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Lipopolysaccharide-induced brain activation of the indoleamine 2,3-dioxygenase and depressive-like behavior are impaired in a mouse model of metabolic syndrome

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Summary Although peripheral low-grade inflammation has been associated with a high incidence of mood symptoms in patients with metabolic syndrome (MetS), much less is known about the potential involvement of brain activation of cytokines in that context. Recently we showed in a mouse model of MetS, namely the *db/db* mice, an enhanced hippocampal inflammation associated with increased anxiety-like behavior (Dinel et al., 2011). However, depressive-like behavior was not affected in *db/db* mice. Based on the strong association between depressive-like behavior and cytokine-induced brain activation of indoleamine 2,3-dioxygenase (IDO), the enzyme that metabolizes tryptophan along the kynurenine pathway, these results may suggest an impairment of brain IDO activation in *db/db* mice. To test this hypothesis, we measured the ability of *db/db* mice and their healthy *db/+* littermates to enhance brain IDO activity and depressive-like behavior after a systemic immune challenge with lipopolysaccharide (LPS).

Here we show that LPS (5 μg/mouse) significantly increased depressive-like behavior (increased immobility time in a forced-swim test, FST) 24 h after treatment in *db/+* mice, but not in *db/db* mice. Interestingly, *db/db* mice also displayed after LPS treatment blunted increase of brain kynurenine/tryptophan ratio compared to their *db/+* counterparts, despite enhanced induction of hippocampal cytokine expression (interleukin-1β, tumor necrosis factor-α). Moreover, this was associated with an impaired effect of LPS on hippocampal expression of the brain-derived neurotrophic factor (BDNF) that contributes to mood regulation, including under inflammatory conditions.

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Collectively, these data indicate that the rise in brain tryptophan catabolism and depressive-like behavior induced by innate immune system activation is impaired in *db*/*db* mice. These findings could have relevance in improving the management and treatment of inflammation-related complications in MetS.

1. Introduction

Over the last decades, clinical (Evans et al., 2005; Raison et al., 2010; Capuron and Miller, 2011) and experimental (Castanon et al., 2002; Frenois et al., 2007; Moreau et al., 2008) research focusing on the intricate relationship between the innate immune system and the brain have supported a main role for dysregulated production and/or brain action of cytokines in mood disorders (Dantzer et al., 2008; Zunszain et al., 2012). This is particularly relevant to people with severe obesity or metabolic syndrome (MetS) who display a high incidence of mood symptoms concomitant with low-grade inflammation (Dandona et al., 2005; Capuron et al., 2008; Viscogliosi et al., 2013).

Interestingly, development of mood symptoms in patients with inflammatory conditions is often associated with reduced circulating tryptophan levels and concomitant increased serum or cerebrospinal fluid concentrations of one of its main metabolite, kynurenine (Glaser and Kiecolt-Glaser, 2005; Myint et al., 2007; Raison et al., 2010; Capuron and Miller, 2011). These clinical findings suggested a possible link between inflammation-related mood disorders and cytokine-induced activation of the indoleamine 2,3-dioxygenase (IDO) that is the first and rate-limiting enzyme that catabolizes tryptophan along the kynurenine pathway in activated monocytes, macrophages and brain microglia (Moroni et al., 1991; Takikawa et al., 1984). Sustained brain IDO activation resulting from chronic cytokine production may be deleterious because of its negative impact on monoaminergic neurotransmission (by changing serotonin synthesis and catabolism) and on neuronal survival (by increasing production of several kynurenine derivatives with neuroactive properties) (Corona et al., 2013; Dantzer et al., 2011; Guillemin, 2012; Liu et al., 2013; Stone et al., 2013). Actually, increased production of kynurenine derivatives has been related to the stretch of both hippocampus damages and mood disorders in humans (Schiepers et al., 2005; Maletic et al., 2007), and with depressive-like behavior in rodents (Walker et al., 2013). Experimentally, we demonstrated in mice challenged with a cytokine inducer such as lipopolysaccharide (LPS) that cytokine-induced brain IDO activation, particularly in the hippocampus, parallels development of depressive-like behaviors (Frenois et al., 2007; André et al., 2008; Moreau et al., 2008). This association between brain IDO activity and depressive-like behavior was also demonstrated in aged mice (Godbout et al., 2008; Kelley et al., 2013) and in mice deficient for the microglial fractalkine receptor (CX_3CR1) (Corona et al., 2010), which normally allows neurons to control microglial activation (Cardona et al., 2006). Both models display sustained hippocampal microglial activation and cytokine production after a LPS challenge, together with protracted brain IDO expression and depressive-like behavior (Godbout et al., 2008; Wynne et al., 2010; Corona et al., 2013). Other studies have confirmed the key role of hippocampal cytokine activation in inducing brain IDO activity and depressive-like behavior (André et al., 2008; Wang et al., 2009; Fu et al., 2010). Interestingly, direct peripheral administration of kynurenine dose-dependently increases depressive-like behavior in non immune-stimulated mice (O'Connor et al., 2009c). Moreover, pharmacological or genetic inhibition of brain IDO activation prevents the induction of depressive-like behavior by systemic immune challenges (Henry et al., 2009; O'Connor et al., 2009a, 2009b, 2009c). Taken together, these findings demonstrate the causal role of brain IDO activation by cytokines in mediating inflammation-related depressive-like behavior.

Although peripheral low-grade inflammation (Capuron et al., 2008) and IDO activation (Brandacher et al., 2006, 2007; Oxenkrug, 2010) have been associated with a high incidence of mood symptoms in patients with MetS, much less is known about the potential involvement of brain cytokine and IDO activation in that context. Interestingly, we recently reported enhanced expression of inflammatory cytokines in the hippocampus of *db*/*db* mice, which display several features of MetS as a consequence of an inactivating mutation in the leptin receptor, compared to their healthy counterparts (*db*/+ mice) (Dinel et al., 2011). This increased hippocampal inflammation is related to some particular mood symptoms: *db*/*db* mice display in those basal conditions increased anxiety-like behavior but similar depressive-like behavior than *db*/+ mice (Dinel et al., 2011). Based on the strong association between brain IDO activation and induction of depressive-like behavior, these results may suggest an impairment of cytokine-induced brain IDO activation in *db*/*db* mice. Testing this assumption is particularly relevant in order to fully understand the regulation of brain IDO activity in the context of the MetS and its inflammation-related complications.

