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Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: Focus on brain indoleamine 2,3-dioxygenase activation

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ABSTRACT

Obesity is associated with a high prevalence of mood symptoms and cognitive dysfunctions that emerges as significant risk factors for important health complications such as cardiovascular diseases and type 2 diabetes. It is therefore important to identify the dynamic of development and the pathophysiological mechanisms underlying these neuropsychiatric symptoms. Obesity is also associated with peripheral low-grade inflammation and increased susceptibility to immune-mediated diseases. Excessive production of proinflammatory cytokines and the resulting activation of the brain tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) have been shown to promote neurobehavioral complications, particularly depression. In that context, questions arise about the impact of diet-induced obesity on the onset of neuropsychiatric alterations and the increased susceptibility to immune-mediated diseases displayed by obese patients, particularly through brain IDO activation.

To answer these questions, we used C57Bl/6 mice exposed to standard diet or western diet (WD; consisting of palatable energy-dense food) since weaning and for 20 weeks. We then measured inflammatory and behavioral responses to a systemic immune challenge with lipopolysaccharide (LPS) in experimental conditions known to alter cognitive and emotional behaviors independently of any motor impairment. We first showed that in absence of LPS, 9 weeks of WD is sufficient to impair spatial recognition memory (in the Y-maze). On the other hand, 18 weeks of WD increased anxiety-like behavior (in the elevated plus-maze), but did not affect depressive-like behavior (in the tail-suspension and forced-swim tests). However, 20 weeks of WD altered LPS-induced depressive-like behavior compared to LPS-treated lean mice and exacerbated hippocampal and hypothalamic proinflammatory cytokine expression and brain IDO activation. Taken together, these results show that WD exposure alters cognition and anxiety in unstimulated conditions and enhances activation of neurobiological mechanisms underlying depression after immune stimulation. They suggest therefore that obesity, and possibly obesity-associated inflammatory priming, may represent a vulnerability state to immune-mediated depressive symptoms.

1. Introduction

Over the past decades, obesity has continuously increased at alarming rates throughout the world fostering the rise in serious obesity-related outcomes, particularly cardiovascular diseases and metabolic alterations such as type 2 diabetes (Malnick and Knobler, 2006). In addition, obesity is often associated with a high prevalence of altered emotional reactivity and cognitive dysfunctions that frequently evolves in neuropsychiatric disorders (Francis and Stevenson, 2013; Luppino et al., 2012; Sellbom and Gunstad, 2012). Moreover, neuropsychiatric symptoms emerge as additional risk factors for obesity-related systemic pathological complications (Fiedorowicz et al., 2008; Scott et al., 2008). It is therefore important to identify the dynamic of development of such emotional and cognitive alterations and the underlying pathophysiological mechanisms in the context of obesity.

Obesity is presently viewed not only as a metabolic disorder but also as an inflammatory disease affecting both innate and acquired immune system (Gregor and Hotamisligil, 2011). Indeed, obese patients often display basal low-grade systemic inflammation elicited

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by both adipose tissue (Cancello and Clement, 2006) and gut microbiota (Cani et al., 2008), and increased susceptibility to immune-mediated diseases (Kanneganti and Dixit, 2012) and to infections (Falagas and Kompoti, 2006; Huttunen and Syrjanen, 2013). Interestingly, clinical studies report positive associations in obese subjects between peripheral inflammatory status and cognitive decline (Sellbom and Gunstad, 2012; Sweat et al., 2008) or mood symptoms (Capuron et al., 2008). Conversely, surgery-induced weight loss is associated with reduced peripheral inflammation (Cancello and Clement, 2006) and significant improvement in emotional status (Capuron et al., 2010; Emery et al., 2007). In addition, studies performed in rodent models of obesity show that inflammation also exists within the brain, particularly in areas involved in mood regulation and memory formation such as the hippocampus (Dinel et al., 2011, 2014) or the cortex (Pistell et al., 2010). Of note, increased cytokine expression in these structures is associated with increased emotional behavior and cognitive impairments.

Both clinical (Capuron and Miller, 2011; Raison et al., 2010) and experimental (Chess et al., 2009; Frenois et al., 2007; Gold et al., 2011; Henry et al., 2009; Moreau et al., 2008; O'Connor et al., 2009a,b,c; Salazar et al., 2012; Walker et al., 2013) studies have shown that dysregulated production and/or brain action of cytokines promote emotional and cognitive complications through selective activation of the tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO), its metabolite kynurenine or the neuroactive derivatives of kynurenine. Moreover, similar association between brain IDO activation and emotional or cognitive impairments also exists in chronic inflammatory conditions such as aging (Capuron and Miller, 2011; Corona et al., 2012; Godbout et al., 2008; Kelley et al., 2013). As severely obese individuals also display increased peripheral IDO activity (Brandacher et al., 2007; Oxenkrug, 2010), peripheral cytokine production and IDO activation may be relevant to the onset of emotional and cognitive alterations in obesity. However, the impact of diet-induced obesity on brain IDO activation and its role in the onset of emotional and cognitive alterations in that context is still largely unknown.

In the present study, we sought to approach this question by studying in mice the emotional, cognitive and inflammatory impact of chronic consumption of western diet (WD; consisting of palatable energy-dense food). In basal conditions, we first followed the development of obesity-associated behavioral alterations by assessing spatial working memory, anxiety-like and depressive-like behaviors after 9 and 18 weeks of WD exposure. As WD exposure alone did not affect either inflammatory status or depressive-like behavior, we then used the cytokine inducer lipopolysaccharide (LPS) to evaluate the effect of WD on LPS-induced IDO activation and depressive-like behavior, independently of sickness behavior. Indeed, whereas peripheral inflammatory response to immune challenges has been already studied in obese mice (Amar et al., 2007; Lawrence et al., 2012; Naguib et al., 2004; Pini et al., 2013; Pohl et al., 2013), there is still scarce information regarding LPS-induced depressive-like behavior (Aguilar-Valles et al., 2014; Dinel et al., 2014) and hippocampal inflammatory activation (Dinel et al., 2014) in that context. We show here that in unstimulated conditions chronic WD exposure first impaired spatial memory, then increased anxiety-like behavior. Moreover, it altered LPS-induced depressive-like behavior and exacerbated brain inflammation, including IDO activation.

2. Materials and methods

2.1. Animals and diets

All animal experiments were conducted according to the relevant French (Directive 87/148, Ministère de l'Agriculture et de la Pêche) and international (Directive 2010/63, European Community) legislation. They adhered to protocols approved by the Animal Care and Use Committee from Bordeaux University (approval ID: 5012047-A). Every effort was made to minimize suffering and the number of animal used. Three-week old male C57BL/6J mice were obtained from Charles River (France). On arrival, they were randomly allocated to control group (Standard diet: SD; $n = 24$) or western diet group (WD; $n = 24$). No body weight differences existed between both groups at the beginning of the experiment. Control group was fed with standard chow (A04, Safe, France) that provides 2.9 kcal/g of diet (of which 9.3% from fat). Western diet (WD) group was fed with an in-house prepared palatable energy-dense chow providing 4.0 kcal/g of diet (of which 49% from fat) as previously published (Berraondo et al., 2000). Mice were housed 4 per cage in a controlled environment (normal 12 h light/dark cycle; 22 ± 1 °C), with food and water available *ad libitum*. Body weight was measured once a week over a 20-week period. All mice were individually handled for a few minutes once daily for 1 week before behavioral tests were initiated to minimize stress reactions to manipulation. Mice were 21–23 weeks-old by the time of behavioral assessments.

