The Role of Indoleamine 2,3-Dioxygenase in a Mouse Model of Neuroinflammation-Induced Depression


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Abstract. Indoleamine 2,3-dioxygenase (IDO), an enzyme which is activated by pro-inflammatory cytokines, has been suggested as a potential link between neuroinflammatory processes in neurodegenerative diseases (like Alzheimer’s disease) and depression. The present study aimed to determine whether neuroinflammation-induced increased IDO levels in the mammalian brain will lead to depressive-like behavior. Neuroinflammation was initiated in mice by a single intracerebroventricular injection of lipopolysaccharide (LPS). Cerebral inflammation was monitored 1, 2, 3 and 4 days after the injection with small-animal positron emission tomography (PET) using the inflammatory marker \([11C]\)-PK11195. In the presence or absence of systemically applied 1-methyl-tryptophan (1-MT), a competitive IDO-inhibitor, we assessed the development of depressive-like behavioral symptoms in parallel with IDO expression and activity. The PK11195 PET signal reached a highly significant peak 3 days after LPS injection, while these animals displayed a significant increase of depressive-like behavior in the forced swim test compared to vehicle-injected animals. These findings were paralleled by a significant increase of IDO in the brainstem, and an increased kynurenine/tryptophan ratio in the serum. Moreover, we report here for the first time, that inhibition of IDO by 1-MT in centrally induced neuroinflammation under experimental conditions can prevent the development of depressive-like behavior.

Keywords: Depression, indoleamine 2,3-dioxygenase, lipopolysaccharide, neuroinflammation, positron emission tomography

INTRODUCTION

Neuroinflammation is defined as the activation of an immune response in the central nervous system (CNS) [1]. During neuroinflammation, microglia, the most important resident immune cells, become activated [2] and, as a consequence, their morphology starts to change and secretion of pro-inflammatory cytokines, such as interferon \(\gamma (IFN\gamma)\) [3], tumor necrosis factor \(\alpha (TNF\alpha)\) [4], or interleukin 6 [5], is initiated. An important hallmark of activated microglia is the expression of peripheral benzodiazepine receptors (PBRs) on the outer membrane of the mitochondria, which allows the non-invasive detection their activated state by means of labeled PBR ligands. Ligands such as the...
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the development of depression. Depression may thus
vation of the immune system in the brain can lead to
chronic inflammatory changes have been shown to
play a key role in the pathogenesis of neurodegenerative
diseases, like Alzheimer’s disease (AD) [8]. The
typical hallmarks of AD are extracellular depo-
sitions of amyloid-β, intracellular aggregates of the
protein tau, but besides these features, activation of
microglia and elevated cytokine and chemokine levels
have been shown as well [8]. It has been shown that
AD is often accompanied by symptoms of depression,
and patients are often being treated for depression sev-
eral years before clinical diagnosis of AD occurs [9].
Meta-analysis on retrospective and prospective studies
revealed that the history of depression approximately
doubles the risk of developing dementia. Several stud-
ies support the hypothesis that depression is a likely
risk factor for dementia in general and for AD specifi-
cally [10, 11].

Inflammation is an important hallmark of sepsis as
well. It has been recently shown that severe sepsis can
lead to cognitive impairment and functional disability
[12].

During peripheral infection, either acute or chronic,
the immune system is activated, e.g., by macrophage
stimulation, and pro-inflammatory cytokines are
released, which also act on the brain causing sickness
behavior. Sickness behavior shares similar features
with major depression. Both are characterized by
malaise, weakness, loss of appetite, lethargy, or
decreased interest in the surroundings. However,
sickness behavior breaks off when the pathogen is
eliminated. When the peripheral immune system is
continuously activated, like in chronic inflammatory
diseases, such as rheumatoid arthritis, osteoarthritis, or
inflammatory lung disease, the resulting chronic acti-
vation of the immune system in the brain can lead to
the development of depression. Depression may thus
represent a maladaptive version of cytokine-induced
sickness behavior [13]. In 1991, R.S. Smith proposed
the macrophage theory of depression [14], which
triggered a growing body of evidence supporting
the notion that inflammation can increase the risk of
developing major depression [15]. The overall idea
being that the overexpression of pro-inflammatory
agents is associated with increased activity of the
ubiquitous intracellular enzyme indoleamine-2,3-
dioxygenase (IDO), which catalyzes tryptophan
catabolism through the kynurenine pathway [13, 16].

