Exacerbated fatigue and motor deficits in interleukin-10 deficient mice after peripheral immune stimulation

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Abstract

The anti-inflammatory cytokine interleukin (IL)-10 is important for regulating inflammation both in the periphery and brain but whether it protects against infection- or age-related psychomotor disturbances and fatigue is unknown. Therefore, the current study evaluated motor coordination, time to fatigue, and several central and peripheral proinflammatory cytokines in male young adult (3 mo) and middle-aged (12 mo) wild type (IL-10\textsuperscript{+/+}) and IL-10 deficient (IL-10\textsuperscript{-/-}) mice after i.p. injection of lipopolysaccharide (LPS) or saline. No differences were observed due to age so data from the two ages were pooled and analyzed to determine effects of genotype and treatment. Treatment with LPS increased IL-1\textbeta, IL-6, and tumor necrosis factor \alpha (TNF\alpha) mRNA in all brain areas examined in both IL-10\textsuperscript{+/+} and IL-10\textsuperscript{-/-} mice but to a greater extent and for a longer time in IL-10\textsuperscript{-/-} mice. Plasma IL-1\textbeta and IL-6 were increased similarly in IL-10\textsuperscript{+/+} and IL-10\textsuperscript{-/-} mice 4 h after LPS but remained elevated longer in IL-10\textsuperscript{-/-} mice; whereas TNF\alpha was higher in LPS-treated IL-10\textsuperscript{-/-} mice throughout. LPS treatment did not affect motor performance or motor learning in IL-10\textsuperscript{+/+} mice; however, both were reduced in LPS-treated IL-10\textsuperscript{-/-} mice compared to saline. Furthermore, although LPS reduced the time to fatigue in both IL-10\textsuperscript{+/+} and IL-10\textsuperscript{-/-} mice, the effects were exacerbated in IL-10\textsuperscript{-/-} mice. Thus, the increased brain and peripheral inflammation induced by LPS in IL-10\textsuperscript{-/-} mice was associated with increased coordination deficits and fatigue. These data suggest that IL-10 may inhibit motor deficits and fatigue associated with peripheral infections via its anti-inflammatory effects.

Key words: brain, fatigue, interleukin-10, proinflammatory cytokines, motor coordination
Introduction

The proinflammatory cytokines interleukin (IL)-1β, IL-6 and tumor necrosis factor α (TNFα) are well known to induce sickness behavior in response to infection (7). In addition to sickness behavior, proinflammatory cytokines reduce muscle mass and strength (20, 30) and may also affect brain areas involved in motor coordination and fatigue (8). This may be particularly important in the elderly because inflammation tends to increase with age and this is often paralleled by a decline in psychomotor function, largely marked by impaired coordination, balance and strength (16). These motor and balance disruptions are the major cause of admission into hospitals and nursing homes (18) and it is estimated that injuries from falls cause more deaths in the elderly than pneumonia or diabetes (The National Safety Council). It is noteworthy that constitutive expression of several proinflammatory cytokines are increased in both the periphery and brain of old but otherwise healthy animals (32). Moreover, when lipopolysaccharide (LPS) is administered to mimic a peripheral infection, old mice experienced an exaggerated proinflammatory cytokine response in the brain and exhibited signs of behavioral pathology not seen in younger cohorts, including prolonged anorexia (10), depressive-like behavior (11), and cognitive dysfunction (2). Collectively, these studies support a linkage between inflammation and motor deficits and fatigue.

The anti-inflammatory cytokine IL-10 can inhibit the effector functions of macrophages and microglia by blocking proinflammatory cytokine synthesis, suppressing expression of receptors for proinflammatory cytokines, and inhibiting cytokine receptor activation (28). IL-10 therefore, is important for restricting the proinflammatory response during infection and, not surprisingly, mice deficient in IL-10 have higher serum levels of proinflammatory cytokines after LPS administration (1). This too is relevant to the geriatric population because the age-
associated increase in proinflammatory cytokines in the brain is inversely related to IL-10 expression in rats and mice (22, 32).

