

Differential behavioral, physiological, and hormonal sensitivity to LPS challenge in rats

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Abstract: In animals, systemic administration of lipopolysaccharide (LPS) induces a characteristic set of responses that has been termed sickness behavior. In the present study, the effects of a wide dose range of LPS injections on behavior and physiology were examined in rats. Male rats were injected IP with either saline or LPS (1, 5, 15, 50, 125, or 250 µg/kg). Body temperature, body weight, and home-cage activity were monitored over 24 h after injection. Behaviorally the social interaction and the saccharin preference tests were assessed at 2 and 24 h post-injection, respectively. Further animals were treated with LPS to assess hypothalamic–pituitary adrenal (HPA) axis and immune system responses 2 h post-injection. LPS significantly reduced body weight (1 µg/kg and above), increased ACTH (15 µg/kg and above) and serum corticosterone levels (5 µg/kg and above). In addition LPS raised serum interleukins (5 µg/kg and above) and tumor necrosis factor-alpha (1 µg/kg and above). Social behavior and preference for saccharin were significantly decreased at all doses of LPS tested (1–250 µg/kg) while a robust reduction in home-cage activity was observed starting at 15 µg/kg. The present finding suggests that LPS caused profound behavioral alterations and stimulation of the immune system even at very low doses.

Keywords: sickness behavior, lipopolysaccharide, cytokines, ACTH, corticosterone, social interaction

Introduction

Clinical depression is a complex phenomenon reflecting many subtypes and different aetiologies. Beyond the recognized role of monoaminergic systems in the pathophysiology of depression, recent studies have provided a strong link between depression and inflammatory phenomena.^{1–5} Indeed, it has been hypothesized that depressed patients may be suffering from a dysregulation of the immune system with increased production of pro-inflammatory cytokines, such as interleukin-1 β (IL-1 β), IL-6 and tumor necrosis factor-alpha (TNF- α).^{6–9} Moreover, administration of cytokines is well known to induce unfavorable symptoms which have many features in common with major depression.¹⁰ Patients receiving interferon-alpha administration for the treatment of hepatitis C frequently develop major depression symptoms, which can be prevented or treated with antidepressant drugs.^{11,12} Similarly, systemic administration to healthy subjects of the cytokine inducer lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, causes a marked increase in the circulating cytokines (eg, IL-1 β , IL-6, and TNF- α), a concomitant elevation in serum cortisol levels and a significant elevation in negative emotions, including sadness, anxiety, and depressed mood.^{13–18} Accordingly, systemic LPS injection to animals causes effects comparable to those found in humans. In particular, the acute administration of LPS to rats induces depressive-like symptoms known as “sickness behavior” which includes loss of interest or pleasure (anhedonia), impaired cognitive function, retardation of motor activity, reduced interest in exploration, and decrease in social activities.^{19–22} However, results

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obtained so far in animal studies are difficult to interpret, since the “depressive-like” components induced by LPS challenge may overlap with more general psychomotor disturbances. Investigations to date have been limited to doses and time points at which LPS produced a maximal effect on neuroendocrine, inflammatory, and behavioral parameters. The present study was designed to investigate the effects of LPS, from low to high doses, on the behavioral, physiological and neuroendocrine responses in the rat. In particular, it was considered important to explore to what extent a wide dose range of LPS would modulate psychological stress in an attempt to dissect specific behavioral components from less specific psychomotor symptoms.

Materials and methods

Animals

Adult male Sprague Dawley rats (Charles River, Calco, Italy), weighing between 280 and 360 g at the start of testing, were used in these experiments. Singly housed animals were acclimatised for one week in a room controlled for temperature (22 ± 1 °C), humidity (60%), and lighting (12 h light–dark cycle; lights on 0600 h). Food and water were provided ad libitum throughout the experiment. All experiments were performed in accordance with European Community ethical regulations on the care of animals for scientific research (CEE Council 86/609 Italian D.L. 27/01/92 no. 116) and were fully compliant with GlaxoSmithKline ethical standards.

Experimental procedure

The study herein reported consisted of six different cohorts of animals, all of which received the same LPS treatment

(Figure 1). All cohorts of animals received an intraperitoneal (IP) injection of LPS ranging from 1 to 250 µg/kg.

The first two cohorts of animals were used to measure home cage activity and social interaction. Body weight and body temperature were also measured. Eight to 10 rats/group/cohort were used. Evaluation of hormone and cytokine peripheral levels were carried out in other three animal cohorts to avoid confounding effects on these biochemical parameters due to manipulation of the animals ($n = 5$ to 8 rats/group/cohort). An additional set of animals ($n = 9$ to 10 rats/group) was used to carry out the saccharin preference test.

Challenge

LPS derived from *Escherichia coli* O111:B4 serotype was purchased from Sigma-Aldrich (Milan, Italy) and stored at 4 °C until use. The LPS solutions were freshly prepared in sterile pyrogen-free saline and administered IP at the doses of 1, 5, 15, 50, 125, or 250 µg/kg (2 mL/kg body weight). Control animals received IP saline injections (2 mL/kg body weight). Treatments were conducted during the light phase of a 12 h light-dark cycle, between 0800–1000 h, except for animals undergoing the saccharin preference test which received injections 3–4 h prior light-off, between 1400–1500 h.

Behavioral tests

Social interaction

The test arena consisted of a rectangular black perspex box (54 cm × 36 cm) with walls 33 cm high, and a white floor divided in 9 cm squares (4 × 6) for the line crossing read out. Three walls were black and one transparent in order to assist video recording. The day before the test, rats

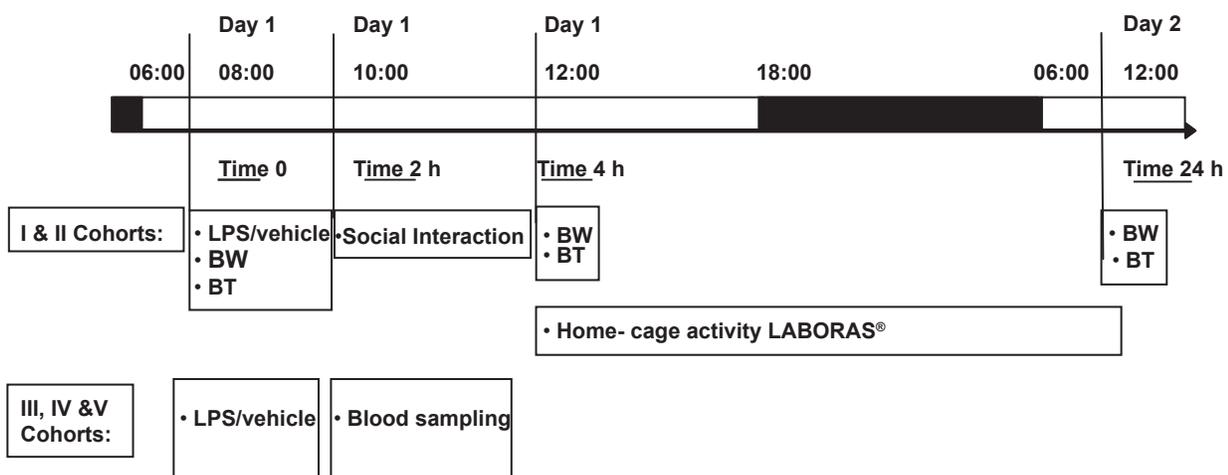


Figure 1 Description of the experimental procedure used in the current study. **Abbreviations:** BW, body weight; BT, body temperature.