In the present study, we sought to approach this question by measuring inflammatory and behavioral responses of *db*/*db* mice to a systemic LPS challenge in experimental conditions that allow LPS to induce IDO activation and depressive-like behavior independently from sickness behavior (Frenois et al., 2007; O'Connor et al., 2009c). Whereas some reports focused on LPS-induced sickness behavior in *db*/*db* mice (Faggioni et al., 1997; O'Connor et al., 2005; Lin et al., 2007), no studies investigated in these mice LPS-induced hippocampal cytokines expression, brain IDO activation and depressive-like behavior. Here we show that *db*/*db* mice displayed blunted brain IDO activation and depressive-like behavior in response to LPS, despite enhanced induction of hippocampal cytokine expression.

2. Materials and methods

2.1. Animals and treatment

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. Male *db*/*db* (C57BLKS/J-*lepr*^{db}/*lepr*^{db}; $n = 32$) and *db*/+ (C57BLKS/J-*lepr*^{db}/+; $n = 32$) mice between 10 and 12 weeks of age were obtained from Charles River Laboratories (France). They were housed individually under a normal 12-h light:dark cycle with food and water available *ad libitum*. Mice were handled daily for 1 week before the experiment onset to minimize stress reactions to manipulation.

LPS was phenol-extracted from *Escherichia coli* (serotype 0127:B8; RBI/Sigma). On the test day, LPS was dissolved in sterile endotoxin-free isotonic saline and administered intraperitoneally (i.p.). The dose of LPS (5 μg/mouse) was selected on the basis of its ability to induce the full spectrum of sickness in both *db*/*db* and *db*/+ mice (O'Connor et al., 2005).

2.2. Experimental procedure

Fig. 1 shows the experimental design and timing of behavioral experiments, body weight measures and tissue collection. Peripheral LPS administration enhances peripheral and brain production of proinflammatory cytokines, which are responsible of physiological and behavioral symptoms of sickness (Dantzer et al., 2008). These symptoms of sickness progressively wane whereas the expression of depression-like behaviors continues (Frenois et al., 2007; Godbout et al., 2008; O'Connor et al., 2009c). Consequently, only protracted depressive-like behaviors remain 24 h after LPS treatment. Therefore, we measured the behavioral and neurobiological reactivity to LPS challenge at different relevant post-treatment time-points. Mice were injected i.p. with sterile physiological saline or LPS (5 μg/mouse) and were immediately returned to their home cage. They were weighed just before and 2, 6 and/or 25 h after this injection in order to use body weight loss as a marker of sickness and as control for LPS efficiency (André et al., 2008). Mice were euthanized by CO_2 inhalation either 2 h or 25 h after LPS injection, 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. Mice were 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 directly stored at −80 °C until IDO activity assay (mice sacrificed 25 h post-LPS) or carefully dissected to immediately collect, dry frozen and store the hippocampus for subsequent determination of mRNA levels (mice sacrificed 2 h post-LPS). Lungs and liver were also rapidly collected and directly stored at −80 °C.

2.3. Behavioral measurements

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). Mice were tested in the activity cage at 6 h and 23 h post-treatment, and in the forced swim test (FST) at 24 h post-treatment. All testing equipment was thoroughly cleaned between each session.

2.3.1. Two-compartment cage

In order to determine the effect of LPS on motor activity, mice were individually placed into a polypropylene cage (30 cm × 11 cm × 12 cm) similar to the home cage but divided into two communicating compartments that were separated by a plexiglas wall containing a small opening (2.5 cm × 3.0 cm). As previously described (Frenois et al., 2007; Moreau et al., 2008), activity was evaluated by counting the number of between-compartments crossings performed during the 6-min test.

2.3.2. Forced swim test (FST)

This standardized test of depressive-like behavior was essentially conducted as previously described (Frenois et al., 2007; Moreau et al., 2008; Dinel et al., 2011). Briefly, each mouse was placed individually in a cylinder (16 cm × 31 cm) containing warm water (25 ± 1 °C) to avoid temperature-related stress response. Mice were tested during a 6-min period. Immobility time was determined by the time a mouse stopped struggling and moved only slowly to remain floating in the water, keeping its head above water. Increased duration of immobility has been proposed to reflect a state of helplessness that is reduced by antidepressants.

2.4. Biochemical measurements

2.4.1. Cytokines and corticosterone assay

Plasma cytokines (IL-1β, IL-6, TNF-α, INF-γ and IL-10) 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,

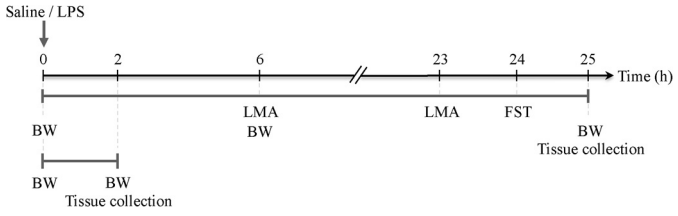

Figure 1 Experimental timeline and design. *db*/*db* and *db*/+ mice ($n = 8$/group) were injected with sterile saline or lipopolysaccharide (LPS; 5 μg/mouse i.p.) and were then tested for body weight changes (BW), locomotor activity (LMA) and/or forced swim test (FST). Tissues were collected after sacrifice 2 h or 25 h after treatment.

France) as previously described (Richard et al., 2010). All samples were run in duplicate.

2.4.2. Reverse transcription and real-time RT-PCR

Total RNA was extracted from the hippocampus using a RNeasy Mini Kit (Qiagen) and reverse-transcribed as previously described (O'Connor et al., 2009c). Real-time RT-PCR for IL-1β, IL-6, TNF-α, INF-γ, IL10, TLR4, CD14, CD11b, CX3CL1, CX3CR1 and BDNF was performed on an ABI Prism 7700 using Taqman gene expression assays for sequence-specific primers purchased from Applied Biosytems (Foster City, CA). 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.