The depletion of tryptophan in brain cells reduces the
production of brain serotonin (5-HT) [13, 16]. The
degradation of tryptophan along the kynurenine
pathway also generates neurotoxins, like quinolinic
acid (QUIN), an NMDA receptor agonist, or 3-
hydroxykynurenine (3-HK), which leads to apoptosis
in neurons, which can add to local excitotoxic neuronal
overstimulation next to modulating serotonergic neu-
rotransmission [17]. Taken together, cytokine-induced
IDO-mediated tryptophan depletion [18] and QUIN-
mediated neurotoxicity [17, 19] are hypothesized to
be involved in the pathophysiology of mood disorders,
like major depression. Nevertheless, it has been also
shown that the metabolism of tryptophan by IDO
through the kynurenine pathway is increased in AD
[20]. Lipopolysaccharide (LPS) injection in rodents
is often used as an experimental model for systemic
immune challenges. LPS-injected mice display imme-
diate sickness behavior after peripheral injection of
LPS whereas depressive-like behavior seems to emerge
in a shifted temporal frame, when sickness is already
abated [13, 21]. Accordingly, IDO activation and
concomitant depressive-like behavior has been shown to
be causally interrelated in this mouse model [22].

Previous studies demonstrated that systemic expo-
sure to LPS induces chronic central neuroinflammation
[23] as well as cerebral IDO activation [24, 25]. In
our study, we measured IDO activity and expres-
sion after intracerebroventricular LPS injection and
assessed the development of depressive-like symptoms
in the presence or absence of 1-methyl tryptophan
(1-MT), a competitive IDO-inhibitor. Neuroinflamma-
tion was monitored over time by small animal positron
emission tomography (PET).

We report here for the first time that the inhibition
of IDO in centrally induced neuroinflammation is suf-
ficent to prevent the development of depressive-like
behavior in mice.

MATERIALS AND METHODS

Ethics statement

All animal care and treatments were reviewed and
approved by the Ethical Committee for the use of
experimental animals of the University of Groningen
under license number DEC5461.
Animals

Three months old male C57Bl/6J mice (n = 8–10/group, groups: Placebo/PBS, 1-MT/PBS, Placebo/LPS, 1-MT/LPS) were obtained from Harlan (Horst, The Netherlands). Animals were individually housed under normal laboratory conditions (air-conditioned, 21 ± 2°C), humidity-controlled room, 12/12 h light/dark cycle (light on from 08:00–20:00). Food and water were available ad libitum. In all cases the weight of the animals was monitored on a daily basis.

Intracerebroventricular LPS injection

Before fixation in a stereotactic apparatus mice were anaesthetized with avertin (250 mg/kg by intraperitoneal injection). Periprocedural analgesia was provided with finadyne (2.5 mg/kg, subcutaneously). The coordinates for the bilateral intracerebroventricular (ICV) injections were −2.5 mm dorsal/ventral, −1.0 mm lateral, and −0.5 mm anterior/posterior from bregma [26]. The holes were drilled perpendicularly to the previously exposed skull. 1/100,000 endotoxin-free phosphate buffered saline (PBS) or 5/100,000 g lipopolysaccharide (LPS, L-6529, serotype 055:B5, Sigma-Aldrich) dissolved in 1/100,000 l PBS was injected into the lateral ventricles using a 1/100 l Hamilton needle (cat. nr. 170431, Omnilabo) fitted to a 25/100 l Hamilton syringe. This dose of LPS was previously shown to induce significant neuroinflammation [27–30]. A syringe pump (TSE system, Bad Homburg, Germany) was used for injection at a constant rate of 0.3/100 l/min. After injection, the needle was kept in the ventricles for an additional 5 min and subsequently slowly removed from the brain. The incision was sealed with dental cement.