(cohorts I and II; Figure 1) were placed individually in the arena for a 5 min familiarization period. Two hours after LPS or saline injection, a social interaction test was performed by placing treated pairs of rats ($n = 8$ to 10 rats/group/cohort), matched for body weight and unfamiliar to each other, into the test arena and their behavior was video-recorded for 15 min. All experiments were carried out in dim light (2 Lux) between 1000–1200 h. At the end of the social interaction test, each animal was returned to its home-cage. The Observer[®] (Noldus, Wageningen, The Netherlands) software for collection and analysis of observational data was used for scoring duration of social interaction (grooming, following and sniffing of the partner body) and frequency of line crossing (number of lines crossed on the base of arena) by an experienced observer blind to experimental groups.

Home cage activity (LABORAS[®])

The spontaneous home-cage activity was monitored with the automated individual cage system LABORAS[®] (Laboratory Animal Behavior Observation, Registration and Analysis System, Metris, Hoofddorp, Netherlands). One hour after the social interaction test (3 h post-LPS injection) each rat (cohorts I and II; Figure 1) was transferred from its home cage and placed individually with its own bedding in a Makrolon type III cage (840 cm²) and behavior was recorded during a 24 h period. Drinking water and food pellets were provided ad libitum for the entire period of the test session. The cumulative time spent (seconds) on each of the following behaviors was assessed automatically: locomotion (activities such as walking and running), immobility (absence of movements while the animal is in a lying position), rearing (the animal standing upright on its hind legs mostly against the wall), grooming (shaking, scratching, or licking). The total distance was also assessed and expressed as total meters traveled by the animal over the time point examined.²³

Saccharin preference test

This experiment was designed to assess the effect of LPS on the preference for a palatable solution using a two-bottle paradigm. Rats were allowed for 24 h to freely choose between a bottle of water and a bottle containing a saccharin solution (0.1% w/v; Sigma-Aldrich) in their home cage with water and food ad libitum. Bottle positions were switched daily with a variable sequence in order to avoid any place preference. For each animal, the total fluid consumption (grams) was measured by weighing bottles before and after each test session (24 h period time) and defined as the sum of water and saccharin intake, whereas the saccharin preference score was

expressed as the percentage of saccharin intake ($\% = \text{saccharin intake} \times 100 / \text{total fluid intake}$). As baseline value for each rat, the total fluid consumption and saccharin preference score were measured over the last 24 h of adaptation. On the day of the test, two pre-weighed bottles, one containing drinking water and another containing saccharin solution, were presented to each rat immediately after the IP injection of either saline or LPS (1–250 $\mu\text{g}/\text{kg}$). The bottles were weighed again 24 h after LPS challenge (1400–1500 h) and the weight difference was considered as the intake from each bottle. The timing of LPS treatment was selected based on earlier studies conducted by Shen and colleagues.²¹

Physiological parameters

Body temperature

Four days prior to the test session, rats (cohorts I and II; Figure 1) were implanted subcutaneously with microchip transponders (IPTT-200; BioMedic Data System, Inc., Seaford, DE, USA), for body temperature recording. Measurements of body temperature were obtained from freely moving animals using a DAS-5007 Pocket Scanner System (BioMedic Data System, Inc.).

To obtain baseline measurements each rat was monitored the day before the test session 0800–1000 h. On the test day, temperature recording occurred immediately before ($T = 0$) as well as at two ($T = 2$), four ($T = 4$), and 24 h ($T = 24$) after injection of either LPS or saline.

Body weight

On the test day, the body weight (BW) of each animal (cohorts I and II; Figure 1) was measured immediately before ($T = 0$) as well as at four ($T = 4$) and 24 h ($T = 24$) after injection of either LPS or saline.

Hormone and cytokine levels

Blood sampling

Rat cohorts III to V (Figure 1) were sacrificed by guillotine decapitation 2 h after LPS administration for blood sample collection. For plasma preparation, trunk blood was collected into 1 ml K3 EDTA tubes (BD Biosciences, Erembodegen, Belgium), centrifuged 10 min at 3000 g at 4 °C and stored at –80 °C until the day of the experiment. Serum was prepared by collecting trunk blood in Microtainer tubes (BD Biosciences), letting the blood clot for 30 min at room temperature and centrifuging at 3000 g for 15 min at 4 °C. A protease inhibitor cocktail (Protease Inhibitor Mix, Amersham Biosciences, Uppsala, Sweden) was added to supernatants and aliquots were stored at –80 °C until measurement.

Plasma ACTH and serum corticosterone levels

Adrenocorticotrophic hormone (ACTH) levels were measured in plasma with an IRMA kit (Nichols Institute Diagnostics, San Clemente, CA, USA). Corticosterone levels were measured in serum, using a RIA kit (RIA Immuchem™ Double antibody ¹²⁵I RIA kit, MP Biomedicals, Irvine, CA, USA). Experiments were carried out following the manufacturers' protocol.

Cytokine serum levels

IL-1 β , IL-6, IL-10, and TNF- α levels were measured in serum with a Luminex instrument, using a multiplex suspension array technology (Bio-Rad, Hercules, CA, USA). In this assay, each specific primary antibody recognizing a cytokine is conjugated to a distinct type of fluorescent bead. All antibody-bearing beads are added simultaneously to the sample, allowing the formation of immunocomplexes. The different immunocomplexes are detected by binding to specific biotinylated antibodies and then to streptavidin-phycoeritrin. The quantification is carried out by the Luminex instrument by identifying the kind of bead, which is specific for each analyte and by evaluating the fluorescence intensity, which is related to the concentration of the analyte in solution. A comparison with a standard curve is carried out to obtain concentration values. The assays were carried out using a Bio-plex instrument (Bio-Rad) and all kits and reagents were obtained from Bio-Rad. Briefly, after rinsing wells with assay buffer, antibody conjugated beads were loaded onto the kit plates. Rat serum samples were diluted 1:4 with rat serum sample diluent and 50 μ L were loaded on the kit plates in duplicate. A standard cytokine lyophilised mix was dissolved in rat serum standard diluent and plates were loaded with 50 μ L/well. After 30 min incubation at room temperature under agitation in the dark, samples were washed three times by filtration. Incubation with biotinylated secondary antibody and phycoeritrin-conjugated streptavidin were carried out following the same scheme. Fluorescence values were collected and analysed with the Bio-Plex Manager program (detection limits: IL-1 β 7.8 pg/mL; IL-6 31.25 pg/mL; IL-10 7.8 pg/mL, and TNF- α 7.8 pg/mL).