2.4.3. Concentrations of kynurenine (KYN) and tryptophan (TRP)

KYN and TRP levels were determined as previously described (Moreau et al., 2005). The KYN/TRP ratio allows to indirectly assess IDO activity in lungs and brain and tryptophan-2,3 dioxygenase (TDO) activity in the liver. 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.

2.5. 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 genotype (*db/+ vs. db/db*) and treatment (saline *vs.* LPS) as between factors and time as within factor. When appropriate, differences between groups were determined using the Fisher's least significant difference *post hoc* multiple pairwise comparisons. Statistical significance was set at $p < 0.05$.

3. Results

3.1. LPS-induced depressive-like behavior and increase of brain KYN/TRP ratio is impaired in *db/db* mice

Duration of immobility measured in the FST was used as an index of depressive-like behavior. This parameter was similar in *db/+* and *db/db* mice 24 h after saline treatment ($p > 0.1$) as previously reported in non-injected *db/+* and *db/db* mice (Dinel et al., 2011; Fig. 2A). We have previously shown that LPS treatment increases the time spent immobile in the FST, 24 h after injection, independently of any motor impairment (Frenois et al., 2007; Godbout et al., 2008; O'Connor et al., 2009c). Here, LPS significantly increased duration of immobility ($F_{(1,22)} = 5.4$, $p < 0.05$) compared to saline-treated mice in *db/+* mice that were 65% more immobile than their saline-treated controls ($p < 0.05$), but not in *db/db* mice ($p > 0.1$) (Fig. 2A). Both LPS-treated *db/+* and *db/db* mice displayed by that time similar locomotor activity levels (similar number of crossings) than their respective saline-treated counterparts (Supplementary Fig. S1). This result showed that 24 h after treatment all mice have recovered from the LPS-induced reduction of locomotor activity observed 6 h after treatment (treatment: $F_{(1,23)} = 11.6$, $p < 0.01$; Supplementary Fig. S1). Moreover, they confirmed that increased immobility in the FST was not just linked to impaired motor activity since *db/+* mice, which were more immobile than *db/db* mice in the FST, were on the contrary significantly more active in the two-compartment cages (genotype: $F_{(1,23)} = 59.7$, $p < 0.001$).

We then measured brain activation of IDO since it mediates LPS-induced increase of the duration of immobility in

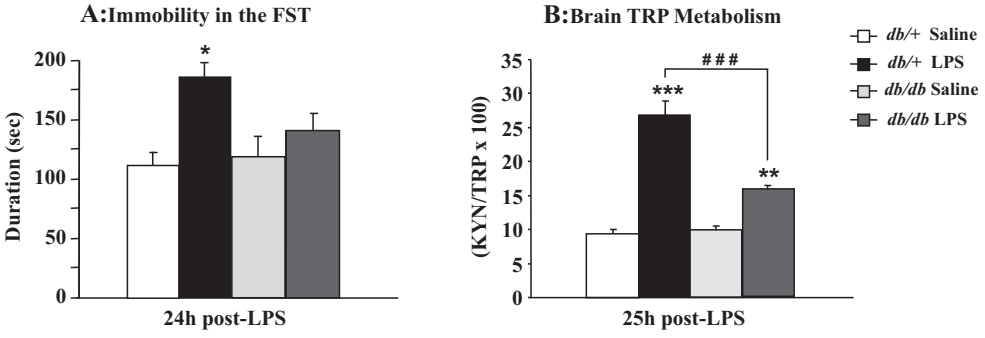

Figure 2 Effect of LPS on depressive-like behavior and brain tryptophan metabolism in *db/db* and *db/+* mice. (A) Immobility time measured over 6 min in the forced swim test (FST) 24 h after saline or LPS (5 μg/mouse, i.p.) administration. Immobility time was determined by the time a mouse stopped struggling and moved only slowly to remain floating in the water, keeping its head above water. (B) Kynurenine (KYN)/tryptophan (TRP) ratio measured in homogenates of whole brains collected 1 h after completion of the FST, within a few seconds after the mice were picked up from their home cage. Brain KYN and TRP concentrations were determined by HPLC and the KYN/TRP ratio was used to assess IDO activity. Data represent means ± SEM ($n = 8$/group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, for saline *vs.* LPS. $^{###}p < 0.001$ for *db/db vs. db/+* mice.

the FST (Fu et al., 2010; Lawson et al., 2013). Brain KYN/TRP ratio, used as an indirect evaluation of IDO activation, was measured 1 h after completion of the FST as previously described (O'Connor et al., 2009a, 2009b, 2009c). The ANOVA analysis revealed a main effect of genotype ($F_{(1,23)} = 18.7$, $p < 0.001$) and treatment ($F_{(1,23)} = 84.6$, $p < 0.0001$), with a significant interaction between both factors ($F_{(1,23)} = 16.5$, $p < 0.001$; Fig. 2B). Basal brain KYN/TRP ratio was similar in both genotypes. As anticipated, LPS significantly increased this ratio in all treated mice, but this increase was significantly blunted in *db/db* mice compared to *db/+* mice (LPS: *db/+ vs. db/db*: $p < 0.001$). Of note, this was due to a significantly smaller increase of brain KYN levels after LPS in *db/db* mice compared to *db/+* mice (treatment: $F_{(1,21)} = 16.1$, $p < 0.001$; genotype × treatment: $F_{(1,21)} = 5.5$, $p < 0.05$), whereas both saline-treated groups displayed similar basal brain KYN levels (Supplementary Table S1). Concurrently, no significant between-genotype differences neither treatment effect were found regarding brain TRP levels. Taken together these results strongly suggest therefore that changes in brain KYN/TRP ratio were likely linked to brain IDO activation, although a potential modulation of this ratio by the KYN transported from the periphery to the brain is still possible. Blunted LPS-induced increase of brain KYN/TRP ratio in *db/db* mice may therefore participate to their absence of LPS-induced increase of depressive-like behavior. A question arose then as to whether the efficiency of LPS treatment is globally impaired in *db/db* mice.