Slow-release pellet implantation

Four days prior to ICV injection, mice were initially anaesthetized by avertin (250 mg/kg by intraperitoneal injection). After anesthetic induction, the animals were placed in an open circuit system and 2% isoflurane was administered via a nose-cone. The competitive IDO inhibitor 1-methyl-DL-tryptophan (1-MT) was applied via a slow release pellet implanted subcutaneously beneath the dorsal skin surface. Pellets were designed to continuously release 5 mg/day of drug for 21 days (Innovative Research of America, Sarasota, FL, USA) [22]. Similar drug free pellets served as placebo.

Synthesis of [11C]-PK11195

The PET tracer [11C]-PK11195 was produced at the University Medical Center Groningen according to the procedure described by Cremer et al. [31]. The product was obtained in 32 ± 10% radiochemical yield as a sterile solution in 10% ethanol in saline. The radiochemical purity was always >95% and the specific activity was 110 ± 69 GBq/μmol.

Micro-PET imaging

Mice were anaesthetized by 5% isoflurane (Pharmachemie, The Netherlands) mixed with medical air in an induction chamber. Anesthesia was maintained with 2% isoflurane in medical air at a flow of 2 ml/min via a nose-cone. Mice were injected intravenously with 7.5 ± 2.5 MBq [11C]-PK11195 via the penile vein and positioned in a small animal PET camera (Focus 220, Siemens Medical Solutions USA, Inc.) in a transaxial position with their heads in the field of view. A transmission scan with a 57Co point source was acquired for the correction of attenuation by tissue. Ten minutes after tracer injection, a 30-min static emission scan was started. After completion of the emission scan, the animals were allowed to recover. Emission sinograms were iteratively reconstructed (OSEM2d, 4 iterations) after being normalized, corrected for attenuation and decay of radioactivity. Regions-of-interest (ROI) were manually drawn over the whole mouse brain in the reconstructed images using the microPET® ASIPro™ VM6.6.2.0 software. Tracer uptake in ROI was obtained in Bq/cm³ and converted into the standardized uptake value (SUV), defined as: [tissue activity concentration (MBq/cm³)]/[(injected dose (MBq)/body weight (g)].

Elevated plus maze

The elevated plus maze test was used to measure anxiety-related exploration [32]. The test consisted of a plus-shaped maze, 50 cm above the floor, with two opposite arms closed by high side walls and two opposite open arms without walls (50 cm long, 5 cm wide). The mouse was placed in the center of the maze and was allowed to explore the maze freely for 8 min. Exploratory activity, time spent in the dark arms, open arms, and center zone were recorded visually by two raters (one of them was blind to the experiment). The ratio of time spent in the open (light) and closed (dark) arms and in the center to total time spent in the maze was calculated for each group. A lower ratio of time
spent in the open arms and total time is indicative for higher anxiety levels.

**Spontaneous alternation**

Short-term spatial memory performance (working memory) was investigated by recording spontaneous (since it is not reinforced) alternation behavior in a Y-maze paradigm [33]. The maze consisted of three tubular and transparent Plexiglas arms forming the Y. All three arms were 5 cm in diameter, 27.5 cm long, and at a 120° angle from each other. The experimental room contained visual cues for spatial orientation. The mouse (naive to the maze) was placed into the center of the Y maze and allowed to explore the maze freely during an 8-min session. The series of arm entries (considered to be completed when all four paws of the animal entered the arm) was recorded visually by two raters (one of them was blind to the experiment). Alternation was defined as successive entries into the three arms on overlapping triplet sets. Working memory was assessed by the alternation percentage, which was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two). Exploratory activity was assessed by counting the total number of arm entries.