Statistical analysis

Statistical analyses were conducted using either Statistica V6.1 (Statsoft, Inc. Tulsa, OK, USA) or SAS® (SAS Institute Inc., Cary, NC, USA).

The data from the separate cohorts of animals including the social interaction, home cage activity and the biochemical read-out, were combined into one dataset and analyzed by one-way ANOVA with cohort as a blocking factor, followed by planned comparisons on the predicted means.

Because the body weight, body temperature and the saccharin preference test were measured repeatedly over time, a repeated measures (treatment \times time) approach was used, with the baseline measurements (T = 0) also included as a covariate in the analysis (ANCOVA approach), followed by planned comparison on the predicted means.

Results from all studies were expressed as the predicted mean \pm standard error of the mean (SEM). We use predicted means rather than the more usual observed means as the predicted means take into account differences between the cohorts and, where covariates were used, baseline values. In all studies, levels of statistical significance were set at $p < 0.05$.

Results

Social interaction test

An overall significant decrease in the time spent in social interaction occurred following LPS treatment compared to vehicle treated animals, $F(6,97) = 4.355$, $p < 0.001$; Figure 2A. LPS administration provoked significant changes ($p < 0.05 - p < 0.001$) in the social interaction from 1 to 125 μ g/kg. Furthermore, LPS caused a significant reduction in the locomotor activity, $F(6,97) = 7.017$, $p < 0.001$; Figure 2B, as assessed by reduction in the line-crossing frequency at all doses tested ($p < 0.05 - p < 0.001$) with the exception of 1 μ g/kg LPS.

Home-cage activity

Home-cage activity parameters were altered by treatment with LPS over a 24 h period time compared to control animals (Table 1). LPS treatment did not affect home-cage activity at any of lower doses tested (1 μ g/kg to 5 μ g/kg) whereas it induced a significant decrease in locomotor activity, expressed both in terms of duration, $F(5,49) = 3.774$, $p < 0.01$, and distance travelled, $F(5,49) = 4.307$, $p < 0.01$, with effective doses of 15, 50, and 125 μ g/kg ($p < 0.05 - p < 0.001$; Table 1). Rearing behavior was significantly decreased by LPS, $F(5,49) = 4.972$, $p < 0.001$, particularly this decrease was observed at doses of 15 μ g/kg and above ($p < 0.01 - p < 0.001$). Accordingly, immobility was significantly increased following LPS treatment $F(5,49) = 4.864$, $p < 0.01$, at the dose of 15 μ g/kg and above ($p < 0.05 - p < 0.01$). In addition, a marked decrease in grooming duration was detected in animals treated with the highest LPS doses 50 and 125 μ g/kg, $F(5,49) = 3.966$, $p < 0.01$, with either doses being effective ($p < 0.01$ for each).

Saccharin preference test

In the baseline session when given a free choice between tap water and saccharin solution all rats displayed a

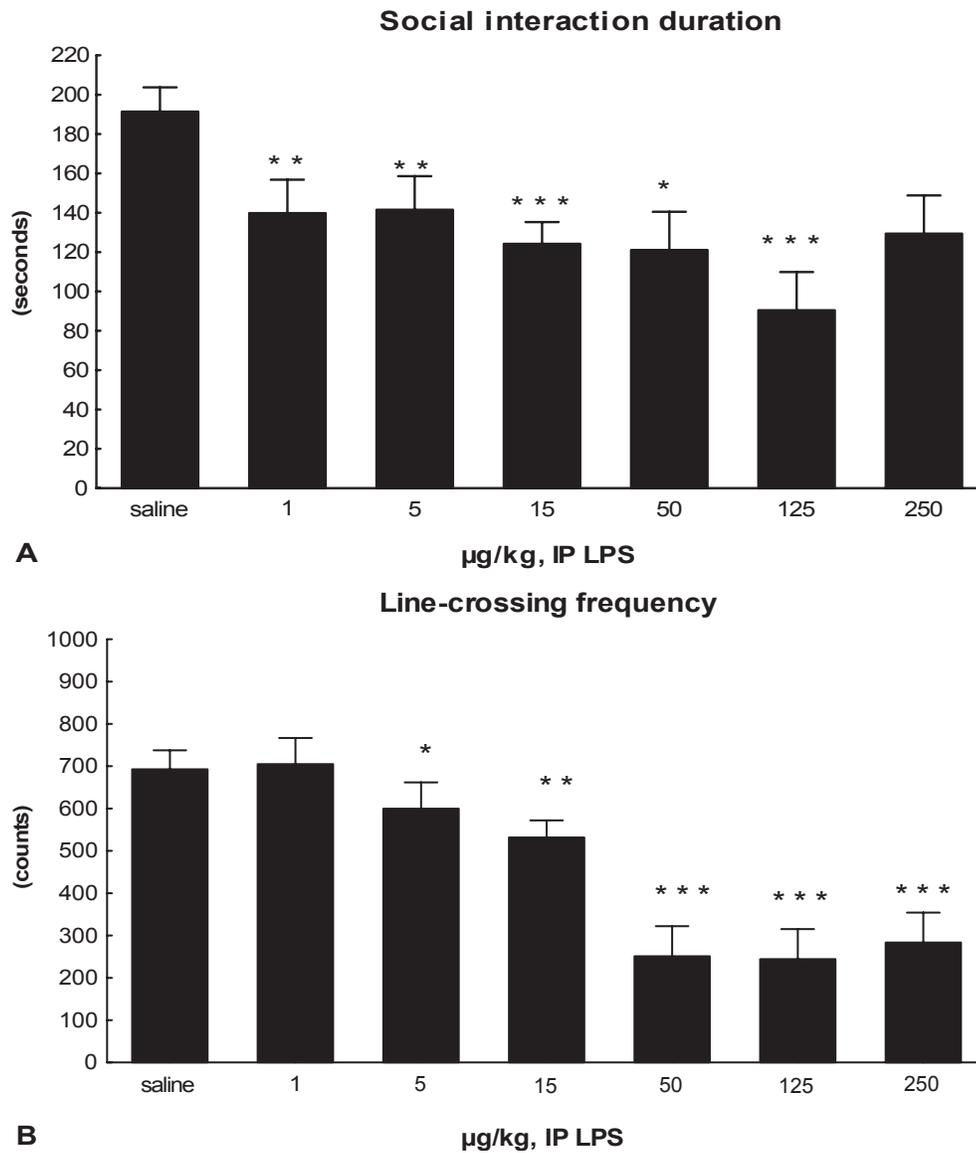


Figure 2 Effects of lipopolysaccharide (LPS) in the rat social interaction test: social interaction duration (**A**) and line-crossing frequency (**B**) LPS was injected IP 2 h before the test session.