Given the purported relationship between proinflammatory cytokines and motor deficits and fatigue, the present study was conducted to determine if IL-10 protects against infection- or age-related psychomotor disturbances and fatigue. Specifically, we evaluated motor coordination, time to fatigue, and several proinflammatory cytokine proteins and mRNAs in plasma and brain, respectively, in young adult and middle-aged wild type and IL-10-deficient mice after i.p. injection of LPS. The hypothesis was that fatigue and motor deficits in older mice and mice challenged with LPS would be exacerbated in the absence of the anti-inflammatory cytokine, IL-10.
Materials and methods

Animals

Male C57BL/6J (WT; IL-10\(^{+/+}\)) and IL-10 knockout B6.129P2-Ii10tm1Cgn (IL-10\(^{-/-}\)) mice were purchased from Jackson Laboratories (Jackson Laboratories, Bar Harbor, Maine) at 2 months of age and maintained under SPF conditions at the University of Illinois to minimize the development of colitis (14). Mice were housed in polypropylene cages and maintained at 23° C under a reverse phase 12h light/dark cycle with ad libitum access to water and rodent chow. So as to test the mice when they are naturally active, all experimental procedures occurred during the dark phase of the light/dark cycle under infrared lighting. Mice were 3-5 or 10-12 months old when studies were conducted. Subjects that exhibited signs of colitis (e.g., perianal ulceration, diarrhea, rectal prolapse, weight loss) were removed from the study (n=2). All procedures were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the University of Illinois Laboratory Animal Care and Use Committee.

Experimental Design

To determine the effect of age, LPS and IL-10 on motor coordination and fatigue, 5- and 11-month old IL-10\(^{+/+}\) and IL-10\(^{-/-}\) mice were injected intraperitoneally (i.p.) with 0.33 mg/kg (~10\(\mu\)g/mouse) *Escherichia coli* LPS (serotype 0127:B8, Sigma) or sterile saline (n=8/group). Mice were tested on the RotaRod treadmill in three consecutive trials at 4 and 24 h post-injection. At the end of the last RotaRod trial at the 24 h time point, mice were allowed to rest for 1 h and then placed on a motorized treadmill and tested for time to fatigue. Immediately after the exhaustive fatigue test, mice were killed by CO\(_2\) asphyxiation, blood was collected by cardiac
puncture, and the brain was quickly dissected to collect the hippocampus, striatum, cortex and cerebellum. Brain tissue was placed in RNAlater and tissue and plasma were stored at -80° C until assaying for peripheral and central proinflammatory cytokines. To determine the effects of LPS on the inflammatory response at a time point corresponding to the behavioral test, a separate study was conducted wherein IL-10+/+ and IL-10−/− mice (n=6/group) were given either LPS or saline and killed 4 h post-injection for collection of blood and brain.

Behavioral Tests

Motor function. A RotaRod treadmill (Med Associates Inc., St Albans, Vermont) was used to assess motor coordination. Mice were placed on a 4 cm diameter horizontal rod positioned 16.5 cm above a cushioned base. Immediately prior to the first test session, mice were allowed a 30 sec acclimation period in which the speed of the RotaRod was held constant at 4 rpm. Mice had to maintain their footing and balance as the rotating drum accelerated. During the test sessions, the speed of rotation was increased by continuous acceleration from 0-40 rpm for 5 min and latency to fall was measured. Animals that reached maximum speed were allowed to stay on for a total of 500 sec and then were removed from the RotaRod. Each test session consisted of 3 trials with a 1 h inter-trial interval.

Exhaustive fatigue. To assess volitional fatigue, mice were placed on a motorized treadmill (Jog-a-Dog®, Ottawa Lake, MI) in individual lanes separated by plastic dividers 24 h after LPS or saline injection. In each test, mice ran for 10 min at 6m/min followed by an 8 min period in which the speed was gradually increased to 18m/min. The final speed corresponded to an intensity of ~80-85% of their maximal oxygen uptake (23). The mice ran until volitional exhaustion which was defined as 10 sec of continual non-running at the back of the treadmill.
lane where a foam sponge was placed. Electrical shock was not used as negative reinforcement
to induce running behavior.

Cytokine Determination

Plasma cytokines. Plasma samples were assayed for IL-1\(\beta\), IL-6, TNF\(\alpha\) and IL-10 using
a multiplex bead-based immunoassay kit combined with a Cytokine Reagent kit as described by
the manufacturer (Bio-Rad, Hercules, CA). The multiplex assay was sensitive to <3pg/ml for
IL-1\(\beta\), IL-6, TNF\(\alpha\) and IL-10. The inter-assay and intra-assay coefficients of variation were
<8%.