**Forced swim test**

The forced swim test is a widely used paradigm to measure antidepressant activity and depressive-like behavior in animal models [34]. A cylinder (22 cm height × 11.5 cm diameter) was filled with water (23–25 °C) up to a height of 17 cm. The animal was placed inside the cylinder and immobility time was recorded over a 7 min test period. Afterwards the mouse was dried and returned to its cage. Between testing sessions the water was refreshed.

**Protein analysis**

Immediately after the PET scans and behavioral experiments, mice were sacrificed by CO₂ inhalation. Brains were quickly dissected, snap-frozen in liquid nitrogen and stored at −80 °C until homogenization and Western blotting [35]. Antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were used for IDO (rat monoclonal antibody), and HPRT (hypoxanthine-guanine phosphoribosyltransferase) as an internal standard to correct for variations in protein content (rabbit polyclonal antibody), and stained with horseradish peroxidase-conjugated secondary antibodies (goat-anti-mouse, donkey-anti-rabbit, respectively). The proteins were detected using Enhanced Chemiluminescence (Pierce ECL, Western Blotting Substrate, Rockford, IL, USA) and the Molecular Image Chemidoc XRS+ System (Biorad). The results were analyzed using Image Lab software (BioRad).

**Serum tryptophan and kynurenine quantification**

We used as an **in vivo** index of IDO activity the ratio of the blood levels of kynurenine/tryptophan. Nevertheless, it is known, that tryptophan 2,3-dioxygenase (TDO) also catabolizes tryptophan into kynurenine, but since 1-MT specifically inhibits IDO, but not TDO, we could use the results of the ratio of kynurenine/tryptophan as a reflection of IDO activity [36]. Tryptophan and kynurenine concentrations in serum were determined by an automated on-line solid-phase extraction-liquid chromatographic-tandem mass spectrometric (XL-Chromatographic mass spectrometry) method with deuterated internal standards as described previously [37].

**Statistical analysis**

The data in Fig. 1F were analyzed by 2-way analysis of variance (ANOVA), and the data in Fig. 4. were analyzed by 3-way ANOVA with repeated measures. All other data were analyzed by one-way ANOVA, followed by the LSD post-hoc test to determine the differences between the selected groups using the program SPSS 16.0 for Windows. *p < 0.05 was considered to be statistically significant (**p < 0.005, highly significant; ***p < 0.0005, extremely significant). Data are presented as a mean value ± the standard error of the mean (SEM).

**RESULTS**

**Intracerebroventricular LPS injection-induced neuroinflammation culminates 3 days after injection**

We evaluated the temporal course of **in vivo** cerebral inflammation in the mouse brain using micro-PET (Fig. 1D). PK11195 binding potential peaked 3 days after the ICV injection of either LPS (F(7,57) = 5.099, p < 0.0005) or, interestingly, also after injection of PBS (p ≤ 0.05) with similar kinetics. Gial activation was significantly higher at day 3 and 4 in LPS injected mice when compared to PBS injected mice. Already on the fourth post-operative day, the inflammatory response started to decline (Fig. 1D).
Fig. 1. Microglia activation in the brain visualized by small animal PET. Representative pictures of a mouse PET scan (A–C). Microglia activation is depicted using colors for high (red) or low (purple) [11C]PK11195 uptake. A) transversal section, B) coronal section, C) sagittal section. Brain (ROI) is indicated by the arrow. D) Standardized uptake value (SUV) versus days after ICV injection. 1 μl endotoxin-free phosphate buffered saline (PBS) or 5 μg lipopolysaccharide (LPS) dissolved in 1 μl PBS was injected into the lateral ventricles. Data represent mean SUV±SEM as a percentage of control, PBS injected animals on day 1. Number of scanned animals: 1. day PBS = 13, LPS = 14, 2. day: PBS = 6, LPS = 7, 3. day: PBS = 8, LPS = 7, 4. day: PBS = 5, LPS = 5, *p<0.05, ***p<0.0005. E) Diagram of the time course of the PET scanning design. F) SUVs 3 days after injection in placebo or 1-MT pretreated, PBS or LPS injected mice. Four days prior to ICV injection, the IDO inhibitor 1-methyl-tryptophan (1-MT) was applied via a slow release pellet (continuously release 5mg/day of drug/placebo) implanted subcutaneously beneath the dorsal skin surface. Data represent mean SUV±SEM as a percentage of the Placebo/PBS group. Number of scanned animals: Placebo/PBS = 6, 1-MT/PBS = 6, Placebo/LPS = 7, 1-MT/LPS = 6 *p<0.05, ***p<0.0005. G) Schematic representation of the time course of the experiment.