Notes: Predicted means \pm SEM are shown ($n = 8$ to 10 rats /group/cohort). Asterisks represent significant differences detected by planned comparisons between individual treatment groups and vehicle group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 1 Effect of lipopolysaccharide (LPS) on home-cage parameters over 24 h after the injection

Home-cage activity parameters					
Group	Locomotion (s)	Immobility (s)	Rearing (s)	Grooming (s)	Total distance (m)
saline	576 \pm 49	53230 \pm 1220	4991 \pm 343	12024 \pm 621	51 \pm 4.2
LPS 1 μ g/kg	536 \pm 80	56148 \pm 2011	4228 \pm 565	10503 \pm 1023	44 \pm 6.8
LPS 5 μ g/kg	554 \pm 80	57397 \pm 2011	4439 \pm 565	10241 \pm 1023	44 \pm 6.8
LPS 15 μ g/kg	351 \pm 80*	61136 \pm 2011*	2994 \pm 565**	9198 \pm 1023	28 \pm 6.8*
LPS 50 μ g/kg	308 \pm 77***	59178 \pm 1939**	2772 \pm 544***	8813 \pm 987**	26 \pm 6.6***
LPS 125 μ g/kg	286 \pm 77***	61257 \pm 1939**	2691 \pm 544***	7598 \pm 987**	24 \pm 6.6***

Notes: Predicted means \pm SEM are shown. Asterisks represent significant differences detected by planned comparisons between individual treatment groups and vehicle group; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ ($n = 8-10$ /group/cohort). Data from 250 μ g/kg LPS group were lost.

preference score for saccharin with mean value of $94\% \pm 0.64$. LPS caused a significant reduction, in saccharin preference at 24 h following challenge $F(6,61) = 4.211, p < 0.01$, with all doses being effective ($p < 0.05 - p < 0.001$; Figure 3A). Water intake was significantly affected by the

LPS treatment $F(6,61) = 3.357, p < 0.01$, being markedly increased at doses of 5 and 15 LPS $\mu\text{g}/\text{kg}$ ($p < 0.05 - p < 0.001$; Figure 3B). Total fluid consumption relative to saline condition was also reduced at all doses of LPS tested $F(6,61) = 6.685, p < 0.001$; Figure 3C.

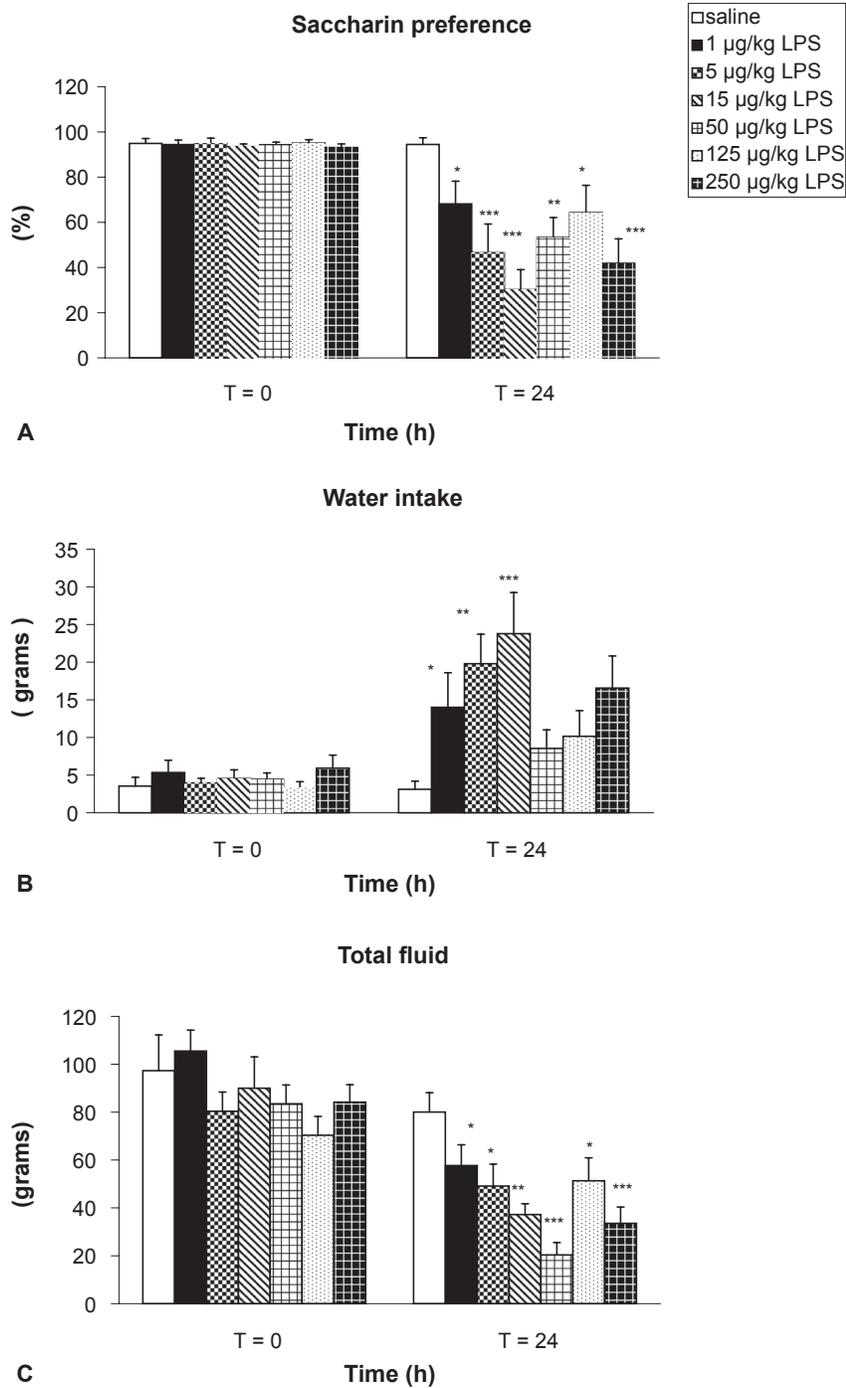


Figure 3 Effects of lipopolysaccharide (LPS) in the saccharin preference. (A) water intake (B) and total fluid intake (C). LPS was injected 3–4 h before lights off (between 1400–1500 h) with the test carried out at 24 h following endotoxin challenge.

Notes: Animals were not fluid-deprived for the entire experiments. Means \pm SEM are shown ($n = 9$ to 10 rats/group). The measurements were analyzed using an ANCOVA approach with baseline values ($T = 0$) included as covariate. Asterisks represent significant differences detected by planned comparisons on the predicted between individual treatment groups and saline group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Body weight

Baseline body weights were fitted as covariate to remove some of pre-treatment animal-to-animal variability which could have contributed to a loss of statistical power in capturing the LPS-related effects on body weight. Consequently, an overall effect of treatment, $F(6,78) = 9.40$, $p < 0.001$, and a considerable interaction between the treatment and time, $F(6,75) = 2.97$, $p < 0.01$, were observed. At both 1 $\mu\text{g}/\text{kg}$ and 15 $\mu\text{g}/\text{kg}$ LPS significantly ($p < 0.05$ and $p < 0.01$, respectively; Table 2), reduced BW 4 h after the injection, but not 24 h after the injection. LPS at doses of 50, 125, or 250 $\mu\text{g}/\text{kg}$ significantly decreased ($p < 0.001$ for each) BW at both 4 and 24 h following injection.