3.2. LPS-induced body weight loss and peripheral inflammatory activation is similar in both *db/+* and *db/db* mice

In order to control the efficiency of the response to LPS, we measured first body weight changes induced by LPS 2 h, or 6 and 25 h after its injection, compared to pre-treatment body weight. As expected, *db/db* mice were heavier than *db/+* mice whatever the treatment (genotype: $F_{(1,23)} = 6.0$, $p < 0.05$; data not shown). Body weight was reduced 2 h after LPS in both genotypes (treatment: $F_{(1,33)} = 7.6$, $p < 0.01$; Supplementary Fig. S2A). Similarly, LPS progressively decreased body weight compared to saline-treated controls 6 h and 25 h after its injection (treatment: $F_{(1,23)} = 36.1$, $p < 0.0001$; treatment × time: $F_{(1,23)} = 13.0$, $p < 0.01$; Supplementary Fig. S2B) in both *db/+* and *db/db* mice (treatment × genotype: $p > 0.1$).

We then compared the effect of LPS on peripheral cytokine production between *db/+* and *db/db* mice. The increase in plasma cytokine levels observed in response to peripheral LPS administration results from the activation of both peritoneal and tissue pathogen-associated molecular patterns (*PAMPs*)-responding cells. Therefore, this measure provides an accurate indication of the entire systemic peripheral cytokine response to LPS. Plasma levels of IL-1β ($F_{(1,20)} = 13.5$, $p < 0.01$; Fig. 3A), IL-6 ($F_{(1,23)} = 196.8$, $p < 0.0001$; Fig. 3B), TNF-α ($F_{(1,20)} = 16.3$, $p < 0.001$; Fig. 3C) and the anti-inflammatory cytokine IL-10 ($F_{(1,23)} = 79.1$, $p < 0.001$; Fig. 3D) significantly increased in

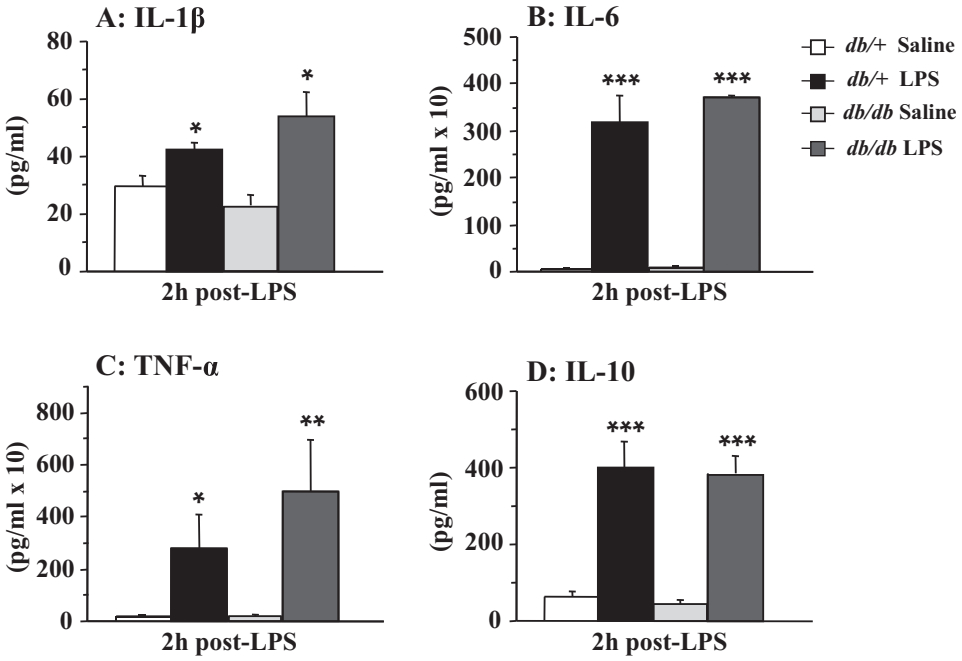

Figure 3 Effect of LPS on plasma concentrations of cytokines in *db/db* and *db/+* mice. Concentrations of (A) interleukin-1 β (IL-1β), (B) IL-6, (C) tumor necrosis factor-α (TNF-α) and (D) the anti-inflammatory cytokine IL-10 measured with a Milliplex kit (Merk-Millipore, France) in plasma collected 2 h after saline or LPS (5 μg/mouse, i.p.) administration. All mice were sacrificed within a few seconds after being picked up from their home cage. Data represent means ± SEM ($n = 8$/group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, for saline *vs.* LPS.

all LPS-treated mice 2 h after treatment, regardless their genotype. As interferon-γ (IFN-γ) only increases between 6 h and 12 h post-LPS (André et al., 2008), no significant effect of LPS was observed for plasma INF-γ levels at 2 h post-treatment, these levels being low and similar in all groups (data not shown).

A well-known consequence of LPS-induced systemic immune stimulation is activation of the hypothalamic-pituitary adrenal (HPA) axis (Castanon et al., 2003). Plasma levels of corticosterone were therefore measured in both *db/+* and *db/db* mice 2 h after LPS treatment. In addition, this measure was also particularly interesting because high levels of basal plasma corticosterone are part of the classical features characterizing *db/db* mice (Stranahan et al., 2008; Dinel et al., 2011). In accordance with their expected phenotype, saline-treated *db/db* mice displayed higher plasma levels of corticosterone than *db/+* mice (genotype: $F_{(1,32)} = 7.1$, $p < 0.05$; Fig. 4A). Moreover, LPS increased plasma corticosterone release 2 h after treatment in all treated mice ($F_{(1,32)} = 75.5$, $p < 0.001$), this effect being not anymore significant at 25 h post-LPS (data not shown). In basal conditions, peripheral TRP is metabolized along the kynurenine pathway in the liver by the TDO enzyme, whose activity is mainly regulated by circulating levels of corticosterone (Morgan and Badawy, 1989). Here, *db/db* mice displayed 25 h after treatment higher liver KYN/TRP ratio than *db/+* mice (genotype: $F_{(1,23)} = 6.8$, $p < 0.05$; Fig. 4B), whatever the treatment ($p > 0.2$). Lastly, changes in lung KYN/TRP ratio that depend on the effects of circulating cytokines on lung IDO activity (André et al., 2008; O'Connor et al., 2009c) were measured 25 h after LPS. In unstimulated conditions, both *db/+* and *db/db* mice displayed similar lung KYN/TRP ratio (Fig. 4C). Moreover, this ratio was significantly increased in all LPS-treated mice ($F_{(1,21)} = 31.8$, $p < 0.0001$), regardless their genotype. Consequently, LPS induced in both genotypes similar increase in lung KYN levels (treatment: $F_{(1,21)} = 33.9$, $p < 0.001$; Supplementary Table S1) and decrease in TRP levels (treatment: $F_{(1,21)} = 13.2$, $p < 0.01$). In summary, LPS-treated *db/db* mice displayed similar peripheral levels of KYN than their *db/±* counterparts but lower brain KYN levels. Peripheral KYN unlikely played therefore a major role in controlling brain levels of KYN in the present case, although the possibility of a differential effect of LPS on the KYN transport across the blood brain barrier cannot be excluded. Whatever the case, these results strongly suggest that both *db/+* and *db/db* mice displayed similar peripheral inflammatory activation in response to LPS.

