 2005), particularly when neurogenesis ablation occurs during juvenility (Martinez-Canabal et al., 2013), and we recently showed that jHFD decreases hippocampal neurogenesis (Boitard et al., 2012). Similarly, acute or chronic pro-inflammatory cytokines overexpression in the brain impairs long-term, but not short-term, hippocampal-dependent memory (Rachal Pugh et al., 2001; Hein et al., 2010). Moreover, we show spatial reversal acquisition is delayed in jHFD fed rats suggesting they are less flexible. Again, blockade of hippocampal neurogenesis as well as enhancement of brain pro-inflammatory cytokines induced by acute inflammation (in aged animals) impairs the ability to use previously learned information in a novel situation in order to complete a hippocampus-dependent task (Burghardt et al., 2012; Chen et al., 2008; Dupret et al., 2008; Garthe et al., 2009). It is therefore possible that jHFD impairs spatial memory consolidation and flexible memory expression through decreased neurogenesis, as previously suggested (Boitard et al., 2012), but also through increased hippocampal inflammation.

Obesity is a disease in which low grade inflammation is described at the periphery both in humans and rodent models (Clement et al., 2004; Cottam et al., 2004; Everard and Cani, 2013). In rodents, obesity-induced inflammation is also described at the brain level and, of interest for spatial memory, in the hippocampus (Dinel et al., 2011; Lu et al., 2011; Thirumangalakudi et al., 2008). In the present study, we do not find any effects of HFD exposure on peripheral or brain pro-inflammatory cytokines production at basal state. This discrepancy between our work and previous studies may be due to the species (mice versus rats) or the type of HFD used. Whereas a very HFD (60% Kcal from fat) induces an increase in basal cytokine expression in the brain, a HFD similar to ours (40% Kcal from fat) does not modify these levels (Maric et al., 2013; Pistell et al., 2010).

Despite revealing no diet effects on basal inflammatory status, we further explored if HFD exposure could exacerbate the pro-inflammatory cytokine production in response to systemic immune challenge. Bacterial LPS stimulation, classically used to induce and assess the immune system response, results in a similar increase of the circulating pro-inflammatory cytokines in control and HFD groups. However, jHFD but not aHFD exposure induces a potentiated expression of central pro-inflammatory cytokines IL-1β and TNFα after peripheral immune LPS stimulation. This exaggerated inflammatory response is specific to the hippocampus, as it is not found in the hypothalamus or the frontal cortex. Yet, in such conditions, pro-inflammatory cytokine expression is only assessed 4 h after systemic LPS injection. This could have masked some earlier HFD effects on the levels of circulating and hypothalamic cytokines as previously described (Pohl et al., 2009). Nevertheless, we recently obtained similar results in a genetic model of obesity, the *db*/*db* mice, for which overweight starts very early after weaning. In these obese mice, stronger inflammation is described specifically in hippocampus (Dinel et al., 2011) and heightened inflammation after LPS challenge occurrs only at the central, but not peripheral, level (Dinel et al., 2014).

It is not yet clear whether hippocampal inflammation induced by the combination of LPS and jHFD intake originates in endothelial cells, neurons, astrocytes, microglia or leukocytes. However, some recent evidences suggest that at least microglia and leukocytes infiltration could be involved in this effect. Amplified central inflammatory response following immune challenge may be related to microglial priming, which induces stronger cytokine production and/or impairments in resolving the inflammation (Chen et al., 2008; Field et al., 2012; Murray et al., 2012). This could also be mediated by the transmigration of cytokines-expressing leukocyte to the brain following systemic LPS injection (Rummel et al., 2010). As this process is regulated by circulating leptin, the high levels of endogenous leptin occurring with obesity exacerbate neutrophile recruitment into the brain after LPS challenge (Aguilar-Valles et al., 2013). The increased blood-brain barrier permeability specifically observed at the hippocampal level in obese animals (Davidson et al., 2012; Kanoski et al., 2010) could also favor leukocyte recruitment into the hippocampus following systemic inflammation.

In addition to immune challenge, some physiological conditions can enhance brain cytokines expression. For example, contextual fear conditioning or spatial working memory leads to increased IL-1β expression in the hippocampus of lean mice (Goshen et al., 2007; Labrousse et al., 2009). Here we demonstrate that reversal spatial learning in the MWM is associated with an enhanced IL-1β expression specifically in the hippocampus of jHFD fed, but not in control, rats. According to previous studies (Goshen et al., 2007; Labrousse et al., 2009), one would have expected an enhancement of hippocampal IL-1β expression in control rats after reversal learning. This discrepancy could be due to the fact we used well-trained rats whereas naïve mice were used in previous studies. It is a possibility that hippocampal IL-1β production may decrease from trial to trial during spatial training in control rats, this decrease being mitigated after jHFD intake. If physiological IL-1β expression is important for memory, high cytokine expression can rapidly be deleterious (Goshen et al., 2007). Even if the higher IL-1β expression in the hippocampus of jHFD rats could be related to their delayed reversal acquisition, future work will be needed to explore the causal link between potentiated hippocampal inflammation in jHFD rats and their spatial memory deficit. One suggestion would be to block the action of IL-1β in the hippocampus of jHFD animals, using the antagonist IL-1RA, and to evaluate if it prevents their spatial memory deficits as successfully used in obese *db*/*db* mice (Erion et al., 2014).

Altogether, our results demonstrate that jHFD exposure leads to spatial memory impairment and to an exaggerated increase of pro-inflammatory cytokines expression specifically in the hippocampus, either under pathological (immune challenge) or physiological conditions (reversal spatial learning). These results extend our previous work (Boitard et al., 2012) demonstrating that jHFD, but not aHFD, intake decreases hippocampal neurogenesis and impairs spatial relational memory in mice. As enhanced hippocampal inflammation can decrease hippocampal neurogenesis (Goshen et al., 2008; Ekdahl et al., 2009), it remains to be determined in jHFD-fed rats how hippocampal inflammation affects neurogenesis and spatial memory. Our results indicate the juvenile period is particularly vulnerable to the adverse effects of HFD on hippocampal function. Concerns should be raised on this issue, knowing the increased prevalence of obesity in youth.

Conflict of interest

Nothing to report.

Authorship and contributorship

C.B. and A.C. performed the behavioral experiments, C.B., J.S. and A.A. performed the PCR and A.A. the hormone measurements. C.B., N.C., S.L. and G.F. participated in experimental design, interpretation of data and preparation of the manuscript.

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