Body temperature

The body temperature of rats at pre-injection time was taken as baseline level and the mean value was 36.89 ± 0.1 °C. The analysis of covariance revealed that there was no evidence of an overall effect of treatment, $F(6,83) = 0.6$, $p = 0.73$, but an effect of time $F(2,85) = 33.38$, $p < 0.001$, and a considerable difference for the main effect of treatment X time, $F(12,125) = 3.07$, $p < 0.001$ were detected. Planned comparison analysis showed that the highest dose of 250 $\mu\text{g}/\text{kg}$ LPS produced a significant decrease on thermal response at 2 h post injection when compared to saline treated rats ($p < 0.01$; Figure 4). Doses of 5, 15, 125, and 250 $\mu\text{g}/\text{kg}$ LPS showed little changes in the body temperature in the 4 h following challenge. At the last time point examined of 24 h, only the dose of 125 $\mu\text{g}/\text{kg}$ LPS induced a marked hyperthermia reaching statistical significance when compared to saline-treated rats ($p < 0.001$; Figure 4).

Table 2 Effect of lipopolysaccharide (LPS) on body weight

Body weight (g)		
Group	4 h	24 h
saline	294 \pm 1.0	295 \pm 2.6
LPS 1 $\mu\text{g}/\text{kg}$	290 \pm 1.6*	293 \pm 3.8
LPS 5 $\mu\text{g}/\text{kg}$	291 \pm 1.6	291 \pm 3.8
LPS 15 $\mu\text{g}/\text{kg}$	289 \pm 1.0**	289 \pm 2.4
LPS 50 $\mu\text{g}/\text{kg}$	285 \pm 1.8***	277 \pm 3.4***
LPS 125 $\mu\text{g}/\text{kg}$	284 \pm 1.8***	271 \pm 3.4***
LPS 250 $\mu\text{g}/\text{kg}$	283 \pm 1.4***	280 \pm 3.4***

Notes: Measurements were taken immediately before ($T = 0$) and at 4 and 24 h after the injection. The results presented in this table are based on predicted means \pm SEM from the analysis (8–10/group/cohort). Asterisks represent significant differences detected by planned comparisons between individual treatment groups and vehicle group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Plasma ACTH and serum corticosterone levels

Injection of LPS caused a significant increase in plasma ACTH levels within 2 h of injection, $F(6,56) = 4.509$, $p < 0.001$, and this effect was particularly evident at doses from 15 to 250 $\mu\text{g}/\text{kg}$ ($p < 0.05 - p < 0.001$, Figure 5B).

Serum corticosterone levels were also altered following LPS challenge, $F(6,56) = 7.792$, $p < 0.001$, being significantly affected at doses from 5 to 250 $\mu\text{g}/\text{kg}$ ($p < 0.01 - p < 0.001$; Figure 5A).

Cytokine serum levels

There was an overall effect of LPS treatment in the IL-1 β , IL-6 and IL-10 cytokine levels in rat serum 2 h after LPS injection, $F(6,55) = 14.243$, $p < 0.001$; $F(6,55) = 16.411$, $p < 0.001$; $F(6,55) = 15.943$, $p < 0.001$, respectively. IL-1 β and IL-6 levels were significantly increased starting at the 5 $\mu\text{g}/\text{kg}$ LPS treatment, ($p < 0.001$, Figures 6A and 6B) and IL-10 by the 1 $\mu\text{g}/\text{kg}$ LPS treatment ($p < 0.01 - p < 0.001$, Figure 6C). TNF- α levels also showed a very large increase after LPS challenge, $F(6,49) = 32.975$, $p < 0.001$, which was statistically significant at all doses ($p < 0.01 - p < 0.001$, Figure 6D).

Discussion

The present investigation demonstrated that systemic administration of LPS elicited profound effects on rat behavior, physiology, circulating stress hormones and cytokines, revealing differential reactivity thresholds for these parameters.

LPS caused a reduction in social interaction at all doses tested. LPS also impaired locomotor activity at all doses tested except 1 $\mu\text{g}/\text{kg}$, indicating that the reduction in social interaction at doses above 1 $\mu\text{g}/\text{kg}$ could be at least partly ascribed to a general LPS induced decrease of activity. Therefore the decrease in social behavior could be considered independent from sedative-like effects of the endotoxin treatment at 1 $\mu\text{g}/\text{kg}$ LPS. Previous studies have shown that peripheral administration of LPS severely depresses social exploration and locomotor activity in the rat.^{24–26} Our results extend these findings and suggest that the decrease in social interaction can be specifically targeted by LPS challenge, and that this decrease may be independent of a general impairment of motor behavior. In addition, a persistent reduction in general activity was also revealed in home-cage conditions. LPS doses of 15 $\mu\text{g}/\text{kg}$ and above resulted in a decrease of locomotion, rearing, grooming and mobility. Furthermore, LPS (doses of 15 $\mu\text{g}/\text{kg}$ and above) produced a marked reduction in body weight, detectable at the first time