3.3. Hippocampal inflammatory response to LPS challenge is not impaired in *db/db* mice

We then evaluated central inflammatory response to LPS by measuring expression of cytokines and related inflammatory markers in the hippocampus that plays a key role in both

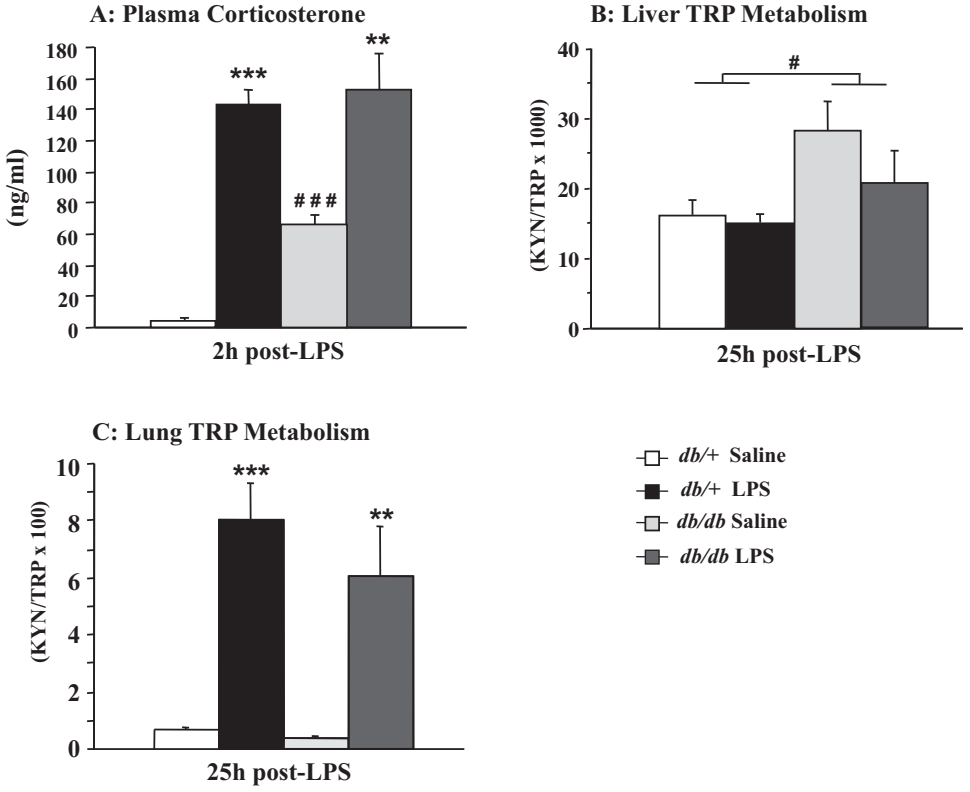

Figure 4 Effect of LPS on plasma concentrations of corticosterone, and liver and lung tryptophan metabolism in *db/db* and *db/+* mice. (A) Concentrations of corticosterone measured by an in-house RIA in plasma collected 2 h after saline or LPS (5 μg/mouse, i.p.) administration. All mice were sacrificed within a few seconds after being picked up from their home cage. Kynurenine (KYN)/tryptophan (TRP) ratios respectively measured in homogenates from (B) liver and (C) lungs collected 25 h after LPS treatment. KYN and TRP concentrations were determined by HPLC and the KYN/TRP ratio was used to assess liver TDO and lung IDO activity. Data represent means ± SEM ($n = 8$/group). $^{**}p < 0.01$, $^{***}p < 0.001$, for saline *vs.* LPS. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ for *db/db vs. db/+* mice.

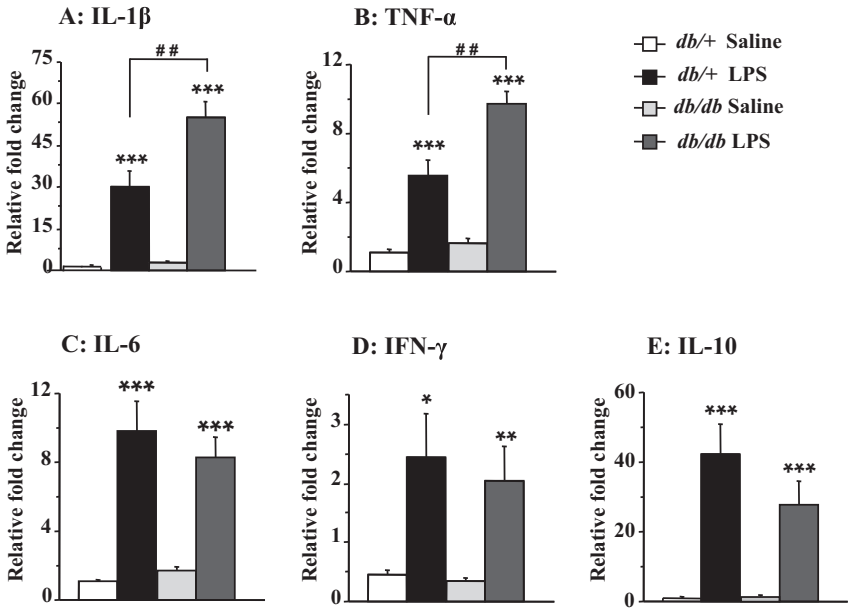

Figure 5 Effect of LPS on mRNA expression levels of cytokines in the hippocampus of *db/db* and *db/+* mice. Relative fold changes in the levels of (A) interleukin-1 β (IL-1β), (B) tumor necrosis factor-α (TNF-α) (C) IL-6, (D) interferon-γ (IFN-γ) and (E) the anti-inflammatory cytokine IL-10 mRNA expression measured by real-time RT-PCR 2 h after saline or LPS (5 μg/mouse, i.p.) administration and calculated in relation to the averaged value for control saline group. Data represent means ± SEM ($n = 8$/group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, for saline *vs.* LPS. $^{\#\#}p < 0.01$ for *db/db vs. db/+* mice.