Cytokine mRNA in brain. Total RNA was isolated from homogenized brain regions using
the Tri Reagent protocol (Sigma, ST. Louis, MO). A QuantiTect Reverse Transcription Kit
(Qiagen, Valencia, CA) was used for cDNA synthesis with integrated removal of genomic DNA
contamination according to the manufacturer’s protocol. In brief, RNA samples were mixed
with gDNA Wipeout Buffer and RNase-free water and incubated at 42°C for 2 min. Quantiscript
Reverse Transcriptase, Quantiscript RT Buffer, and RT Primer mix were added to samples and
incubated at 42°C for 15 min, followed by incubation at 95°C for 3 min to inactivate
Quantiscript Reverse Transcriptase. Quantitative real time PCR was performed using the
Applied Biosystems (Foster, CA) Assay-on Demand Gene Expression protocol. In short, cDNA
was amplified by PCR where a target cDNA (IL-1\(\beta\), Mm00434228_ml; TNF\(\alpha\), m00443258_ml;
IL-6, Mm00446190_ml) and a reference cDNA (glucose-3 phosphate dehydrogenase,
Mn99999915_gl) were amplified simultaneously using an oligonucleotide probe with a 5’
fluorescent reporter dye (6-FAM) and a 3’ quencher dye (NFQ). PCR reactions were performed
at the following conditions: 50° C for 2 min, 95° C for 10 min, followed by 40 cycles of 95° C
for 15 sec and 60° C for 1 min. Fluorescence was determined on an ABI PRISM 7900HT-sequence detection system (Perkin Elmer, Forest City, CA). Data were analyzed using the comparative threshold cycle (Ct) method as previously described (5), and results are expressed as fold change compared to saline controls.

Data Analysis

All data were analyzed using Statistical Analysis Systems (SAS) General Linear Model procedures. Data were subjected to two-way (genotype x treatment) or three-way (genotype x treatment x age) ANOVA or a repeated-measures ANOVA in which test day was a within-subjects measure (i.e., repeated measure), and LPS (0 μg or 10 μg), genotype (IL-10+/+ or IL-10−/−) and age (adult and middle-aged) were between-subjects measures to determine significant main effects as well as interactions between these main effects. All data are expressed as the means ± SEM.
Results

**Psychomotor deficits in IL-10^{+/+} and IL-10^{-/-} mice after LPS administration**

To determine the effects of age, peripheral immune stimulation and IL-10 deficiency on psychomotor performance and fatigue, 5 and 11-month old IL-10^{+/+} and IL-10^{-/-} mice were treated i.p. with LPS. Although increased brain cytokine production and a more severe sickness behavior syndrome had been reported in older rodents (22-24-month old) injected with LPS (6, 9, 10, 12, 29) contrary to our hypothesis, no effects of age were observed for any measurement. Therefore, data from the two age groups were pooled and analyzed to assess genotype and treatment effects.

Motor coordination was assessed on the RotaRod treadmill 4 and 24 h after injection and the average latency to fall during the three trials conducted at each time point was determined (Figure 1A). ANOVA of latency to fall from the RotaRod revealed main effects of Time (F(1,28)=9.19; *P*=0.003) and Treatment (F(1,28)=13.47; *P*=0.0004) and a Treatment × Genotype (F(1,28)=28.51; *P*<0.0001) interaction. There was a tendency for a Treatment × Time (F(1,28)=2.81; *P*=0.0962) and Treatment × Genotype × Time (F(1,28)=3.27; *P*=0.0731) interaction. Posthoc analysis indicated LPS treatment did not affect performance of IL-10^{+/+} mice on the RotaRod treadmill at 4 h (*P*=0.2643) while IL-10^{-/-} mice performed worse after LPS than did saline treated controls (*P*=0.0069). Moreover, IL-10^{+/+} mice showed improvement in motor coordination 24 h after LPS but motor deficits were still apparent in IL-10^{-/-} mice (*P*<0.0001). Furthermore, only LPS-treated IL-10^{-/-} mice failed to demonstrate motor learning by improving performance over consecutive trials conducted at 4 and 24 h (Figure 1B). Consecutive training on an accelerating Rotarod can be regarded as a valid paradigm for motor skill learning (4). Interestingly, both the saline and LPS treated IL-10^{+/-} mice and the saline
treated IL-10\(^{-/-}\) mice improved performance on the RotaRod, however, the IL-10\(^{+/+}\) mice that received saline seem to exhibit the greatest improvement out of all the treatment groups. These results indicate that the psychomotor disturbances associated with LPS treatment were more pronounced and persistent in IL-10-deficient mice.