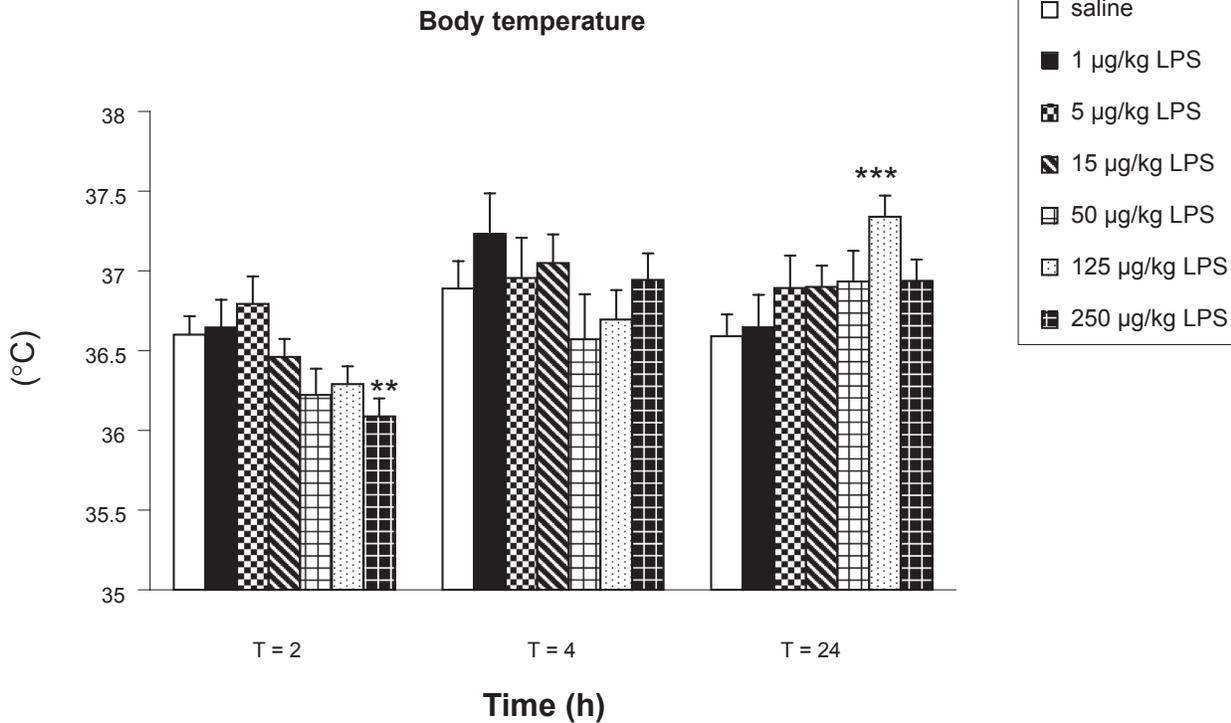


Figure 4 Effects of lipopolysaccharide (LPS) on body temperature at 24 h post-challenge: the measurements were analyzed using an ANCOVA approach with baseline values (T = 0) included as covariate.

Notes: The results presented in this figure are based on the predicted means \pm SEM (n = 6 to 10/group/cohort) from the analysis. Asterisks represent significant differences detected by planned comparisons between individual treatment groups and saline group. ** $p < 0.01$; *** $p < 0.001$.

point of 4 h. However this effect was maintained 24 h after LPS injection only for the highest doses tested, whereas a complete recover was observed in animals treated with lower endotoxin doses. We did not observe any mortality during our experimental procedure and additional studies conducted in our laboratory confirmed a full recovery of rats treated at 250 $\mu\text{g}/\text{kg}$ LPS after 72 h (data not shown). This finding can be explained considering that rats exhibiting sickness behavior may not have the energy to eat and drink during the recovery period as control animals as well as animals exposed to a lesser challenge of LPS. This effect is in part in agreement with previous reports, showing that relatively high doses of LPS induced weight loss assessed at the same time points as those observed in the current study.²⁷⁻³²

LPS did not produce a reliable body temperature response. In particular, a hypothermic effect was observed at the earlier time point of 2 h for the 250 $\mu\text{g}/\text{kg}$ LPS dose, whereas the sole hyperthermic effect was displayed at 24 h after 125 $\mu\text{g}/\text{kg}$ LPS challenge. These findings confirmed the result obtained in previous studies conducted inside our laboratory and directed towards investigating the effect of LPS on BT. We repeatedly observed the same phenomenon: a trend toward either hypothermic or hyperthermic effects that was

depending on the dose and timing without reaching a robust or significant effect. The inconsistencies between the results on LPS thermal response in our experiments and literature data could be explained by one or more of the following factors: the serotype and/or doses of LPS, the time point as well as body temperature parameter assessment (ie, rectal versus core versus subcutaneous).³³⁻³⁶ For instance, these researchers have discussed the validity of the observed effects of LPS on body temperature, suggesting that it may be extremely variable and time dependent when assessed using a rectal temperature probe. It is also worth noting that a continuous monitoring of the body temperature was not feasible since repeated handling could influence the behavioral/physiological parameters assessed in the present studies.

Nevertheless, the hypothermia observed in the present study at the highest dose of LPS, may be considered as an adaptive thermoregulatory or a survival response to a systemic inflammation.

Findings from our study have shown that LPS produced a reliable decrease in both the total fluid intake and the preference for saccharin in comparison to saline-injected animals over a 24 h time point. However at doses of 1–15 $\mu\text{g}/\text{kg}$, LPS significantly increased water consumption, suggesting

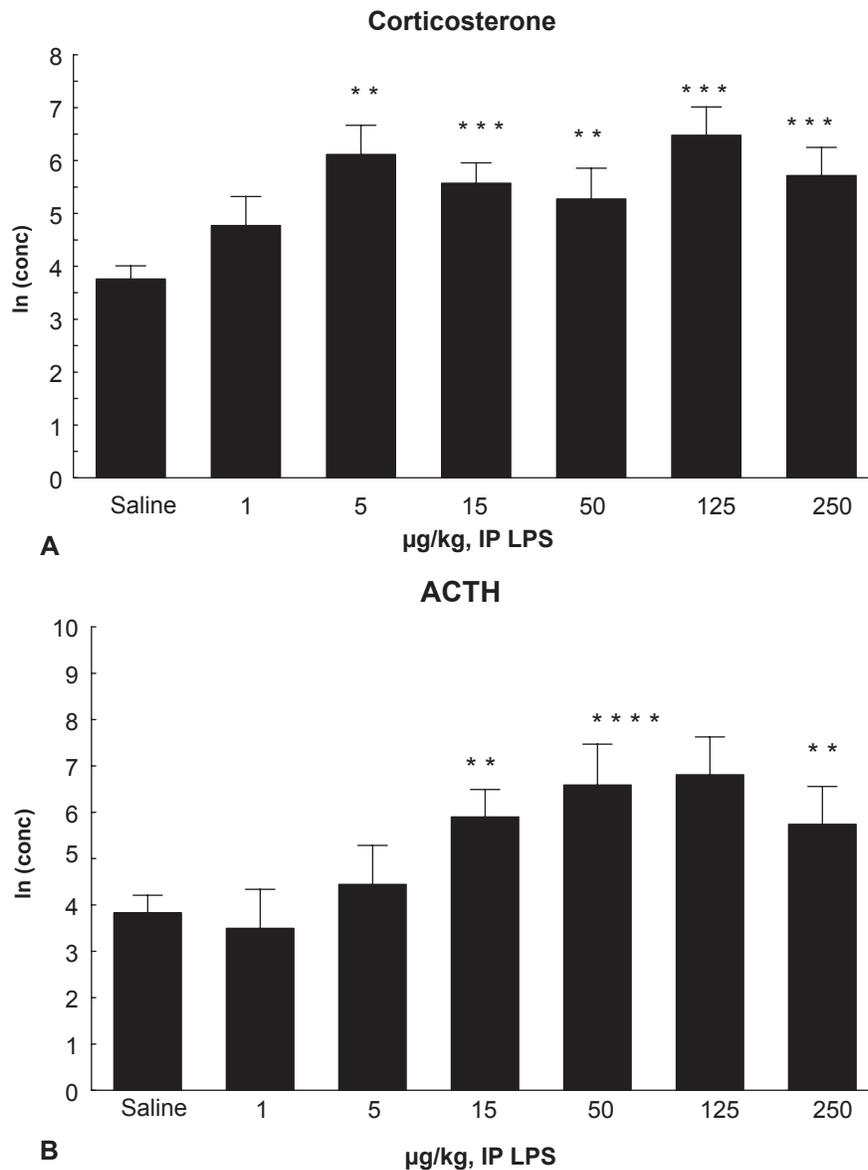


Figure 5 Corticosterone serum levels (A) and adrenocorticotrophic hormone (ACTH) plasma levels (B) 2 h following lipopolysaccharide (LPS) challenge.