brain IDO activation and behavioral changes in the FST (Frenois et al., 2007; André et al., 2008; Fu et al., 2010). 2 h after treatment, LPS significantly increased mRNA expression of IL-1β ($F_{(1,32)} = 94.0$, $p < 0.001$), TNF-α ($F_{(1,34)} = 115.1$, $p < 0.0001$), IL-6 ($F_{(1,32)} = 52.0$, $p < 0.0001$), IFN-γ ($F_{(1,31)} = 14.8$, $p < 0.001$) and IL-10 ($F_{(1,30)} = 33.1$, $p < 0.001$) in both *db/+* and *db/db* mice (Fig. 5A–E), although this induction was higher in *db/db* mice for IL-1β (genotype: $F_{(1,32)} = 10.0$, $p < 0.01$; treatment × genotype: $F_{(1,32)} = 7.9$, $p < 0.01$) and TNF-α (genotype: $F_{(1,34)} = 15.9$, $p < 0.001$; treatment × genotype: $F_{(1,34)} = 9.7$, $p < 0.01$).

In addition to cytokines, we also measured the effect of LPS on the hippocampal expression of different molecules playing a key role in microglial activation, firstly TLR4 and CD14 that form the LPS receptor complex. The expression of TLR4 mRNAs was similar in all groups and unaffected by LPS (Fig. 6A). On the contrary, LPS increased mRNA expression of CD14 ($F_{(1,32)} = 88.9$, $p < 0.001$; Fig. 6B) regardless the genotype. Additionally, mRNA expression of CD11b, a marker of microglial activation, was smaller in *db/db* than *db/+* mice (genotype: $F_{(1,33)} = 4.1$, $p = 0.05$; Fig. 6C) and was reduced by LPS in both genotype (treatment: $F_{(1,33)} = 10.6$, $p < 0.01$). We also measured the hippocampal expression of the neuronal chemokine CX_3CL1 that negatively regulates the activation of microglia, and its microglial receptor CX_3CR1. Basal expression of CX_3CL1 and CX_3CR1 mRNAs was similar in both *db/+* and *db/db* mice (Fig. 6D and E). LPS reduced the mRNA expression of CX_3CR1 ($F_{(1,32)} = 41.6$, $p < 0.001$) and CX_3CL1 ($F_{(1,32)} = 7.4$, $p < 0.05$) in both genotypes. The brain-derived neurotrophic factor (BDNF) is another important hippocampal molecule that contributes to mood regulation, particularly under inflammatory conditions (Barrientos et al., 2004). In basal conditions, BDNF mRNA expression was significantly smaller in *db/db* than *db/+* mice ($p < 0.05$; Fig. 6F). Moreover, it was reduced by LPS in *db/+* mice but not in *db/db* mice (genotype × treatment: $F_{(1,31)} = 3.9$, $p = 0.05$).

Taken together, these results show that LPS-induced expression of inflammatory markers in the hippocampus was either similar or higher in *db/db* mice than in *db/+* mice 2 h after treatment. Blunted brain IDO activation and induction of depressive-like behavior occurring in *db/db* mice after a systemic LPS challenge cannot be therefore attributed to a globally reduced inflammatory activation within the hippocampus. They are however associated with an impaired effect of LPS on hippocampal expression of BDNF.

4. Discussion

Although there are but few studies reporting some alterations of sickness behavior induced by systemic LPS challenge in *db/db* mice (Faggioni et al., 1997; O'Connor et al., 2005; Lin et al., 2007), much less is known about brain IDO activation and the resulting induction of depressive-like behaviors. The current study shows for the first time that LPS-induced increase of brain KYN/TRP ratio and depressive-like behavior in the FST is impaired in *db/db* mice. These results suggest that MetS might alter the ability of brain IDO, the enzyme responsible of brain TRP catabolism along the KYN pathway in inflammatory conditions, to appropriately respond to innate immune system activation.

Recently, we developed preclinical animal models in which it is possible to experimentally dissociate inflammation-induced depressive-like behavior from sickness behavior (Frenois et al., 2007; Godbout et al., 2008; Moreau et al., 2008; O'Connor et al., 2009a, 2009b, 2009c). These models have provided very useful tools to thoroughly study the