2.2. Experimental procedures

The present study first aimed at assessing the impact of chronic WD exposure on emotional behaviors (anxiety-like and depressive-like behaviors), as well as spatial working memory performances in unstimulated conditions. Mice were therefore tested in the elevated plus-maze (anxiety-like behavior), the tail suspension test (depressive-like behavior) or the Y-maze (hippocampal-dependent memory test) after 9 and 18 weeks of exposure to either regular chow or WD. Each mouse was randomly exposed to one behavioral test only at each time-point and never tested in the same test twice in order to avoid potential interferences.

One week after completion of these behavioral tests, inflammatory and behavioral responses to a systemic immune challenge with LPS were then measured in order to assess the potential consequences of chronic WD exposure on brain cytokine and IDO activation and related behavioral alterations. Peripheral LPS administration enhances peripheral and brain production of inflammatory cytokines, which are responsible of physiological and behavioral symptoms of sickness (Dantzer et al., 2008). These symptoms progressively wane whereas the expression of depressive-like behaviors remains up to 24 h after treatment (Frenois et al., 2007; Godbout et al., 2008; O'Connor et al., 2009c). Consequently, it is possible to experimentally dissociate emotional behaviors from sickness behavior (particularly motor impairment) by choosing relevant post-treatment time-points. Here, mice fed with SD or WD for 20 weeks were intraperitoneally injected with sterile endotoxin-free saline or freshly prepared LPS (*Escherichia coli*, serotype 0127:B8, Sigma). The dose used (830 µg/kg) was selected on the basis of its ability to induce the full spectrum of sickness response and a reliable increase of brain IDO activity (Frenois et al., 2007; Lestage et al., 2002; O'Connor et al., 2009c). Mice were then divided in two subgroups sacrificed either 2 h (group 1) or 25 h after treatment (group 2). Mice from this last group were respectively tested 23 h and 24 h after treatment for their locomotor activity, in order to verify that they had fully recovered from LPS-induced locomotor impairment, and for their depressive-like behavior. LPS-induced body weight loss was also assessed just before and 3, 6 and 25 h after treatment.

By the time of sacrifice, mice were euthanized by CO_2 inhalation within a few seconds after being picked up from their home cage. Blood samples were immediately collected via cardiac puncture into EDTA (10%)-coated chilled tubes. After centrifugation (10 min, 3000*g*, 4 °C), aliquots of plasma were stored at −80 °C.

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Mice were immediately perfused with chilled PBS via the ascending aorta to remove all traces of blood from tissues. Brains were rapidly extracted from the skulls and either carefully dissected to immediately collect, dry frozen and store the hippocampus and the hypothalamus for subsequent determination of mRNA levels (group 1) or directly stored at −80 °C until IDO activity assays (group 2). Lungs were also rapidly collected from these last mice and directly stored at −80 °C.

2.3. Behavioral measures

All behavioral experiments were performed in the morning, under conditions of dim light and low noise. Behavior was videotaped to be scored later by a trained observer blind to drug treatments, using the "Observer Basic" software (Noldus, Netherlands). All testing equipment was thoroughly cleaned between each session.

2.3.1. Elevated plus maze (EPM)

The EPM was a plus shaped maze made of dark gray plastic with two opposing open arms (30 × 8 cm) and two opposing closed arms (30 × 8 × 15 cm) connected by a central platform (8 × 8 cm) and elevated 120 cm above the floor. Each mouse was placed in the center of the maze facing an open arm and the number of arm entries, as well as the percent of time spent in open arms, was assessed during a 5-min period. An entry was scored as such only when the mouse placed all four limbs into any given arm. A reduction of the percent of time spent and number of entries into the open arms is considered as an anxiety-like index, independent of locomotor activity (Belzung and Griebel, 2001).

2.3.2. Y-maze

Spontaneous spatial recognition in the Y-maze was used as a hippocampal-dependent test as previously described (Dellu et al., 2000; Labrousse et al., 2009). The apparatus was a Y-shaped maze made of dark gray plastic with three identical arms (34 × 8 × 14 cm). Corncob litter covered the floor and was mixed between each trial in order to remove olfactory cues. Visual cues were placed in the testing room and kept constant during the whole test. Discrimination of novelty versus familiarity was based on the different aspects of the environment that the mouse can perceive from each arm of the Y-maze. In the first trial of the test (acquisition), one arm was closed with a door and mice were allowed to freely visit the two other arms for 5 min. After a 30-min inter-trial interval (ITI), mice were again placed in the start arm for the second trial (retrieval) and allowed free access to all three arms for 5 min. Start and closed arms were randomly assigned to each mouse. Arm entries were defined as all four paws entering the arm. Preference for novelty was also measured using a short 2-min ITI between acquisition and retrieval in order to control for potential motivational disturbances (Dellu et al., 2000; Labrousse et al., 2009). Analyses were based on the time spent exploring the novel and the familiar arms during the second trial.

2.3.3. Tail suspension test (TST)

The TST was carried out as previously described (Moreau et al., 2008). Briefly, an adhesive tape was fixed to the mouse tail (distance from the tip of the tail = 2 cm) and hooked to a horizontal ring stand bar placed 30 cm above the floor. The test was conducted for a period of 6 min in a visually isolated area. The apparatus was cleaned thoroughly after each mouse. Mice demonstrated several escape attempts interspersed with immobility periods during which they hung passively and completely motionless.

2.3.4. Motor activity

The motor effects of LPS were assessed as previously described (Frenois et al., 2007; Moreau et al., 2008) in a cage (30 × 11 × 12 cm) divided into 2 communicating compartments. Motor activity was evaluated by counting the total number of between-compartments crossings and rearings performed over the 6-min test. The cage was cleaned thoroughly between each session.

2.3.5. Forced swim test (FST)

Experimental procedure used was essentially similar to that described previously (Frenois et al., 2007; Moreau et al., 2008). Briefly, mouse was placed individually in a cylinder (diameter: 16 cm; height: 31 cm) filled with 25 °C water for a 6-min test. Duration of swimming, climbing and immobility was determined during the 5 last minutes of the test. A mouse was judged to be immobile when it moved only slowly to remain floating, keeping its head above water.

2.4. Biochemical measures

2.4.1. Hormones, cytokines and LPS assays

As previously described (André et al., 2008; Moreau et al., 2008), leptin, insulin, resistin, IL-1β, IFN-γ, TNF-α and IL-6 were measured with a Milliplex kit (Merk-Millipore, France) following the manufacturer's instructions. Total plasma corticosterone was measured with an in-house RIA using a highly specific antibody provided by H. Vaudry (University of Rouen, France) as previously described (Richard et al., 2010). Plasma glucose levels were measured using a One Touch Ultra glucometer per the manufacturer's instructions as previously described (O'Connor et al., 2005). Plasma LPS concentrations were measured using the Endosafe-MCS detection system (Charles River Laboratories, Lyon, France), as previously described (Cani et al., 2008). All samples were run in duplicate.

2.4.2. Measurements of kynurenine (KYN) and tryptophan (TRP)

KYN and TRP levels were determined as previously described (Dinel et al., 2014; Moreau et al., 2005). The KYN/TRP ratio allows indirectly assessing IDO activity in lungs and brain. Briefly, tissues were homogenized using ice cold potassium 0.14 M KCl, 20 mM phosphate buffer pH 7.0 with an UltraTurrax T25 homogenizer at 1000 rpm. Homogenates were then centrifuged at 14,000*g* for 30 min at 4 °C. 200 µl of supernatants were precipitated in trichloroacetic acid (2 mM) and then centrifuged twice (15 and 5 min) at 1300*g* at 4 °C. Supernatants were injected onto a 5-µm C_{18} HPLC column (Lichrospher, Alltech, Deerfield, IL, USA) at a flow rate of 1.0 ml/min with mobile phase containing 0.1 M ammonium acetate/acetic acid buffer and 5% acetonitrile (pH 4.65). Levels of KYN were evaluated by UV absorbency at 360 nm. Levels of TRP were detected by fluorescent detector at 285 nm excitation and 365 nm emission wavelengths.