**Fatigue in IL-10\(^{+/+}\) and IL-10\(^{-/-}\) mice after peripheral LPS injection**

To determine if IL-10 affected exhaustive fatigue following peripheral immune activation, time to fatigue on a motorized treadmill was determined for IL-10\(^{+/+}\) and IL-10\(^{-/-}\) mice 24 h after LPS injection (Figure 2). ANOVA of time to fatigue revealed a significant main effect of LPS (F(1,28)=14.44; \(P=0.0007\)) as well as an LPS × Genotype (F(1,28)=7.46; \(P=0.0108\)) interaction. Moreover, in IL-10\(^{+/+}\) mice LPS treatment tended to reduce time to fatigue compared to saline treated IL-10\(^{+/+}\) (\(P=0.0950\)), whereas in IL-10\(^{-/-}\) mice time to fatigue was markedly depressed (\(p<0.0001\)). Although not statistically significant, LPS treatment reduced time to fatigue in IL-10\(^{+/+}\) mice by 59%, whereas time to fatigue in IL-10\(^{-/-}\) mice was reduced 94%. The reduced time to fatigue in IL-10\(^{-/-}\) mice after LPS was particularly striking because saline-treated IL-10\(^{-/-}\) mice resisted fatigue. Thus, in the absence of peripheral immune stimulation, IL-10 deficiency seemed to enhance motor function and time to fatigue. But when given LPS to mimic a peripheral infection, the findings indicate that IL-10 deficiency leads to gross motor impairments and fatigue.

**Proinflammatory cytokines in IL-10\(^{+/+}\) and IL-10\(^{-/-}\) mice after peripheral LPS injection**

Plasma IL-1\(\beta\), IL-6 and TNF\(\alpha\) levels were measured in IL-10\(^{+/+}\) and IL-10\(^{-/-}\) mice 4 and 24 h after injection of saline or LPS (Table 1). Furthermore, because the striatum, cerebellum,
and motor cortical regions of the frontal lobe are important for the acquisition and/or retention of skilled motor behaviors (21), proinflammatory cytokine mRNA levels were measured in the cerebellum, cortex, and striatum (Table 1). Proinflammatory cytokine mRNA levels in the hippocampus were also determined because this area is associated with LPS-induced deficits in learning and memory. Plasma and brain proinflammatory cytokine data were subjected to ANOVA and the significance of the main effects (Genotype and Treatment) and interaction of main effects (Genotype × Treatment) at each time point are presented in Table 1.

In IL-10−/− mice plasma IL-10 concentration was below assay sensitivity both in the absence and presence of LPS stimulation. However, IL-10 was detectable in plasma of saline-treated IL-10+/+ mice and levels increased after LPS injection. Treatment with LPS increased plasma IL-1β and IL-6 in IL-10+/+ and IL-10−/− mice 4 h after peripheral immune stimulation compared to saline controls. Plasma TNFα, however, was increased in LPS-treated IL-10−/− mice but not significantly increased in IL-10+/+ mice at 4 h, probably because in wild type mice TNFα levels peak 1-2 h after LPS treatment and return to baseline shortly thereafter (1). At 24 h post injection, cytokine levels were returning to baseline in LPS-treated IL-10+/+ mice, yet the proinflammatory cytokines remained elevated in similarly treated IL-10−/− mice. Thus, the LPS-induced elevation in plasma proinflammatory cytokines was prolonged in the absence of IL-10.

Treatment with LPS increased IL-1β, TNFα, and IL-6 mRNA at 4 h in all brain areas in both the IL-10+/+ and IL-10−/− mice, however, the increase was often greater in IL-10−/− mice. For example, in IL-10−/− mice treated with LPS, mRNA levels for the three proinflammatory cytokines were markedly higher in three of the four brain areas sampled compared to IL-10+/+ mice that received LPS. In LPS-treated mice, IL-1β and TNFα mRNA levels in all brain regions examined returned toward baseline at 24 h but were still higher than saline-treated mice.
irrespective of genotype. Interestingly, IL-6 mRNA level in the four brain regions returned to
baseline at 24 h in LPS-treated IL-10\(^{+/+}\) mice but was still higher in IL-10\(^{-/-}\) mice, especially in
the cerebellum. Taken together, these data indicate that in brain areas important for skilled
motor behaviors, IL-10 mediates the magnitude and duration of the proinflammatory cytokine
response during peripheral immune activation.
Discussion