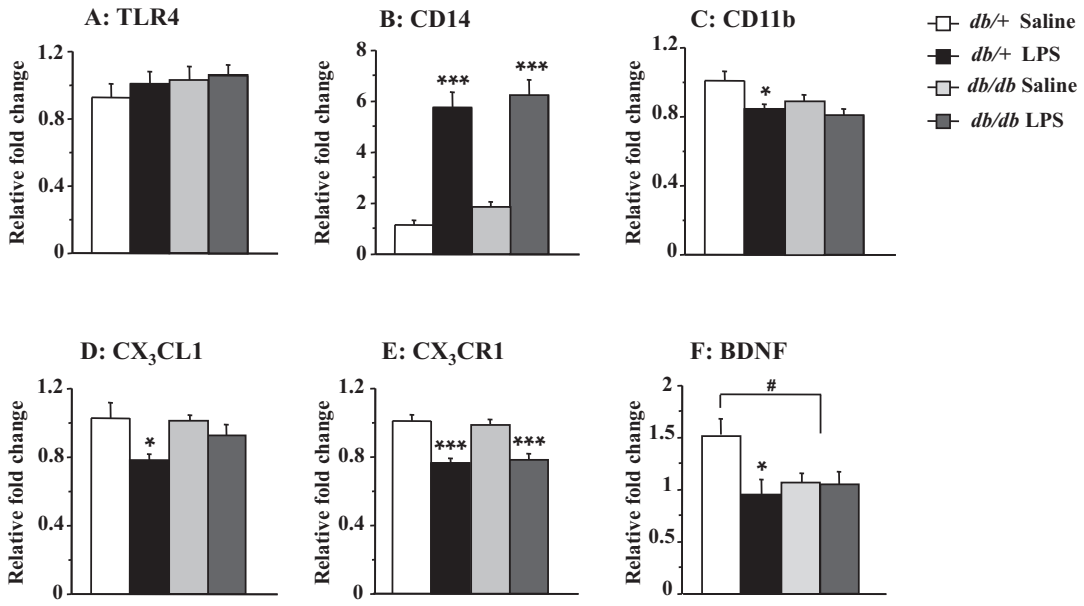

Figure 6 Effect of LPS on mRNA expression levels of different targets of cytokines in the hippocampus of *db*/*db* and *db*/+ mice. Relative fold changes in the levels of mRNA expression of (A) toll-like receptor-4 (TLR4), (B) cluster of differentiation 14 (CD14), (C) CD11b, (D) fractalkine (CX_3CL1), (E) fractalkine receptor (CX_3CR1) and (F) brain-derived neurotrophic factor (BDNF) measured by real-time RT-PCR 2 h after saline or LPS (5 μg/mouse, i.p.) administration and calculated in relation to the averaged value for control saline group. Data represent means ± SEM (n = 8/group). *$p < 0.05$, ***$p < 0.001$, for saline *vs.* LPS. $^{\#}p < 0.05$ for *db*/*db* *vs.* *db*/+ mice.

mechanisms of brain IDO activation by cytokines (André et al., 2008; O'Connor et al., 2009a, 2009b, 2009c; Fu et al., 2010), including in conditions of chronic low-grade basal inflammation such as aging (Godbout et al., 2008; Henry et al., 2009; Corona et al., 2013; Kelley et al., 2013). Applying this experimental approach to *db*/*db* mice allowed us to assess for the first time the induction of brain cytokines and tryptophan catabolism by LPS in a murine model of MetS. In agreement with the findings pointing to the relationship between brain IDO activity and duration of immobility in the FST (Frenois et al., 2007; Godbout et al., 2008; Lawson et al., 2013; Moreau et al., 2008; O'Connor et al., 2009a, 2009b, 2009c), saline-treated *db*/+ and *db*/*db* mice displayed here similar levels of brain KYN/TRP ratio and similar immobility in the FST. These results are akin to our previous findings showing that, in unstimulated conditions, *db*/*db* mice did not differ from *db*/+ mice by their behavioral reactivity in this test (Dinel et al., 2011). Surprisingly, *db*/*db* mice showed after LPS blunted increase of brain KYN/TRP ratio and immobility in the FST compared to their healthy *db*/+ controls. 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. Based on these findings, it might be tempting to conclude that *db*/*db* mice are somehow protected from LPS-induced depressive-like behavior. Such a conclusion needs to be supported by additional experimental data assessing depressive-like behavior in other paradigms, in particular those with higher face validity for clinical depression than the FST (Nestler and Hyman, 2010). Meanwhile, the lack of significant increase of immobility in the FST displayed by LPS-treated *db*/*db* mice fits with blunted induction of brain IDO activity.

It could be argued that a general alteration of LPS efficiency in *db*/*db* mice accounts for the blunted increase of brain KYN/TRP ratio or that *db*/+ and *db*/*db* mice received a fixed dose of LPS (5 μg/mouse) instead of a weight-adjusted dose. However, several data, including ours, argue against this interpretation. First, similar sensitivity to LPS-induced lethality has been reported in both genotypes (Faggioni et al., 1999). Second, the fixed dose of LPS we used has been shown to induce protracted reduction of social interaction in *db*/*db* mice compared to *db*/+ mice (Johnson et al., 2005; O'Connor et al., 2005). Besides, this dose of LPS induces greater increase of core body temperature in obese *ob*/*ob* mice that lack functional leptin than in their lean controls (Lawrence et al., 2012). Third, attempts made to compare fixed *vs.* weight-based strategies of LPS treatment in *db*/*db* mice reveal no major changes in the amplitude of their sickness behavior (O'Connor et al., 2005). Fourth, we reported here that the effect of LPS on peripheral release of inflammatory cytokines (IL-1β, TNF-α, IL-6, INF-γ), as well as lung levels of KYN and TRP, were similar in both *db*/+ and *db*/*db* mice. A higher LPS-induced elevation of plasma TNF-α and IL-6 levels has been previously reported in *db*/*db* mice (Rummel et al., 2010), but after administration of a septic dose of LPS much higher than ours, and by using C57BL/6J mice as controls instead of *db*/+ mice (as we used). This can be important since *db*/+ mice have a similar genetic background (C57BL/6J × DBA/2J) and a similar perinatal environment as their littermate *db*/*db* mice, and it is well known that innate immune system activation can differ from one strain of mice to the others (Nikodemova and Watters, 2011; Painsipp et al., 2011). In the present study, LPS also increased plasma corticosterone release at a similar level

in both genotypes, even if *db*/*db* mice displayed higher basal levels of corticosterone than *db*/+ mice, as previously shown (Stranahan et al., 2008; Dinel et al., 2011). Therefore, impaired increase of brain KYN/TRP ratio and immobility in the FST displayed by *db*/*db* mice is unlikely linked to a reduced effect of LPS on corticosterone. The dissociation between corticosterone levels and IDO activity/depressive-like behavior is also supported by the results obtained in unstimulated conditions, where basal levels of corticosterone are elevated in *db*/*db* mice, whereas basal lung KYN/TRP ratio and immobility in the FST are similar in both *db*/+ and *db*/*db* mice. Corticosterone is also known to stimulate liver TDO activity (Morgan and Badawy, 1989), which in turn can alter brain levels of TRP and KYN (Bano et al., 2010). It could therefore be argued that corticosterone may indirectly affect brain KYN/TRP levels and consequently depressive-like behavior after LPS treatment by differentially modulating liver TDO activity between *db*/*db* and *db*/+ mice. However, this is unlikely the case since LPS similarly affected in both genotypes plasma corticosterone and liver KYN/TRP ratio reflecting TDO activity. Lastly, post-LPS brain KYN/TRP levels may also be influenced by peripheral KYN and TRP concentrations, which mainly depend on lung IDO activation in conditions of immune stimulation, and/or on their transport through the blood brain barrier. Although the possibility that LPS treatment may differentially influence this transport according to the genotype cannot be totally excluded in the present case, the actual dissociation between peripheral and brain KYN levels (respectively similar and different between both *db*/+ and *db*/*db* mice) strongly supports the assumption that increased brain KYN/TRP ratio is mainly due to local IDO activation.