2.4.3. Reverse transcription and real-time RT-PCR

Total RNA was isolated from hippocampus and hypothalamus using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions and reverse-transcribed as previously described (André et al., 2008). Resultant first-strand cDNA was amplified by the Taqman® Universal PCR Master Mix with sequence-specific primers for IL-1β, IL-6, TNF-α, INF-γ, Socs3 and IDO and the FAM-labeled Taqman MBG probe assay mix (Applied Biosystems, Foster City, CA) as previously described (André et al., 2008; Godbout et al., 2008; O'Connor et al., 2009c). Reactions were performed in duplicate according to manufacturer instructions. Relative expression levels were calculated according to the methods of Schmittgen and Livak (2008) and plotted as fold change relative to the appropriate control condition.

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2.5. Statistical analysis

Experiments were conducted as a completely randomized design. Results are presented as mean ± SEM and were analyzed using a two-way ANOVA with diet (standard diet: SD *vs.* western diet: WD) and treatment (saline *vs.* LPS) as between factors and with repeated measurement on the time factor where appropriate, followed by a *post hoc* pair wise multiple comparison procedure using the Fischer's LSD method, if the interaction was significant. Spatial memory performances of each group were compared to chance level through one sample *T*-tests. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Chronic WD intake increased body weight and leptin production

WD induced a significant and sustained increase in body weight, whereas body weight of mice fed with standard diet (SD) started to stabilize from 15 weeks after the experiment onset (diet: $F_{1,828} = 5.4$, $P < 0.05$; time: $F_{18,828} = 311.7$, $P < 0.0001$; diet × time: $F_{18,828} = 14.9$, $P < 0.001$) (Fig. 1). At the end of the experiment, WD mice were approximately 26% heavier than SD mice. Moreover, WD led to a marked increase in the proportion of adipose tissue compared to SD mice (data not shown), which was associated with a significant increase of leptin levels in both adipose tissue (1.3 ± 0.2 *vs.* 2.6 ± 0.3 ng/ml for SD and WD mice respectively; $F_{1,20} = 14.4$, $P < 0.001$) and plasma ($F_{1,16} = 43.8$, $P < 0.001$; Table 1). This result agrees with those showing that plasma release of leptin from adipose tissue is proportional to adipose tissue mass and correlated with body mass index (Friedman and Halaas, 1998). On the other hand, WD had no significant effect on plasma levels of insulin and resistin (Table 1). Moreover, levels of glucose are similar in both non-fasted lean and obese mice (Table 1), suggesting that 20 weeks of WD intake was not associated with hyperglycemia or hyperinsulinemia in our experimental conditions.

3.2. Chronic WD intake first impaired spatial working memory performances and then increased anxiety-like behavior but not depressive-like behavior

Spatial working memory performances were measured after 9 and 18 weeks of SD or WD using a spatial recognition test designed as a hippocampus-dependent task (Dellu et al., 2000). During the acquisition session mice were allowed to visit only two arms out of three in the Y-maze for 5 min. The number of visits and the time spent in each open arm were similar in all mice regardless their diet and the time of test indicating similar spontaneous exploration (data not shown). Spatial memory performances were assessed 30-min later with free access to all 3 arms. SD mice spent more time exploring the novel arm than the familiar arm after either 9 or 18 weeks ($P < 0.05$; Fig. 2A and B), whereas WD mice randomly explored the different arms ($P > 0.2$; Fig. 2A and B). Importantly, these data likely reflect a working memory deficit rather than motor, sensory or motivational disturbances since both SD and WD mice preferentially explored the novel arm compared to familiar arms when tested with a short 2-min ITI (Fig. 2C) that corresponds to a minimal mnemonic demand.

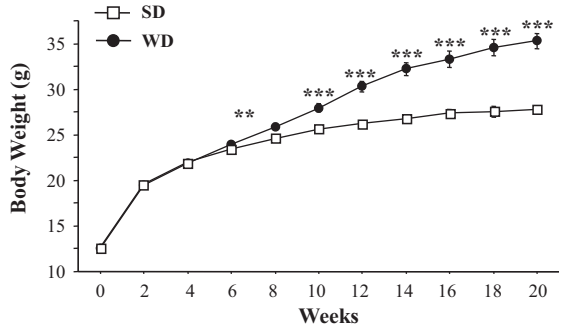

Fig. 1. Chronic WD exposure progressively increases body weight. Time course of body weight changes measured in mice exposed to standard diet (SD) or western diet (WD) for 20 weeks. Data represent means ± SEM ($n = 24$/group). $^{**}P < 0.01$; $^{***}P < 0.001$ for SD *vs.* WD.

Anxiety-like behavior was assessed in the EPM after 9 and 18 weeks of SD or WD exposure. When tested after 9 weeks of SD or WD, all mice displayed similar behavior in the EPM, i.e. similar entries ($P > 0.1$; data not shown) and percent of time spent into open arms ($P > 0.1$; Fig. 2D). By 18 weeks of WD exposure, although all mice equally visited the closed arms of the EPM, WD mice displayed less entries (3.7 ± 0.4 *vs.* 5.8 ± 0.9; $F_{1,40} = 4.3$, $P < 0.05$; data not shown) and reduced percent of time spent into the open arms than control mice ($F_{1,40} = 7.1$, $P < 0.01$; Fig. 2E). Moreover, these behavioral differences were unlikely due to locomotor impairment since both groups exhibited similar number of total arm entries in the EPM (data not shown) and similar number of crossings when tested in two-compartment cages (Fig. 3B). Thus, 18 weeks of WD exposure enhanced anxiety-like behaviors.

In contrast, assessment of depressive-like behaviors in the TST revealed similar duration of immobility in both groups of mice whatever the duration of WD exposure, suggesting that WD mice did not display greater depressive-like behavior than SD mice (Fig. 2F). In summary, chronic exposure to WD induced selective behavioral alterations affecting hippocampal-dependent memory and anxiety-like behavior, but not depressive-like behavior in absence of any stimulation.

3.3. Chronic WD intake altered LPS-induced body weight loss and behavioral reactivity in the FST

In order to determine whether chronic WD exposure may change LPS-induced behavioral reactivity in the FST, an LPS challenge was performed after 20 weeks of SD or WD exposure. The efficiency of LPS treatment was first assessed by measuring its effect on body weight changes 25 h after LPS administration compared to pretreatment body weight. LPS decreased body weight (treatment: $F_{1,20} = 181.8$, $P < 0.001$; Fig. 3A) in all treated mice compared to their saline counterparts. However, a significant attenuation of body weight loss was found in LPS-treated WD mice compared to LPS-treated SD mice (diet: $F_{1,20} = 17.8$, $P < 0.001$; diet × treatment: $F_{1,20} = 14.3$, $P < 0.01$).

Most of the rodent tests aiming at evaluating behavioral reactivity to an emotional challenge such as the FST are based on changes in motor activity, whereas LPS-induced motor retardation is one of the main symptoms of sickness behavior (Dantzer et al., 2008). Therefore, we checked whether all mice displayed similar locomotor activity before being testing in the FST. Shortly after LPS treatment, all treated mice presented most of the classical visual indicators of sickness including piloerection, curl up body posture, listlessness, and a relative immobility (data not shown). However, all mice exhibited 23 h after treatment similar motor activity regardless their treatment as assessed by the number of crossings performed in two-compartment cages (Fig. 3B). Duration of immobility measured in the FST 1 h later, i.e. 24 h after treatment, was similar in all saline-treated mice regardless their diet (Fig. 3C), as previously displayed by untreated SD and WD mice in the TST (Fig. 2F). The analysis revealed a significant effect of diet ($F_{1,21} = 9.9$, $P < 0.01$) and treatment ($F_{1,21} = 10.8$, $P < 0.01$), with a trend towards a significant interaction between both factors ($F_{1,21} = 3.4$, $P = 0.07$). Actually, LPS significantly reduced immobility

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Table 1
Plasma concentrations of metabolic hormones in SD- or WD-fed mice treated with saline or LPS. Plasma concentrations were measured 25 h after ip administration of saline or lipopolysaccharide (LPS, 830 μg/kg) in mice fed with standard diet (SD) or western diet (WD) for 20 weeks. Data represent means ± SEM (n = 6/group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for saline *vs.* LPS; $^{\#}P < 0.05$ for WD *vs.* SD.