Pretreatment with 1-MT does not have a significant influence on LPS-induced neuroinflammation

Microglia activation was measured by PET analysis on the third post-operative day. The results analyzed by 2-way ANOVA, showed a significant effect of pretreatment (placebo or 1-MT) (F(4,827), p<0.05) and treatment (PBS or LPS) (F(74,309), p<0.0005). These results corroborate previous findings that ICV injection of LPS indeed induces central neuroinflammation. Most interestingly, there was no significant interaction between pretreatment and treatment (F(1,393), p>0.05), which means 1-MT does not have a significant influence on LPS-induced neuroinflammation (Fig. 1F).

LPS induces depressive-like behavior 3 days after ICV injection

A group of animals was tested 3 days after ICV PBS or ICV LPS injection, when neuroinflammation peaked. Depressive-like behavior was significantly increased after ICV LPS injection as tested in the forced swim test (F(1,11)= 5,323, p<0.05, Fig. 2G). Inhibition of IDO abrogates the development of LPS-induced depressive-like behavior

On day 4 after ICV injection, LPS increased the immobility time in the forced swim test (F(3,26)= 3.36, p<0.05) in placebo/LPS mice, compared to either placebo/PBS (p>0.05) or 1-MT/PBS
Fig. 2. The effect of neuroinflammation on behavior and the consequences of IDO inhibition. A) Forced swim test, a widely used paradigm to measure antidepressant activity and depressive-like behavior in animal models. The animal was placed inside the cylinder and immobility time was recorded over a 7 min test period. Data represent mean values ± SEM, *p < 0.05. B) Elevated plus maze test was used to measure anxiety-related exploration. The mouse was placed in the center of the maze and was allowed to explore the maze freely for 8 min. Exploratory activity, time spent in the dark arms, open arms and center zone were recorded visually. The ratio of time spent in the open and closed arms and in the center was calculated for each group. A lower ratio of time spent in the open arms and total time is indicative for higher anxiety levels. Data represent mean values ± SEM in percentage. C, D) Spontaneous alternation test. Short-term spatial memory performance (working memory) was investigated by recording spontaneous alternation behavior in a Y-maze paradigm. The mouse was placed into the center of the Y maze and allowed to explore the maze freely during an 8 min session. The series of arm entries was recorded visually. Alternation was defined as successive entries into the three arms on overlapping triplet sets. Working memory was assessed by the alternation percentage, which was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two). Exploratory activity was assessed by counting the total number of arm entries. Data represent mean values ± SEM. *p < 0.05. E) Spontaneous alternation test 3 days after ICV PBS or ICV LPS injection. Data represent mean values ± SEM in percentage. F) Forced swim test 3 days after ICV PBS or ICV LPS injection. Data represent mean values ± SEM, *p < 0.05.
Fig. 3. A) IDO protein expression in different regions of the brain. Mice were sacrificed, brains were quickly dissected, snap-frozen and Western blotting was done. Data represent mean values ± SEM as a percentage of the Placebo/PBS group. *p < 0.05, **p < 0.005. B) IDO activity in serum. Tryptophan and kynurenine concentrations in serum were determined by an automated on-line solid-phase extraction-liquid chromatographic-tandem mass spectrometric (XLC-MS/MS) method. IDO activity is defined as the ratio of the concentration of kynurenine and tryptophan. Data represent mean values ± SEM as a percentage of the Placebo/PBS group. ***p < 0.0005.