Converging evidence indicates that LPS-induced brain IDO activation depends on brain expression of cytokines, particularly IFN-γ, TNF-α and/or IL-6 (André et al., 2008; O'Connor et al., 2009c; Fu et al., 2010). Although LPS broadly stimulates cytokine expression and IDO activity within the brain (Castanon et al., 2004; André et al., 2008), the hippocampus appears as a key area for IDO activation and induction of depressive-like behavior (Frenois et al., 2007; Henry et al., 2009; Wang et al., 2009; Fu et al., 2010). Although differential hippocampal induction of cytokine expression by LPS existed between *db*/+ and *db*/*db* mice in the present study, it cannot explain the blunted brain IDO activation. Indeed, *db*/*db* mice exhibited 2 h after LPS either similar (as for IL-6, IFN-γ and IL-10) or exacerbated (IL-1β and TNF-α) mRNA expression compared to *db*/+ mice. A complete time course of hippocampal mRNA expression of cytokines in response to LPS would help to better understand the activation of IDO in the context of MetS. However, it is noteworthy that this time-point (2 h post-LPS) corresponds to the peak of hippocampal cytokine expression that precedes local increase of IDO mRNA expression (6—12 h post-LPS) (André et al., 2008).

Measuring changes in cytokine mRNA expression provides early indicators of the activation of the inflammatory response. However, their translation is ultimately required for inducing downstream neurobiological and behavioral modulations. Although we only measured cytokine transcripts, LPS-induced changes in cytokine mRNAs are likely to be reflected in changes of protein levels (van Dam et al., 1998) and correlations between brain mRNA expression of cytokines and corresponding protein levels have been reported in *db*/*db* mice after cerebral hypoxia/ischemia (Kumari et al., 2007). In addition, IDO activation and behavioral changes displayed by LPS-treated *db*/+ mice in the present study confirm that brain cytokines have been induced at the protein levels and were able to act on their targets. This assumption is also supported by the increased CD14 mRNA expression displayed by LPS-treated mice, as well as the reduced expression of other hippocampal targets of cytokines including CD11b, BDNF, the chemokine CX_3CL1, and its receptor CX_3CR1. These results agree with those showing that LPS negatively regulates CX_3CL1 and/or CX_3CR1 in order to thwart the protective control that neuronal CX_3CL1 exerts on microglial activation by acting on CX_3CR1 (Cardona et al., 2006; Wynne et al., 2010). Interestingly, LPS reduced hippocampal BDNF mRNA expression in *db*/+ mice, as previously shown in rats (Barrientos et al., 2004; Tanaka et al., 2006; Tong et al., 2008), but not in *db*/*db* mice. Similarly, Lawrence et al. (2012) recently reported that LPS-induced Fos expression in several brain nuclei is either reduced or absent in obese mice (leptin deficient *ob*/*ob* mice or mice submitted to high-fat diet) compared to lean controls.

Mounting evidence show that CX_3CL1/CX_3CR1 interactions are particularly important when microglial cells become activated by an inflammatory challenge (Cardona et al., 2006). Of note, recent findings report a link between LPS-induced brain IDO activation, behavioral reactivity in the FST, and impaired ability for CX_3CL1 to regulate microglia activation (Godbout et al., 2008; Corona et al., 2010; Wynne et al., 2010; Corona et al., 2012, 2013). Indeed, impaired regulation of microglia by CX_3CL1 because of a genetic deletion of the CX_3CR1 gene is associated with prolonged and exaggerated microglial activation, IDO induction, and depressive-like behavior after LPS challenge (Corona et al., 2010, 2013). Similarly, protracted IDO activation and depressive-like behavior is also reported in aged mice that display reduced brain CX_3CL1 levels (Godbout et al., 2008; Wynne et al., 2010). Interestingly, *db*/*db* mice displayed after LPS challenge exactly the opposite profile of response, namely reduced brain IDO activation and blunted induction of depressive-like behavior. This mirror image may suggest that CX_3CL1/CX_3CR1 interactions are also impaired after LPS challenge in *db*/*db* mice. However, in that case this impairment would lead to sustained CX_3CL1-induced inhibition of microglial activation despite LPS-induced stimulation, associated with reduced IDO activation and depressive-like behavior. Of note, this assumption fits with the observation that the effect of LPS on CX_3CL1 mRNA expression seemed to be slightly stronger in *db*/+ mice than in *db*/*db* mice, although the difference between the two genotypes was not significant. Any conclusion cannot be drawn until these results are confirmed, but they already point to the necessity of deeply investigating the interactions between microglia and neurons in the context of MetS, particularly in conditions of immune stimulation.

In conclusion, the present study constitutes a first important step toward a better understanding of the mechanisms of IDO activation by LPS in the context of MetS. The reasons for the impact of MetS on activation of the innate immune system are likely complex and still need to be identified. Our study also suggests exploring more thoroughly the relationship between MetS and mood. Although clinical data indicate

high incidence of mood disorders in MetS (Dandona et al., 2005; Capuron et al., 2008; Viscogliosi et al., 2013), our experimental results suggest a more complex relationship between MetS and specific mood symptoms: *db*/*db* mice display increased anxiety-like behavior in basal conditions (Dinel et al., 2011) but impairment of depressive-like behavior in immune-stimulated conditions (present study). Although further clinical and experimental studies are still needed, these findings could have relevance in improving the management and treatment of inflammation-related complications in MetS.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2013.10.014.

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