Plasma	Standard diet (SD)		Western diet (WD)	
	Saline	LPS	Saline	LPS
Leptin (ng/ml)	2.0 ± 0.4	2.2 ± 1.0	9.8 ± 2.4$^{\#}$	20.2 ± 2.2*
Resistin (ng/ml)	2.8 ± 0.3	1.8 ± 0.1	3.3 ± 0.4	10.9 ± 1.3***
Insulin (ng/ml)	1.6 ± 0.2	1.3 ± 0.4	2.5 ± 1.0	2.6 ± 0.3
Glucose (mg/dl)	278.7 ± 23.9	212.4 ± 16.0**	256.3 ± 18.6	124.0 ± 17.9**
Corticosterone (ng/ml)	24.5 ± 3.0	48.1 ± 10.8*	25.2 ± 2.7	124.6 ± 12.3**
LPS (EU/ml)	9.9 ± 1.9	23.3 ± 5.7*	5.9 ± 2.0	21.6 ± 6.1*

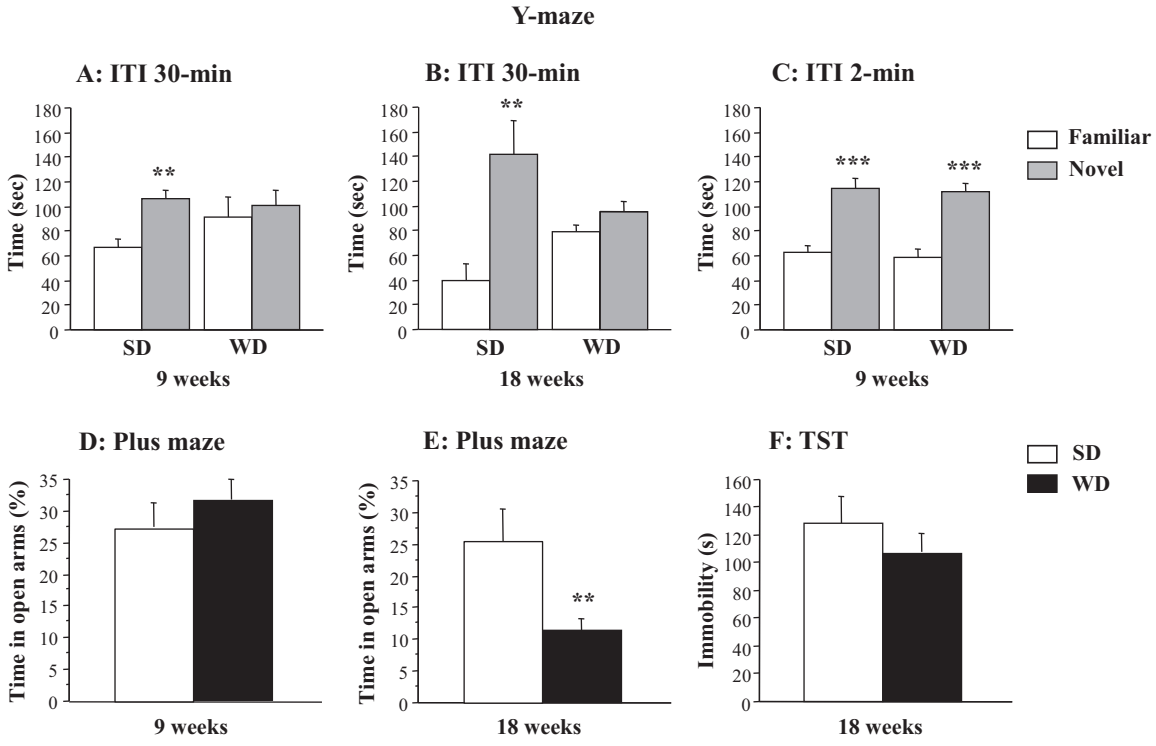

Fig. 2. Time-dependent effect of chronic WD exposure on spatial working memory performances, anxiety-like and depressive-like behavior. Spatial recognition was evaluated in the Y-maze by measuring the time spent exploring novel and familiar arms after a 30-min inter-trial interval (ITI) in mice fed with standard diet (SD) or western diet (WD) for (A) 9 weeks or (B) 18 weeks. (C) Spatial recognition measured after a 2-min ITI (corresponding to a minimal mnemonic demand) in mice fed with SD or WD for 9 weeks. Anxiety-like behavior was assessed by measuring the percent of time spent into open arms of the elevated plus maze at (D) 9 weeks and (E) 18 weeks. (F) Depressive-like behavior was evaluated in the tail suspension test (TST) by measuring immobility time. Data represent means ± SEM (n = 8/group). $^{**}P < 0.01$, $^{***}P < 0.001$ for SD *vs.* WD.

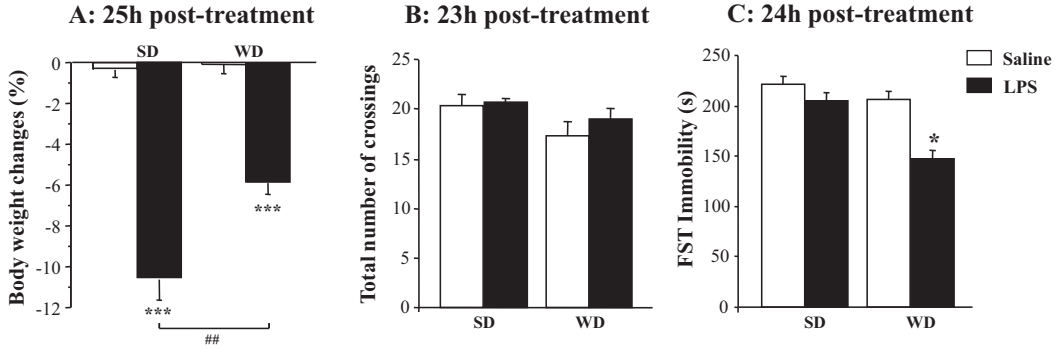

Fig. 3. Exposure to chronic WD alters LPS-induced body weight loss and depressive-like behavior. (A) Body weight changes measured 25 h after ip administration of saline or lipopolysaccharide (LPS, 830 μg/kg) in mice fed with standard diet (SD) or western diet (WD) for 20 weeks. (B) Locomotor activity assessed 23 h after treatment as the total number of between-compartment crossings performed in activity cages over the 6-min test. (C) Immobility time measured over 6 min in the forced swim test (FST) 24 h after treatment. Data represent means ± SEM (n = 6/group). $^{*}P < 0.05$, $^{***}P < 0.001$ for saline *vs.* LPS; $^{\#\#}P < 0.01$ for WD *vs.* SD.

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time in WD mice only (saline *vs.* LPS: −28.5%; $P < 0.05$), in agreement with previous results obtained in collectively-housed C57BL/6J mice (Painsipp et al., 2011). A question arose then as to whether these behavioral changes were related to altered peripheral and/or brain inflammatory responses to LPS.