(p < 0.05) mice. This effect of LPS was completely inhibited by 1-MT pretreatment (p < 0.05) (Fig. 2A). These results demonstrated that IDO inhibition prior to ICV LPS injection was sufficient to prevent the development of LPS-induced depression-like behavior.

**ICV administered LPS did not affect anxiety-related behavior or working memory**

ICV administered LPS did not affect anxiety behavior in the elevated plus maze on day 4 (time spent in the open arms (F(3,31) = 0.41, p > 0.05), in the center (F(3,31) = 0.84, p > 0.05), in the dark (F(3,31) = 0.82, p > 0.05) when compared to placebo/PBS injected mice (Fig. 2B). 1-MT also did not affect anxiety behavior (time spent in the open arms (F(3,31) = 0.41, p > 0.05), in the center (F(3,31) = 0.84, p > 0.05), in the dark (F(3,31) = 0.82, p > 0.05) when compared to placebo/PBS injected animals. Working memory, measured in the spontaneous alternation task (number of alternations (F(3,33)) = 0.89, p > 0.05, Fig. 2C) and exploratory behavior (number of entries (F(3,33)) = 2.08, p > 0.05, Fig. 2D) were not affected by LPS treatment nor by application of 1-MT. Overall, these results showed that neither ICV injected LPS nor concurrent IDO inhibition could affect anxiety-like behavior and working memory performances.

Also, a separate group of animals was tested 3 days after ICV PBS or ICV LPS injection. No abnormalities were found in the working memory (number of alternations: F(1,13) = 1.117, p > 0.05, Fig. 2E) and exploratory behavior (number of entries: F(1,13) = 1.859, p > 0.05, data not shown) as tested in spontaneous alternation test.

**LPS treatment increases IDO levels in the brainstem 4 days after injection**

A significant increase of IDO (F(3,131) = 6.388, p < 0.05) was detected in the brainstem of placebo/LPS (p < 0.05) and 1-MT/LPS (p < 0.005), compared to both PBS groups (Fig. 3A). In the other brain regions, no significant differences among the four groups were observed (cerebellum (F(3,49) = 0.37, p > 0.05), cortex (F(3,52) = 0.24, p > 0.05), hippocampus (F(3,93) = 0.36, p > 0.05), hypothalamus (F(3,51) = 0.70, p > 0.05), olfactory bulb (F(3,109) = 0.69, p > 0.05), and striatum (F(3,56) = 0.815, p > 0.05).

**1-MT inhibits IDO activity in the serum after ICV LPS injection**

ICV LPS injection led to increased IDO activity, defined as a ratio of kynurenine and tryptophan, measured in the serum, which could be completely inhibited by 1-MT (F(3,26) = 10.611, p < 0.0005) (Fig. 3B).

**ICV injection of LPS results in a robust reduction in body weight**

Cerebral inflammation was paralleled by considerable weight reductions in both LPS groups after injection, but was normalized by the time of
behavioral assessment (Fig. 4). The data were analyzed by 3-way ANOVA with repeated measures, showing a significant effect of time (days) (F(36,101), p < 0.0005) and also, there was a significant interaction between time and treatment (PBS or LPS) (F(22,041), p<0.0005). There was no interaction effect between pretreatment (placebo or 1-MT) and treatment (PBS or LPS) (F(6,000), p>0.05). Source of significance was analyzed by univariate measures. No significant difference was observed on day −4 (F(0,454), p>0.05), day −3 (F(0,453), p>0.05), day 0 (F(0,777), p>0.05), and day 4 (F(4,022), p>0.05). There was a significant difference on day 1 (F(8,661), p<0.05), day 2 (F(10,662), p<0.05), and day 3 (F(7,162), p<0.05).