Anti-inflammatory cytokines play an important role in regulating inflammation both in the periphery and brain (27, 32). Because IL-10 has been reported to be decreased in the brain of aged humans and mice allowing inflammatory cytokines to increase (22, 32), one aim of the present study was to determine if IL-10 deficiency would hasten the onset of age-associated psychomotor deficits. If older IL-10−/− mice performed poorer than younger IL-10−/− mice in fatigue and motor coordination tasks, we thought this strain might be useful for investigating psychomotor aging. Unfortunately, we found no age effect on any measurement taken, perhaps because the groups differed in age by just 6 months. In some instances we saw improved performance in the older IL-10+/− mice when compared to the younger IL-10+/− mice (e.g., fatigue test in saline treated mice). It was difficult to expand the age difference further because of the age-associated risk of IL-10−/− mice developing colitis even while maintained under SPF conditions (14). Clinical signs of chronic inflammatory bowel disease are diarrhea, perianal ulceration, rectal prolapse and intestinal bleeding (3, 14). Indeed, two animals exhibited rectal prolapses and were removed from the study. Thus, the IL-10−/− mice did not prove to be useful for studying age-related psychomotor deficits as we had hoped.

That there were no differences attributed to age does not lessen the importance of the dissimilar responses of IL-10+/+ and IL-10−/− mice to peripheral immune stimulation. In the current study, IL-10−/− mice administered LPS had increased motor deficits and a shorter time to fatigue than similarly treated IL-10+/+ mice. The fatigue and motor deficits in LPS-treated IL-10−/− mice were associated with higher plasma inflammatory cytokine levels and increased expression of inflammatory cytokine genes in brain areas important for skilled motor behaviors. We interpret these findings to suggest that IL-10 can inhibit psychomotor deficits related to
peripheral infections via its anti-inflammatory effects. The results are less clear about the role of
IL-10 in motor performance and fatigue in healthy animals as IL-10-deficient mice treated with
saline seemed to perform better. Nonetheless, these findings are significant because
psychomotor functions used to perform day-to-day tasks that are necessary for independent
living (e.g., stair climbing and walking) are often impaired in chronically infected patients. By
extension, the current findings are also relevant to the geriatric population because the age-
related increase in inflammation is associated with a decrease in anti-inflammatory cytokines
including IL-10 (22, 32). It has been estimated that approximately a third of community-
dwelling elderly people suffer at least one fall each year, and this figure increases with
institutionalized patients (15). In addition, functional and cognitive deterioration entails a greater
risk of falls (24). There is evidence of decreased motor performance by people with cognitive
impairment and dementia when performing an additional cognitive task (26).

In the present study, the RotaRod apparatus tested balance and coordination and motor
learning whereas the motorized treadmill tested time to fatigue. The fact that IL-10\(^{-/-}\) mice
performed poorly compared to IL-10\(^{+/+}\) mice when challenged with LPS is consistent with a
linkage between inflammatory cytokines and loss of motor function. In one study, higher
cytokine levels were associated with lower muscle mass and lower muscle strength (30).
Specifically, IL-6 and TNF\(\alpha\) have been shown to cause a loss in muscle mass and strength (20,
30) and these two proinflammatory cytokines, along with IL-1\(\beta\), were still elevated in peripheral
blood of IL-10\(^{-/-}\) mice 24 h after LPS injection. In a related study, we recently found IL-6 to be
higher after LPS treatment in skeletal and cardiac muscle of IL-10\(^{-/-}\) mice, too (17).