3.4. Chronic WD intake exacerbated peripheral inflammatory responses to LPS

In order to compare systemic inflammatory responses to LPS between SD and WD mice, we first measured the effect of LPS on plasma cytokine levels 2 h and 25 h after either saline or LPS injection. Basal plasma levels of cytokines were similar in both SD and WD mice after saline treatment. Plasma levels of TNF-α were similarly increased 2 h after LPS in both SD and WD groups (LPS effect: $F_{1,12} = 17.6$, $P < 0.001$; with no diet or diet × treatment effect, $P > 0.1$; Fig. 4A) and returned to basal levels the next day (data not shown). On the contrary, plasma levels of IL-6 were higher in LPS-treated WD mice compared to SD mice 2 h after LPS (treatment: $F_{1,12} = 178.9$, $P < 0.001$; diet × treatment: $F_{1,12} = 12.1$, $P < 0.01$; Fig. 4B). Moreover, the IL-6 levels decreased one day later but were still significantly higher in WD mice than SD mice (diet × treatment: $F_{1,12} = 9.4$, $P < 0.05$; data not shown). Finally, plasma levels of IL-1β and IFN-γ were not affected by LPS treatment, diet and time (data not shown) as previously reported (Lawrence et al., 2012).

Well-known consequences of LPS-induced systemic immune stimulation are activation of the hypothalamic-pituitary adrenal (HPA) axis and increase of circulating levels of adipokines (Dantzer et al., 2008). Corticosterone levels were low and similar in both SD and WD mice after saline injection ($P > 0.1$; Table 1). LPS increased corticosterone levels in both groups 25 h after injection but the levels were significantly higher in WD than in SD mice (treatment: $F_{1,19} = 55.5$, $P < 0.001$; diet × treatment: $F_{1,19} = 21.1$, $P < 0.001$; Table 1). Similarly, the stimulating effect of LPS on circulating levels of leptin ($F_{1,16} = 7.0$, $P < 0.05$) and resistin ($F_{1,16} = 19.7$, $P < 0.001$) was restricted to WD mice (diet × treatment: $F_{1,16} = 7.4$, $P < 0.05$ and $F_{1,16} = 38.4$, $P < 0.001$ for leptin and resistin, respectively; Table 1). On the contrary, glucose levels were reduced in both SD and WD mice 25 h post-LPS (treatment: $F_{1,16} = 12.0$, $P < 0.01$ with no diet × treatment effect), whereas insulin levels were similar in all groups (Table 1). Moreover, plasma levels of LPS were also compared between SD and WD mice 25 h after treatment since signs of endotoxemia have been reported in other rodent models of obesity, independently of any immune challenge (Cani et al., 2008). Here, no significant effect of diet was found on circulating levels of LPS, but only the expected increase in LPS-treated mice ($F_{1,11} = 9.4$, $P < 0.05$; Table 1).

In conditions of systemic immune activation, changes in lung KYN/TRP ratio depend on the effects of circulating cytokines on the activity of the lung tryptophan-catabolizing enzyme IDO (André et al., 2008; O'Connor et al., 2009c). In basal conditions, all saline-treated mice displayed similar lung KYN/TRP ratio ($P > 0.1$; Fig. 4C). In agreement with the increase of cytokine production displayed by LPS-treated SD and WD mice, lung KYN/TRP ratio also increased 25 h after treatment in both groups ($F_{1,20} = 46.8$, $P < 0.001$), this increase being however significantly higher in WD mice than in their SD counterparts (diet: $F_{1,20} = 5.2$, $P < 0.05$; diet × treatment: $F_{1,20} = 4.1$, $P = 0.05$). In summary, mice chronically fed with WD displayed exacerbated metabolic and peripheral inflammatory responses to LPS compared to SD mice as particularly manifested by higher IL-6 production and lung IDO activation.

3.5. Chronic WD intake exacerbated brain inflammatory responses to LPS

In order to evaluate if chronic exposure to WD also interfered with brain induction of cytokines and the resulting brain IDO activation, cytokine mRNA expression was measured 2 h after LPS in both the hypothalamus and the hippocampus. In the context of inflammation, both brain areas had already been shown to exhibit a marked increase in cytokine expression shortly after LPS that allows coordination of the neurochemical, neuroendocrine and behavioral responses (André et al., 2008; Castanon et al., 2004). Basal mRNA expression of cytokines and related targets measured in both the hypothalamus and the hippocampus was low and similar regardless the diet.

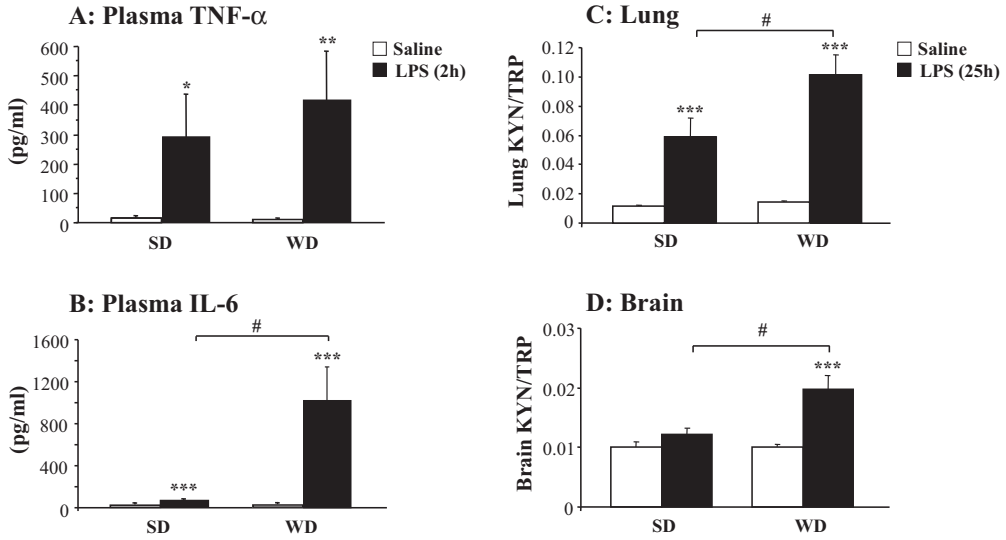

Fig. 4. Exposure to chronic WD increased peripheral inflammatory response to LPS treatment. Plasma concentrations of (A) TNFα and (B) IL-6 measured 2 h after ip administration of saline or lipopolysaccharide (LPS, 830 μg/kg) in mice fed with standard diet (SD) or western diet (WD) for 20 weeks. Kynurenine (KYN) and tryptophan (TRP) concentrations were determined by HPLC in homogenates of (C) lung or (D) whole brain collected 25 h after treatment. The KYN/TRP ratio was used to assess indoleamine 2,3-dioxygenase (IDO) activity. TNF-α, tumor necrosis factor-α; IL-6, interleukin-6. Data represent means ± SEM ($n = 6$/group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for saline *vs.* LPS; $^{\#}P < 0.05$ for WD *vs.* SD.

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In the hypothalamus, LPS similarly increased mRNA expression of TNF-α ($F_{1,19} = 123.9$, $P < 0.001$; Fig. 5A), IFN-γ ($F_{1,19} = 5.3$, $P < 0.05$; Fig. 5B) and IL-1β ($F_{1,16} = 109.1$, $P < 0.001$; Fig. 5C) 2 h after injection in both SD and WD mice (no diet effect or diet × treatment interaction). In contrast, the increase of hypothalamus IL-6 mRNA expression induced by LPS ($F_{1,19} = 133.2$, $P < 0.001$; Fig. 5D) was exacerbated in WD mice compared to SD mice (diet: $F_{1,19} = 34.7$, $P < 0.001$; diet × treatment: $F_{1,19} = 37.3$, $P < 0.001$). Moreover, LPS-induced increase of SOCS-3 mRNA expression ($F_{1,19} = 97.6$, $P < 0.001$; Fig. 5E), an indicator of signaling pathway activation by IL-6 (Lebel et al., 2000), was also significantly amplified in WD mice (diet: $F_{1,19} = 10.3$, $P < 0.01$; diet × treatment: $F_{1,19} = 10.5$, $P < 0.01$). Similarly, LPS-treated WD mice displayed significantly higher levels of IDO mRNA expression after LPS than SD mice (treatment: $F_{1,19} = 38.7$, $P < 0.001$; diet: $F_{1,19} = 5.7$, $P < 0.05$; diet × treatment: $F_{1,19} = 5.8$, $P < 0.05$; Fig. 5F), although basal expression was similar in both WD and SD groups.