DISCUSSION

In the study presented here we used an animal model for depression in neuroinflammatory conditions, such as AD. Our results provide some evidence that LPS-induced neuroinflammation leads to increased IDO protein levels in the brainstem, which is accompanied by the onset of depressive-like behavior. This behavioral phenomenon can be completely blocked by IDO inhibition suggesting a causal relation between the behavioral expression and IDO activation.

Several studies have shown that peripheral inflammation can cause central neuroinflammatory responses which might be causal for depressive-like behavior. Although the question still remains whether central inflammation alone is sufficient to lead to depressive-like behavior.

O’Connor and colleagues showed that the inhibition of IDO by 1-MT in the context of systemic LPS administration can normalize plasma and brain kynurenine/tryptophan ratios, while only kynurenine levels were altered [22], which is consistent with the data presented here. We also found a significant increase of the kynurenine/tryptophan ratio in the serum of ICV LPS-injected animals, whereas tryptophan levels remained unchanged.

We used microPET to monitor the ongoing cerebral innate immune responses and dissociate cytokine-induced sickness from depressive-like behavior. Our findings clearly showed that cerebral inflammation was considerably higher, as expected, in ICV LPS injected mice and reached its peak 3 days after the ICV injection. The 4th post-injection day (when sickness-behavior is imperceptible) was chosen to perform behavioral studies to measure depressive-like behavior. On the 4th post-injection day (when the placebo/LPS treated animals exhibited increased depressive-like behavior in the forced swim test), cerebral inflammation was almost gone. This was confirmed by reduced body weight in both LPS groups during the first two post-injection days and subsequent restoration by the time of behavioral assessment (Fig. 4). The reduced food intake might affect the tryptophan levels in the blood, but that cannot be the reason for the higher kynurenine/tryptophan ratio, since the 1-MT/LPS group showed a dramatic decrease in the body weight as well, but did not show increased kynurenine/tryptophan ratio. No differences were observed in the general activity of the mice when the behavioral tests were done, as seen also in the total arm entries in the elevated plus maze test (data not shown) or in the spontaneous alternation test (Fig. 2). We also did not observe any differences in working
memory, exploratory behavior and anxiety-like behavior between PBS and LPS-treated groups (Fig. 2), indicating that locomotor activity was normal by the time mice were tested in the forced swim test.

Behavioral tests were also performed on day 3 (Fig. 2), showing the same results, namely: working memory and exploratory behavior were not affected, as tested in the spontaneous, alternation test, but significantly increased immobility was observed in the forced swim test in the LPS-treated group. Statistical analysis showed that depressive-like behavior after ICV LPS injection was more robust on the 4th post-operative day than on the 3rd (ICV LPS injection was more robust on the 4th post-operative day than on the 3rd, p values, 0.02 and 0.04, respectively), making the hypothesis stronger, that depressive-like behavior lasts longer after a neuroinflammatory response.

It is important to mention that a limitation of the present study, especially in relation to neurodegenerative diseases, e.g., AD, is that no long-term consequences of ICV LPS-injection and/or IDO inhibition were assessed. Indeed, it has been previously shown that chronic LPS infusion directly in the 4th ventricle in rats can result in impaired cognitive performance [38]. We therefore hypothesize that an acute and transient LPS challenge might not be sufficient to unequivocally confound the elaborate and pleiotropic physiology of brain cytokines normally involved in cognitive processes [39].

Taken together, our findings further support the hypothesis that depression-like behavior remains even when temporally induced sickness behavior has waned. The temporal profile showed a different pattern from that described in previous studies [40, 41], but it should be noted that we used larger doses of LPS to induce the immunological challenge in our study. More importantly, we mainly measured reactivity of resident brain cells in response to ICV LPS injection, which might be delayed when compared to the immediate sickness-inducing vagal afference and mononuclear infiltration to the CNS following systemic LPS exposure.