Notes: Hormone levels are expressed as natural logarithm (ln) of the concentration (ng/mL and pg/mL, respectively) ($n = 5$ to 8 /group/cohort). Mean serum concentration levels for corticosterone: saline = 79 ± 18 ; $1 \mu\text{g/kg}$ LPS = 77 ± 26 ; $5 \mu\text{g/kg}$ LPS = 285 ± 74 ; $15 \mu\text{g/kg}$ LPS = 386 ± 94 ; $50 \mu\text{g/kg}$ LPS = 533 ± 143 ; $125 \mu\text{g/kg}$ LPS = 914 ± 104 ; $250 \mu\text{g/kg}$ LPS = 658 ± 145 . Mean plasma concentration levels for ACTH: saline = 316 ± 169 ; $1 \mu\text{g/kg}$ LPS = 469 ± 443 ; $5 \mu\text{g/kg}$ LPS = 647 ± 553 ; $15 \mu\text{g/kg}$ LPS = 1092 ± 330 ; $50 \mu\text{g/kg}$ LPS = 619 ± 158 ; $125 \mu\text{g/kg}$ LPS = 975 ± 3 ; $250 \mu\text{g/kg}$ LPS = 665 ± 157 . Asterisks represent significant differences detected by planned comparisons performed between individual treatment groups and vehicle group. ** $p < 0.01$; *** $p < 0.001$.

a specific avoidance of the palatable solution. The present results are in part consistent with previous research which has reported profound decreases in the intake of palatable substances, reduction in the total fluid consumption and no alteration in water intake following high dose of LPS challenge: these effects have been proposed to reflect a lower capacity of LPS-treated rats to experience pleasure and being regarded as anhedonic state.^{21,26} As discussed above, in the present study low doses of LPS (1 – $5 \mu\text{g/kg}$) do affect neither locomotion nor the body weight across a 24 h period

following endotoxin challenge. Thus, the reduced preference for saccharin showed by LPS at low doses may be specific to the effects of LPS on reward rather than being the result of a general behavioral inhibition on fluid intake. The aversion to palatable solution observed at higher doses of LPS ($15 \mu\text{g/kg}$ and above) may be difficult to explain due to the fact that the anhedonic component is not distinguishable from major side effects including an overall inhibitory effect of LPS on thirst (adipsia), psychomotor deficit as well as physical debilitation (ie, impaired locomotion and weight loss).

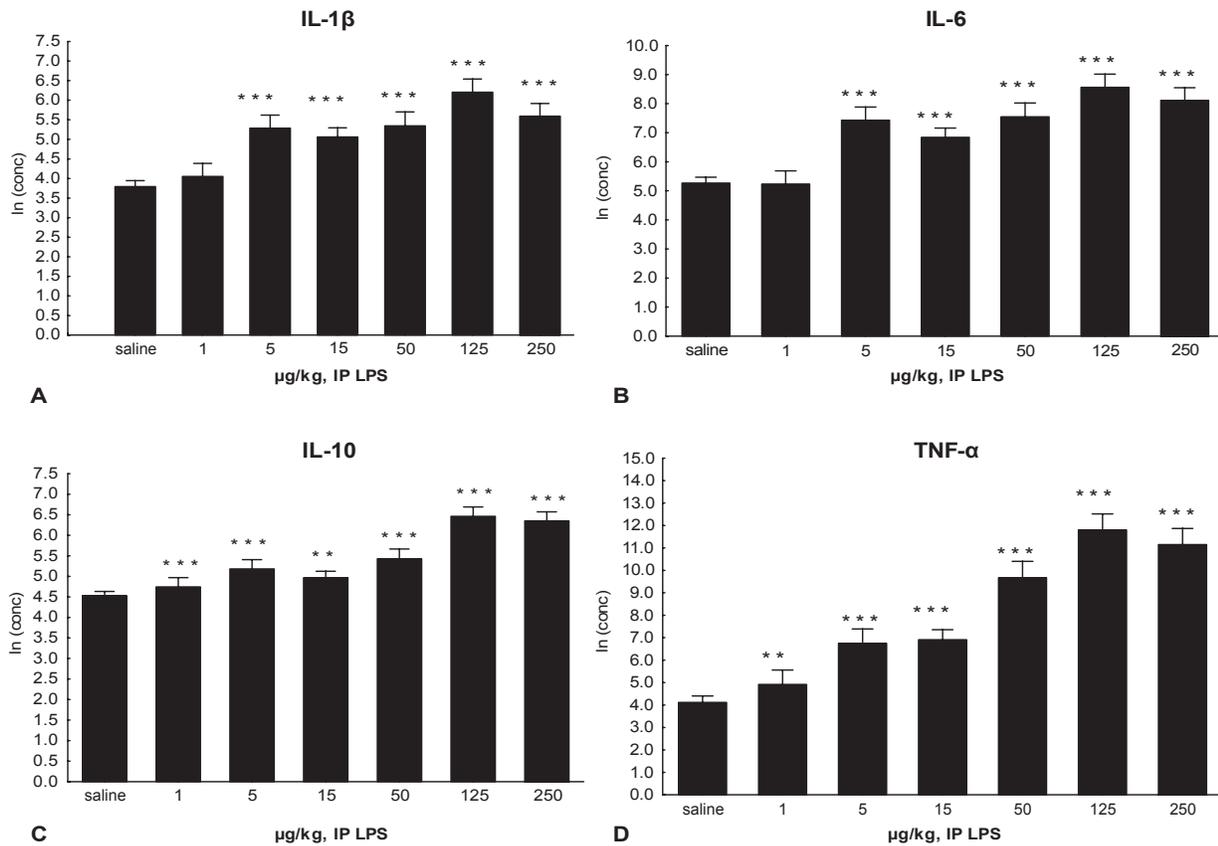


Figure 6 Cytokine levels in rat serum 2 h following lipopolysaccharide (LPS) treatment. Interleukin 1- β (IL1- β) (A), IL-6 (B), IL-10 (C), and tumor necrosis factor alpha (TNF- α) (D).