In the hippocampus, LPS treatment increased mRNA expression of IL-1β ($F_{1,19} = 41.6$, $P < 0.001$; Fig. 6C), IL-6 ($F_{1,18} = 43.3$, $P < 0.001$; Fig. 6D) and SOCS-3 ($F_{1,18} = 19.3$, $P < 0.001$; Fig. 6E) in the same proportion in both SD and WD mice 2 h after treatment. However, LPS-induced increase of mRNA expression of TNF-α ($F_{1,18} = 99.3$, $P < 0.001$; Fig. 6A) and IFN-γ ($F_{1,18} = 16.3$, $P < 0.001$; Fig. 6B) was higher in WD mice than in SD mice (diet: $F_{1,18} = 8.5$, $P < 0.05$ and $F_{1,18} = 9.9$, $P < 0.01$; diet × treatment: $F_{1,18} = 4.7$, $P < 0.05$ and $F_{1,18} = 5.7$, $P < 0.05$ for TNF-α and IFN-γ, respectively). Again, expression of IDO mRNA was exacerbated by LPS in the hippocampus of WD mice (treatment: $F_{1,18} = 50.1$, $P < 0.01$; diet: $F_{1,18} = 5.9$, $P < 0.05$; diet × treatment: $F_{1,18} = 6.1$, $P < 0.05$; Fig. 6F).

Thus, WD selectively exacerbated LPS-induced increase of IL-6 and SOCS-3 mRNA expression in the hypothalamus, TNF-α and IFN-γ in the hippocampus and IDO in both brain areas 2 h after treatment. Increased brain IDO mRNA expression precedes stimulation of its activity (Godbout et al., 2008; Lestage et al., 2002). Accordingly, whereas no group difference was found in unstimulated conditions, WD mice displayed significantly higher brain KYN/TRP ratio than SD mice 25 h after LPS (treatment: $F_{1,20} = 43.3$, $P < 0.001$; diet: $F_{1,20} = 8.9$, $P < 0.01$; diet × treatment: $F_{1,20} = 9.9$, $P < 0.01$; Fig. 4D). Taken together, these results show that chronic WD exacerbated brain IDO activation induced by LPS. These differences of brain cytokine and IDO activation may therefore participate to the differential behavioral and emotional reactivity we reported between WD and SD mice.

4. Discussion

Understanding the pathophysiological mechanisms by which biological dysregulations associated with obesity, particularly low-grade inflammation, could contribute to the pathogenesis of related neuropsychiatric disorders has wide ranging therapeutic implications. The current study shows two main findings. First, chronic consumption of WD provides a brain environment promoting rapid deficit in spatial recognition memory after 9 weeks of WD exposure followed by an enhancement of anxiety-like behavior after 18 weeks of WD exposure. Second, whereas WD did not affect depressive-like behavior and brain inflammatory response at basal state, it interferes with the ability of the organism to appropriately

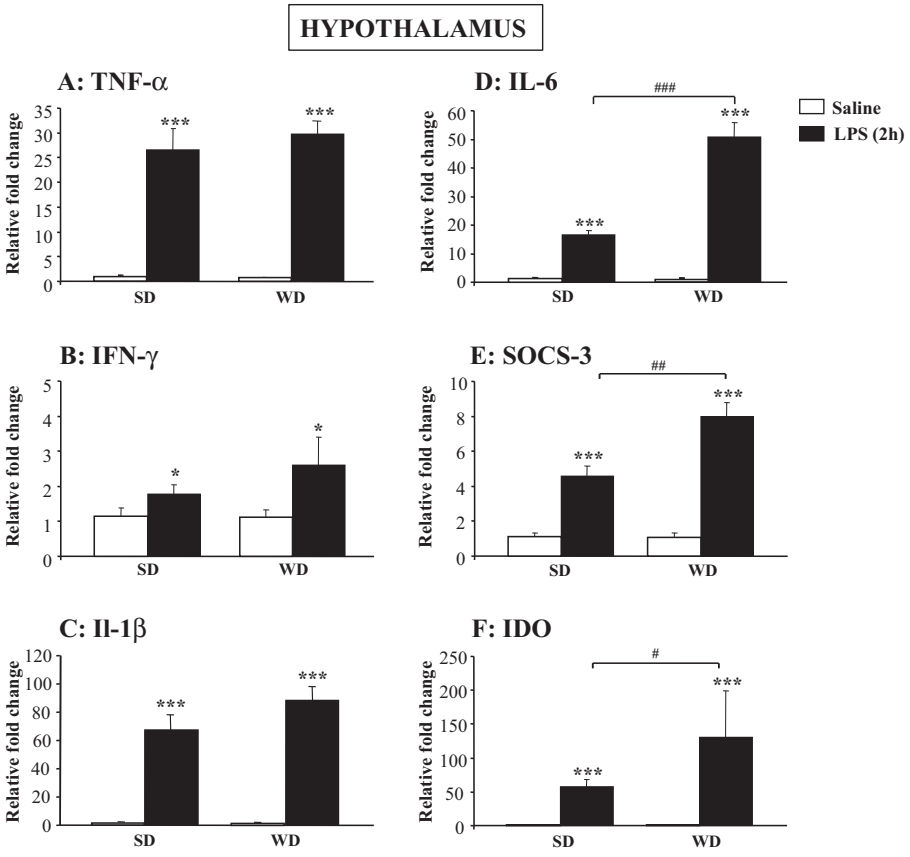

Fig. 5. Chronic WD exposure exacerbates LPS-induced increase of IL-6, SOCS-3 and IDO mRNA expression in the hypothalamus. Relative fold changes in levels of (A) TNF-α, (B) IFN-γ (C) IL-1β, (D) IL-6, (E) SOCS-3 and (F) IDO mRNA expression, as calculated in relation to the averaged value for control saline group. Hypothalamus were collected 2 h after ip administration of saline or lipopolysaccharide (LPS, 830 μg/kg) in mice fed with standard diet (SD) or western diet (WD) for 20 weeks. TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; IL-1, interleukin-1; SOCS-3, suppressor of cytokine signaling-3; IDO, indoleamine 2,3-dioxygenase. Data represent means ± SEM ($n = 6$/group). $^{*}P < 0.05$, $^{***}P < 0.001$ for saline *vs.* LPS; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ for WD *vs.* SD.