We further demonstrate for the first time that the inhibition of IDO by 1-MT pretreatment in the context of centrally LPS-induced neuroinflammation is sufficient to prevent the development of depressive-like behavior. Since IDO in our study was inhibited systemically [22], we cannot ascertain whether this finding is associated with direct central IDO inhibition or reduction of CNS influx of peripheral kynurenine, which has been previously shown to induce depressive-like behavior [22]. It may very well be the result of a combined effect of peripheral and central IDO inhibition. It has been shown that even much lower doses of LPS can cross the brain to blood direction [42], leading to a subsequent peripheral inflammation, as it has been already also shown that lower dose of ICV injected LPS leads to increased production of central and peripheral TNFs [43].

We also measured TNFs expression in the liver and found an increase of TNFs in both LPS treated groups (Results in %: Placebo/PBS: 100 ± 12, 1-MT/PBS: 99 ± 12, Placebo/LPS: 131 ± 15, 1-MT/LPS: 154 ± 15) indicating peripheral inflammation in our paradigm, although this effect is not because of the dose of LPS we used in this study, but because the immune privilege of the brain is not absolute and there is a constitutive communication of the brain and the periphery [44].

It was shown that IDO mRNA expression was reduced in whole brain homogenates of 1-MT/LPS treated mice [22], which, however, was not observed in the context of Bacille Calmette-Guérin-induced infection [45]. In any case, those measurements were conducted in whole brain homogenates, so we cannot exclude the possibility that brain IDO expression or cytokine signaling is regionally altered. Indeed, our data show that IDO protein expression is increased in the brainstem of LPS injected mice, independently of pretreatment with placebo or 1-MT. Although we did not measure cytokine expression levels in the brain, our results clearly argue in favor of a post-translational inhibition of IDO by 1-MT. Down-regulation of IDO expression in other brain regions was not found, but even if it was, this does not necessarily mean it would be a direct effect of 1-MT.

Our PET findings are consistent with a moderate, but not significant anti-inflammatory effect of 1-MT, as shown by the reduction of peripheral benzodiazepine receptors expressed in the brains of 1-MT/LPS relative to placebo/LPS treated mice. O’Connor et al. [22] have shown that the presence of 1-MT did not modify the expression of pro-inflammatory cytokines in mice upon peripheral injection of LPS. However, direct as well as accumulative immune effects of the various neuroactive tryptophan catabolites have to be taken into account. Indeed, Maes and coworkers [19] have shown in vitro that kynurenine and kynurenic acid (KYNA) display anti-inflammatory properties via reduction of stimulated IFNγ and TNFs production. In addition, Doodnauth et al. [6] comment that PBRs are also expressed in activated astrocytes, which preferentially produce KYNA [17, 46]. We therefore hypothesize that IDO inhibition in microglia mitigates the production of pro-inflammatory QUIN, which in combination with a shortage of anti-inflammatory
astrocyte-derived KYNA upon LPS challenge, might leave the brain of I-MT/LPS mice in a neuroinflammatory state lower than placebo/LPS mice, but greater than the placebo/PBS animals.

We have found significantly higher levels of IDO in the brainstem of LPS injected mice. It has been shown that physical stressors, such as trauma or infection, rapidly affect the brainstem, where coordinated processing of autonomic, immune, and neuroendocrine information takes place [47]. Interestingly, injection of mice with LPS selectively increases immediately early gene expression within serotonergic neurons of the interfascicular part of the dorsal raphe nucleus [48]. These neurons comprise a unique, anatomically and functionally distinct immune-responsive subpopulation within the brainstem Raphe complex that essentially differs from the neuronal population responding to anxiety- and stress-related stimuli [49]. This may explain the “paradox” of decreased behavioral activity but increased [50] or unchanged [49] serotonergic neurotransmission following acute immune stimulation.

In conclusion, our findings indicate that neuroinflammation induces depressive-like symptoms in an animal model, which can be abolished by inhibiting the effects of IDO. Moreover, this study not only provides evidence of a pathogenic role of cerebral inflammation in the precipitation of depression, but also suggests that symptoms often concurring with depression, such as anxiety, feelings of misery, and impaired working memory, are the consequences of some other processes and apparently dissociated from brainstem serotonin neurotransmission.

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