Notes: All values are expressed as natural logarithm (ln) of the serum concentration (pg/mL) ($n = 5$ to 8 rats/group/cohort). Mean concentration serum level \pm SEM for IL-1 β : saline = 50 ± 6 ; $1 \mu\text{g/kg}$ LPS = 109 ± 38 ; $5 \mu\text{g/kg}$ LPS = 277 ± 42 ; $15 \mu\text{g/kg}$ LPS = 216 ± 53 ; $50 \mu\text{g/kg}$ LPS = 247 ± 88 ; $125 \mu\text{g/kg}$ LPS = 449 ± 32 ; $250 \mu\text{g/kg}$ LPS = 316 ± 64 . Mean concentration serum level for IL-6: saline = 240 ± 39 ; $1 \mu\text{g/kg}$ LPS = 304 ± 184 ; $5 \mu\text{g/kg}$ LPS = 1617 ± 220 ; $15 \mu\text{g/kg}$ LPS = 1415 ± 382 ; $50 \mu\text{g/kg}$ LPS = 2811 ± 757 ; $125 \mu\text{g/kg}$ LPS = 5822 ± 664 ; $250 \mu\text{g/kg}$ LPS = 6014 ± 1407 . Mean concentration serum level \pm SEM for IL-10: saline = 113 ± 20 ; $1 \mu\text{g/kg}$ LPS = 302 ± 44 ; $5 \mu\text{g/kg}$ LPS = 450 ± 32 ; $15 \mu\text{g/kg}$ LPS = 245 ± 52 ; $50 \mu\text{g/kg}$ LPS = 162 ± 45 ; $125 \mu\text{g/kg}$ LPS = 434 ± 42 ; $250 \mu\text{g/kg}$ LPS = 490 ± 97 . Mean concentration serum level \pm SEM for TNF- α : saline = 91 ± 20 ; $1 \mu\text{g/kg}$ LPS = 334 ± 74 ; $5 \mu\text{g/kg}$ LPS = 2410 ± 649 ; $15 \mu\text{g/kg}$ LPS = 8871 ± 4466 ; $50 \mu\text{g/kg}$ LPS = 40412 ± 26534 ; $125 \mu\text{g/kg}$ LPS = 88375 ± 14214 ; $250 \mu\text{g/kg}$ LPS = 53032 ± 13436 . Asterisks represent significant differences detected by planned comparisons between individual treatment groups and vehicle group. ** $p < 0.01$; *** $p < 0.001$.

LPS challenge also affected the release of peripheral cytokines and HPA axis hormones. Systemic administration of LPS, from 5 to $250 \mu\text{g/kg}$, increased in a dose-dependent manner the production of peripheral cytokines IL-1 β and IL-6 whereas the IL-10 as well TNF- α were found to increase significantly at all doses of LPS tested. Particularly, in agreement with present findings, a rapid increase in serum TNF- α and a slower elevation in IL-1 β and IL-6 were demonstrated after systemic administration of low doses of LPS, while following administration of high doses of LPS, a TNF- α peak was triggered simultaneously to IL-1 β and IL-6 peaks.³⁷

A large increase in the serum or plasma concentrations of both corticosterone and ACTH was also detected starting from the LPS dose of 5 and $15 \mu\text{g/kg}$, respectively. The present results are consistent with previous studies, which have reported increases in HPA axis hormone levels

following LPS administration at comparable doses.^{38,39} HPA axis stimulation following systemic LPS is also produced by the activation of hypothalamic nuclei (ie, paraventricular nucleus) and by the increased expression of CRF as the latter is considered an important mediator for LPS-induced ACTH secretion.⁴⁰⁻⁴⁴ These neuroendocrine effects are not due to a direct pharmacological action of LPS, rather to its function as a potent inducer of synthesis and secretion of numerous cytokines such as IL-1 β , IL-6, TNF- α , which are the most likely mediators of LPS effects on neuroendocrine secretion.⁴⁵

Overall, in the present study we reproduced, at least in part, the symptoms induced by LPS administration in many clinical and preclinical studies, defined generally as "sickness behavior".^{26,46} However it has to be highlighted that the most positive attribute of the present investigation is that a wide range of doses (1 , 5 , 15 , 50 , 125 , and $250 \mu\text{g/kg}$ IP) has

been assessed on a coherent and multifaceted number of measures. Also, for the first time LPS has been behaviorally characterized using the LABORAS[®] system which has several advantages over conventional methodologies as multiple behaviors can be automatically scored in a single experiment and over a much longer duration with continuous monitoring to be undertaken.

Interestingly, these behavioral effects are associated with the stimulation of the immune response and a less pronounced pattern of change in the HPA activity, suggesting that behavioral deficits may not be directly linked to the neuroendocrine activation and reinforcing the hypothesis that cytokines are the key mediators of the sickness behavior.^{24,47} Cytokines may induce changes in the CNS through different mechanisms such as direct diffusion into the brain,^{48,49} peripheral production of pro-inflammatory mediators which in turn may diffuse into the CNS⁵⁰ and activation of specific receptors expressed on the vagus nerve abdominal terminals,^{51–53} thus generating a feedback signal to the brain, ultimately producing behavioral responses.^{54,55} In support to the latter hypothesis, Bluthé and colleagues^{56,57} demonstrated that sickness behavior including a decrease in social interaction induced by administration of either LPS or IL-1 β , was blocked following vagotomy, suggesting a selective involvement of the vagus nerve in the psychological component of the syndrome induced by LPS. Therefore a similar mechanism may be underlying the effects presently observed (ie, low doses interacting selectively with the vagal function). Furthermore other pro-inflammatory mediator not assessed in the current study, such as IL-2, known to induce sickness behavior,⁵⁸ could also have been involved in the behavioral changes observed.

In addition, indirect pharmacological evidence has started to accumulate which classifies sickness behavior as a depressive-like syndrome, that may be relevant to the clinical condition of depression and if confirmed may open new avenues to help our understanding of this debilitating psychiatric condition.⁵⁹ Furthermore, changes in the circulating cytokine levels of TNF- α as well as IL-6 or IL-1 receptor antagonist, and alterations in emotional states such as increased symptoms of anxiety and depression, but not physical sickness symptoms, have been reported in healthy volunteers following low amounts of endotoxin (0.8 ng/kg body weight-intravenous).⁶⁰ Interestingly, the result of this clinical study also demonstrated that endotoxin-induced increases in anxiety and depressed mood are strongly associated with the extent of cytokines secretion rather than HPA axis activation suggesting that these two systems may

be differentially involved in coordinating the behavioral response associated with sickness behavior. However it should be considered that a direct translation of our results into the clinical setting has some limitations. For instance if on the one hand the cytokine dynamic following LPS challenge is reported to be very dose-dependent in human, on the other hand the magnitude of the cytokine response to endotoxin seems to be highly individual. In particular, the scientific literature suggests that either genetic polymorphism or age as well as gender may all play a critical role for variations during endotoxemia.^{61–63}

In conclusion, the present finding reveals that LPS caused a decrease in the social behavior as well as induced profound changes in the hedonic state even at very low doses. Furthermore the administration of high doses of LPS causes a massive release of both cytokines and stress hormones with severe consequences on general behavior and physiological parameters, suggesting that the use of such high doses should be avoided in behavioral studies.

These findings are worthy of further studies to elucidate potential mechanisms involved in LPS-induced syndrome in the rat. Given the possible similarities between the syndrome induced in rodents and humans, such studies may provide a preclinical model for the investigation of potential therapeutic approaches for inflammatory-mediated mood disorders in humans.

Acknowledgments

We gratefully acknowledge C Righetti for technical support in collecting animal blood samples; Dr F Marini for expert assistance and Dr D Wille` for helpful advice on the statistical analysis approach.

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