It is also noteworthy that the central expression of inflammatory cytokines was typically
higher in IL-10\(^{-/-}\) mice after LPS treatment. We were particularly interested in the central
cytokine compartment because of its potential role in the pathogenesis of immunologically
mediated fatigue (25). Chronic fatigue is considered to be the seventh most common symptom
in primary healthcare (13). Chronic fatigue syndrome is often diagnosed for patients who
present six months or more of unexplained fatigue with other characteristic symptoms that are
centrally mediated and known to be inducible by inflammatory cytokines including, cognitive
dysfunction (e.g., impaired memory and concentration, depression, irritability), malaise, poor
sleep, and problems maintaining balance. A recent study of patients with postinfective fatigue
syndrome that examined the relationship between fatigue symptoms and serum cytokines and
cytokines secreted in vitro by isolated PBMCs concluded that fatigue was not associated with
altered cytokine production (31). However, this study was unable to account for inflammatory
cytokines within the CNS where feelings of fatigue are likely to originate. Indeed, the authors
suggested an alternative hypothesis for postinfective fatigue syndrome based on inflammatory
mediators that are produced by activated brain glial cells. Supporting a key role for central
inflammatory cytokines in fatigue is a recent study that examined in mice the perception of
exercise-induced fatigue (19). Inhibiting TNFα in the brain but not in the periphery increased
voluntary exercise, suggesting the perception of fatigue was delayed, whereas injecting
recombinant TNFα intracerebroventricularly reduced voluntary exercise, suggesting the
perception of fatigue was enhanced. Thus, inflammatory cytokines produced within the brain
may be at least as important as those produced in the periphery for inducing fatigue, although
from the current study we cannot distinguish effects of peripheral cytokines from central
cytokines. Nonetheless, to the extent that inflammatory cytokines contribute to exhaustive
fatigue and deficits in motor coordination in elderly subjects or subjects with an infection, the
model described here is useful for exploring ways to prevent or treat fatigue and motor complications.

**Perspectives and Significance**

Fatigue and motor deficits in IL-10-deficient mice given LPS to mimic a peripheral infection were associated with higher plasma inflammatory cytokine levels and increased expression of inflammatory cytokine genes in brain areas important for skilled motor behaviors. These findings are interpreted to suggest that IL-10 can inhibit psychomotor deficits related to peripheral infections via its anti-inflammatory effects. These findings are significant because psychomotor functions used to perform day-to-day tasks that are necessary for independent living are often impaired in chronically infected patients. Thus, strategies to mitigate inflammatory cytokines in either the periphery or brain may be useful for preventing deficits in psychomotor behavior.

**Disclosure statement**

The authors declared no actual or potential competing interests. The experimental procedures involving animals were consistent with PHS guidelines and approved by the campus IACUC.

**Acknowledgements**

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**Figure legend**

**Figure 1A.** RotaRod performance of IL-10<sup>+/+</sup> and IL-10<sup>-/-</sup> mice 4 and 24 h after peripheral injection of saline (open bar) or LPS (black bar). Average latency to fall from the RotaRod was determined for each subject in three trials conducted 4 h postinjection and another three trials conducted 24 h postinjection. Data are presented as the mean ± SEM. Means with different letters are different (*p*<0.05).

**Figure 1B.** Motor learning in IL-10<sup>+/+</sup> and IL-10<sup>-/-</sup> mice 4 and 24 h after peripheral injection of saline (gray line) or LPS (black line). Motor learning was conferred if mice exhibited improved performance over consecutive trials. Data are presented as the mean ± SEM. An asterisk (*) indicates the LPS treatment group was different from the saline treatment group (*p*<0.05).

**Figure 2.** Exhaustive fatigue in IL-10<sup>+/+</sup> and IL-10<sup>-/-</sup> mice 24 h after treatment with saline (open bar) or LPS (black bar). To determine time to fatigue (min) mice ran on a motorized treadmill until volitional exhaustion. Data are presented as the mean ± SEM.
Figure 1

1A. Latency to Fall (Sec) by Genotype and Treatment

- IL-10+/+
- IL-10-/-
- LPS
- Saline

1B. Latency to Fall (Sec) by Trial and Treatment

- IL-10+/+
- IL-10-/-
- LPS
- Saline

Significance: * indicates a significant difference compared to the control group.
Figure 2

![Graph showing Time to Fatigue (min) for IL-10+/+ and IL-10-/- groups. The graph compares LPS and Saline conditions. The IL-10+/+ group shows a 59% difference, while the IL-10-/- group shows a 94% difference.](image-url)
Table 1. Proinflammatory cytokine mRNA and protein in brain and plasma, respectively, of IL-10+/+ and IL-10−/− mice 4 and 24 h after peripheral injection of saline or LPS. Data are presented as the mean ± SEM. Means within a row with different letter superscripts are different (P<0.05).

<table>
<thead>
<tr>
<th></th>
<th>4 Hour</th>
<th>24 Hour</th>
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<tr>
<td></td>
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<td><strong>Cerebellum cytokine mRNA, fold increase</strong></td>
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<td>TNFα</td>
<td>1.65a±0.40</td>
<td>19.36b±2.90</td>
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**SEM. Means**