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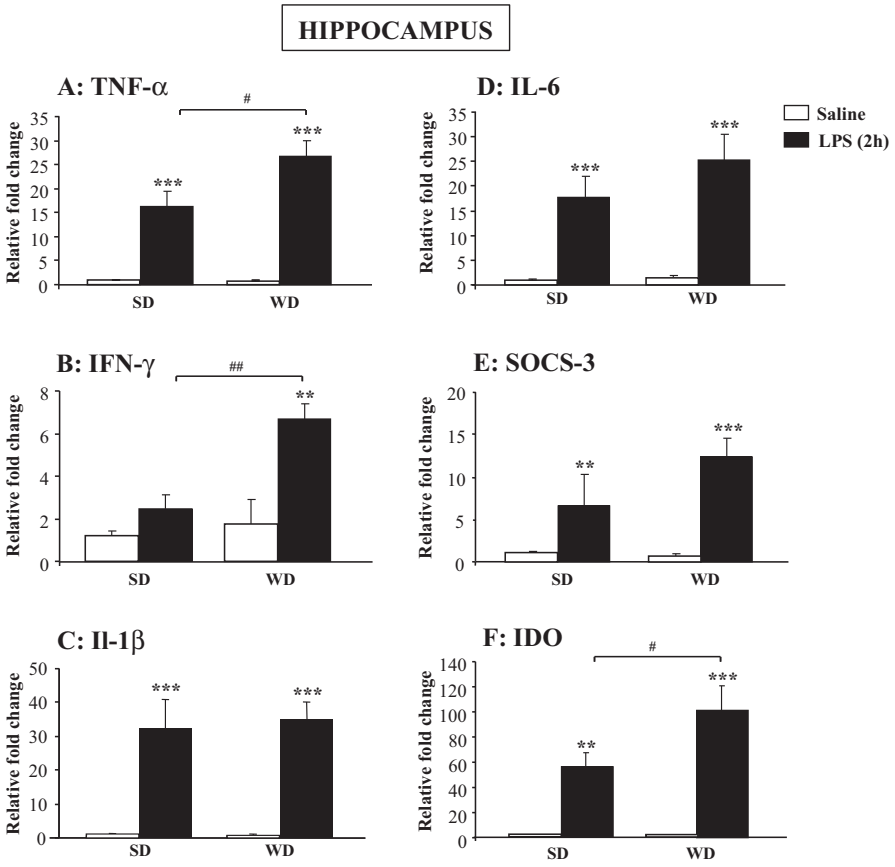

Fig. 6. Chronic WD exposure exacerbates LPS-induced increase of TNF-α, IFN-γ and IDO mRNA expression in the hippocampus. Relative fold changes in levels of (A) TNF-α, (B) IFN-γ, (C) IL-1β, (D) IL-6, (E) SOCS-3 and (F) IDO mRNA expression, as calculated in relation to the averaged value for control saline group. Hippocampus were collected 2 h after ip administration of saline or lipopolysaccharide (LPS, 830 μg/kg) in mice fed with standard diet (SD) or western diet (WD) for 20 weeks. TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; IL-1, interleukin-1; SOCS-3, suppressor of cytokine signaling-3; IDO, indoleamine 2,3-dioxygenase. Data represent means ± SEM (n = 6/group). $^{**}P < 0.01$, $^{***}P < 0.001$ for saline *vs.* LPS; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ for WD *vs.* SD.

respond to a peripheral immune challenge: WD exacerbated activation of the neurobiological mechanisms underlying depressive-like behavior, namely brain cytokine production and IDO activation.

Although obesity is not easily defined in rodents, a number of models resulting from genetic or diet manipulations has been developed over the last decades. In the present study, mice fed with WD displayed significantly higher body weight than mice fed with standard chow, as well as adipose tissue hypertrophy and marked increase in plasma and adipose tissue levels of leptin. However, they did not display significant changes of insulin, glucose or corticosterone plasma levels in basal conditions, suggesting that we likely modeled relatively moderate obesity as previously reported (see for example Amar et al., 2007; Valladolid-Acebes et al., 2013). Accordingly, no detectable basal low-grade inflammation was found in WD mice which displayed similar peripheral and brain cytokine production or IDO activation as control mice, and no significant metabolic endotoxemia. Of note, basal low-grade inflammation is usually reported in models of severe obesity either linked to genetic mutations (Dinel et al., 2011, 2014; Lawrence et al., 2012) or exposure to diets containing much more fat than ours and/or given for a longer period of time (Chen et al., 2005; De Souza et al., 2005; Pistell et al., 2010; Xu et al., 2003). Similarly, low-grade inflammation has been usually reported in humans suffering from severe forms of obesity (Cancello and Clement, 2006; Capuron et al., 2010). Our experimental model provided therefore the opportunity of detecting biological changes and/or behavioral alterations preceding the onset of obesity-related severe comorbidities, in particular metabolic and cardiovascular diseases that can detrimentally affect brain functions (Bruce-Keller et al., 2009).

In particular, our model allowed following the progressive development of related cognitive and emotional alterations according to the severity of obesity. Therefore, an important finding from the present study is that 9 weeks of WD exposure, inducing 5–7% overweight, is sufficient to impair hippocampal-dependent memory whereas 18 weeks of WD exposure, corresponding to 25–30% overweight, is necessary to increase anxiety-like behavior. Importantly, spatial deficit in the Y-maze after 30 min of retention interval was not due to any motor, sensory or motivational problems as spatial performances of WD mice were intact with a 2-min retention interval corresponding to a minimal mnemonic demand. We recently obtained similar impairment in hippocampal-dependent memory, but not anxiety-like behavior, in rats exposed to high-fat diet for 8–12 weeks starting at weaning (Boitard et al., 2012, 2014). Although brain inflammation, in particular increased IL-1β, is one of the potential mechanisms proposed to explain the detrimental effect of WD consumption on spatial memory (Lu et al., 2011; Pistell et al., 2010; Thirumangalakudi et al., 2008; Yirmiya and Goshen, 2011) and anxiety-related behavior (Dinel et al., 2011), it unlikely plays a major role in the present study since no detectable basal inflammation was found in the hippocampus of WD mice. We need however to still measure potential inflammatory changes in other brain areas also involved in controlling emotional behavior, particularly the amygdala complex (Tye et al., 2011), before drawing a

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definitive conclusion. IDO activation has also been reported to participate in cognitive alterations and anxiety-like behavior in other inflammatory conditions than obesity (Chess et al., 2009; Gold et al., 2011; Salazar et al., 2012). However, it seems unlikely to mediate WD-induced behavioral alterations as IDO activation was similar in both WD and control mice. The earlier onset of spatial memory deficits reported in WD mice, compared to their delayed increase of anxiety-like behavior, suggests that different neurobiological mechanisms and/or brain areas may underlie these behavioral alterations. As previous studies indicate that dorsal hippocampus is involved in spatial memory whereas ventral hippocampus is more important for anxiety behavior (for review: Bannerman et al., 2004), it would be of interest to determine whether the dorsal part of the hippocampus is more vulnerable than the ventral part to WD exposure.

Whereas WD exposure altered spatial memory and anxiety-like behavior, it did not change depressive-like behaviors, as assessed in the TST or FST. Similarly, we recently found alterations of spatial memory and anxiety-like behavior without any effect on depressive-like behavior in a genetic model of obesity (Dinel et al., 2011). However, increased basal depressive-like behavior has been previously reported in other diet-induced obesity models compared to lean controls but only when very high-fat diets associated with important metabolic dysfunctions, and presumably low-grade inflammation, were used (Sharma and Fulton, 2012; Yamada et al., 2011). Moreover, in one of these reports, alterations were obtained in the FST one day after a 15-min pre-test session during which no effect of high-fat diet was observed (Yamada et al., 2011). Such differences between these experimental conditions and ours can likely explain this apparent discrepancy.

The present study provides for the first time in a model of diet-induced obesity information regarding activation of the neurobiological mechanisms underlying depressive-like behavior induced by a systemic immune challenge (Fu et al., 2010; Lawson et al., 2013; O'Connor et al., 2009a,b,c). Interestingly, WD consumption resulted in exacerbated immune responses to LPS, suggesting the development of molecular changes underlying an increased vulnerability to immune challenges, although the possibility that this exacerbation might be also partly due to an interaction between the stress of behavioral testing and LPS treatment cannot be totally excluded. Interestingly, only selective parameters of the systemic and brain responses to LPS were affected and they differed between the hypothalamus and hippocampus, strongly suggesting therefore that exacerbated responses were not simply due to a general alteration of LPS efficiency. Compared to mice fed with standard chow, WD mice displayed after LPS higher and/or longer increase of plasma levels of IL-6, corticosterone and leptin, as previously reported (Lawrence et al., 2012; Naguib et al., 2004; Pini et al., 2013; Pohl et al., 2009, 2013), higher hippocampal TNF-α and IFN-γ and hypothalamic IL-6 expression, but blunted body weight loss. Such a dissociation between increased expression of cytokines and reduced body weight loss could appear somehow contradictory and in contrast with the enhanced LPS effect previously reported on sickness symptoms, including changes of body weight (Lawrence et al., 2012; Pohl et al., 2009, 2013). However, a recent study reports that obese mice lose less weight than lean controls 1 day after LPS treatment, as found here, but continue to lose weight 3 days later whereas control mice have started to recover (Pini et al., 2013). The possibility that in the present study WD mice would have lost more weight later on cannot therefore be totally excluded. On the other hand, anorectic adipose tissue derived signals such as leptin may also contribute to LPS-induced body weight loss (Aguilar-Valles et al., 2014). However, the leptin resistance that is classically reported in human and rodent obesity (Friedman and Halaas, 1998) and likely exists in WD mice, as suggested by the efficiency of WD in promoting obesity despite elevated levels of circulating leptin, does not support this hypothesis.

Numerous studies have highlighted the role of brain IDO activation by cytokines in the development of depressive-like behavior associated with inflammation (Frenois et al., 2007; Godbout et al., 2008; Moreau et al., 2008; O'Connor et al., 2009a,b,c). In that context, it can appear somehow puzzling that in the present study, LPS increased brain IDO activation but reduced immobility in the FST instead of increasing it, as we and others previously reported in other strains of mice, particularly CD1 mice (Frenois et al., 2007; Mello et al., 2013; O'Connor et al., 2009c). Of note, differences of innate immune system activation (Babri et al., 2014; Nikodemova and Watters, 2011) and behavioral reactivity in the FST (Lucki et al., 2001) have been already reported between CD1 and C57BL/6J mice (as we used here). Basal immobility displayed by C57BL/6J mice in the present study was for example similar to immobility measured in CD1 mice after LPS (Frenois et al., 2007). Moreover, LPS-induced behavioral changes displayed by C57BL/6J mice in the FST appear to be affected by environmental conditions. Actually, LPS enhances immobility in the FST in single-housed C57BL/6J mice (Lawson et al., 2013; Painsipp et al., 2011; Walker et al., 2013) whereas, in agreement with the present study, it reduces immobility in collectively-housed C57BL/6J mice when the same dose of LPS and the same time of test, i.e. 24 h post-injection, are used (Painsipp et al., 2011, but see Aguilar-Valles et al., 2014 for different results with a much higher dose of LPS and a test 48 h post-injection). Elevated corticosterone levels displayed by LPS-treated WD mice compared to their SD counterparts when the FST took place may contribute to their behavioral responses. Indeed, chronic stress-induced increase of brain cytokine expression has been reported to mediate stress-induced depressive-like behavior in mice *via* HPA axis activation (Goshen et al., 2008). Moreover, stressed-C57BL/6J mice reduce their immobility in the FST compared to unstressed controls (Thoeringer et al., 2007). More studies are necessary to clarify the potential contribution of corticosterone to the behavioral responses displayed by LPS-treated WD mice in the FST, and more importantly to help interpreting these responses.

Due to its predictive validity for clinical depression (Nestler and Hyman, 2010), the FST is classically used to screen pharmacological molecules for their potential antidepressant properties, although it models only some core symptoms of depression rather than the entire syndrome. It would be therefore important to support the present data by additional experiments assessing depressive-like behavior in other paradigms, in particular those with higher face validity for clinical depression than the FST (Nestler and Hyman, 2010). Similarly, it would be of great interest to measure the impact of an antidepressant treatment. Meanwhile, the neurobiological changes displayed by WD mice after LPS, particularly exacerbated IDO activation, support the assumption that WD exposure may alter depressive-like behavior after an immune challenge. Indeed, these findings fit with those highlighting the causal relationship between IDO activation and depressive-like behavior reported in different inflammatory conditions (Frenois et al., 2007; Godbout et al., 2008; Moreau et al., 2008; O'Connor et al., 2009a,b,c). Moreover, enhanced brain cytokine and IDO expression occurred in particular in the hippocampus that is a key brain area for mood control (Maletic et al., 2007). Interestingly, WD exacerbated LPS-induced increase of brain KYN levels, but did not change the effect of LPS on brain TRP levels that was similar in both groups (data not shown). These results suggest therefore that depressive-like behavior may be due in WD mice to the neurotoxic actions of KYN derivatives rather than to reduced brain TRP availability for serotonin synthesis as recently demonstrated in lean mice (Walker et al., 2013). Of note, lung IDO activity was also slightly more elevated after LPS treatment in WD mice than in

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SD mice, as reported in severely obese humans displaying basal peripheral low-grade inflammation (Brandacher et al., 2007; Oxenkrug, 2010). A potential contribution of peripheral KYN, which can cross the brain–blood barrier, to the behavioral changes reported in LPS-treated WD mice cannot therefore be totally excluded.

Converging evidence indicates that LPS-induced brain IDO activation depends on brain expression of cytokines, particularly TNF-α and IL-6 in the hypothalamus and IFN-γ in the hippocampus (André et al., 2008; Fu et al., 2010; O'Connor et al., 2009c). Interestingly, such a structure-dependent dissociation seems to exist concerning the effect of WD on LPS-induced cytokine expression, although LPS broadly stimulates cytokine expression and IDO activity within the brain (André et al., 2008; Castanon et al., 2004). Indeed, WD selectively exacerbated LPS-induced increase of IFN-γ expression in the hippocampus, IL-6 in the hypothalamus and IDO in both brain areas. The mechanisms underlying such a selective enhancement of inflammatory response to LPS by WD exposure, as well as the respective role of hypothalamus and hippocampus in the increase of brain IDO activity, cannot be determined based on the present study. It is noteworthy however that similar exaggerated neuroinflammatory and behavioral responses to innate immune challenge have already been reported in animal models of aging or chronic neurodegenerative diseases, and have been shown to facilitate development of neurobehavioral complications (Cunningham et al., 2009; Godbout et al., 2008; Henry et al., 2009; Perry et al., 2007). This facilitation has been related to an atypical over-reactive or "primed" state of brain microglia that switches their silent phenotype when exposed to immune challenges to produce excessive cytokines (Perry et al., 2007). Although the cellular source of exacerbated neuroinflammation was not directly identified in the present study, our results strongly suggest that obesity may constitute another situation in which a "primed" state of microglia, potentially revealed by a systemic inflammatory challenge, may determine increased vulnerability to the negative consequences of immune-mediated events.

In conclusion, the present study constitutes a first important step towards a better understanding of the onset of neuropsychiatric alterations associated with obesity, in particular cognitive impairments and increased anxiety, and the role of brain cytokine expression and IDO activation in this phenomenon. The results provide evidence that obesity, and possibly obesity-associated inflammatory priming, may represent a vulnerability state to immune-mediated depressive symptoms. These findings might therefore have relevance in improving the management and treatment of inflammation-related complications in obesity.

5. Contributors

Conceived and designed the experiments: N.C., C.A. Performed the experiments: C.A., A.-L.D., N.C. Analyzed the data: N.C., C.A., A.-L.D. Wrote the paper: N.C., C.A., G.F., S.L.

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Conflict of interest

The authors declared that no competing interests exist.

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