Thesis

Influence of immune stimulation on emotional-affective behaviour: an experimental study

submitted by

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Zusammenfassung

Hintergrund:

Präklinische Daten und klinische Studien zeigen, dass Immunstimulation, entweder durch systemische E. coli Lipopolysaccharid (LPS) Gabe oder Zytokin-Therapie zu einer akuten Krankheitssymptomatik (Sickness Response) führt und verzögert eine Depression auslösen kann. In der Pathogenese spielt die Induktion pro-inflammatorischer Zytokine eine wichtige Rolle. Es war daher das Hauptziel dieser experimentellen Diplomarbeit, Kurz und Langzeiteffekte von LPS auf das emotional-affektive Verhalten von Mäusen zu untersuchen. Die spezifischen Fragestellungen waren:

a) Führt LPS induzierte Immunstimulation zu protrahierter Depression?
b) Ist der soziale Kontext für diesen Effekt maßgeblich?
c) Ist die Hypothalamus-Hypophysen-Nebennieren (HHN) - Achse daran beteiligt?
d) Sind verschiedene Mäusestämme für diese Untersuchung gleich gut geeignet?

Methoden:

Die spezifischen Fragestellungen wurden anhand von zwei Mausstämmen untersucht, CD1 und C57/B6J. Angst ähnliches und depressives Verhalten wurden nach erfolgter Immunstimulation mittels intraperitonealer E. coli LPS- oder Vehikelgabe (VEH) ermittelt. Die Verhaltenstests wurden einen Tag sowie vier Wochen nach Behandlung durchgeführt. Angst ähnliches Verhalten wurde mittels Open field test (OF) erhoben während depressives Verhalten mittels Forced swim test (FST) eruiert wurde.

Resultate:

Die Hauptergebnisse dieser Studie können folgendermaßen zusammengefasst werden: Abhängig vom Mäusestamm beeinflusst LPS ängstliches Verhalten und die Fortbewegungsfähigkeit einen Tag nach Behandlung. LPS verursachte variable Auswirkungen auf Depression ähnliche Zustände und bewirkte keine verzögerte Depression. Es zeigte sich, dass Gruppenhaltung (sozialer Kontext) den negativen

Effekt der Intervention auf Angst ähnliches und depressives Verhalten verhindert. Diese Aussage wird durch die Messung der Aktivierung der HHN Achse anhand der Corticosteron Plasmaspiegel unterstützt.

Schlussfolgerung:

Die Daten zeigen, dass

a) ein sozialer Kontext (Gruppenhaltung) einen schützenden Effekt gegen den Anstieg von Ängstlichkeit nach einer akuten Intervention hat;
b) dieser Effekt vom Mäusestamm abhängt;
c) akute Immunstimulation mit LPS verschiedene Effekte auf ängstliches und depressives Verhalten in Bezug auf Kurz oder Langzeit hat;
d) systemische LPS Gabe anscheinend nicht das am besten geeignete Model ist um die Auswirkungen von Immunstimulation auf ängstliches und depressives Verhalten zu ergründen.

Abstract

Background:

Preclinical data and clinical experience indicate that immune stimulation, either by systemic administration of bacterial lipopolysaccharide (LPS) or by cytokine therapy, elicits an acute sickness response and may give rise to long-term depression. The hypothesis has been put forward that systemic inflammation associated with elevated levels of proinflammatory cytokines plays a role in the pathophysiology of depression. As it is known that people with major depression and/or chronic fatigue syndrome suffer under a proinflammatory condition, it was the aim of this experimental study to address the acute and long-term effects of LPS on emotional-affective behaviour. The specific questions under study were:

a) Does LPS-induced immune stimulation cause prolonged depression?
b) Is social context relevant to this effect?
c) Is the hypothalamic-pituitary-adrenal (HPA) axis involved?
d) Are different mouse strains equally suited for behavioural testing?

Methods:

The questions under study were addressed by studying two mouse strains, CD1 and C57BL/6J. Anxiety-related and depression-like behaviour was estimated after immune stimulation by intraperitoneal administration of E. coli LPS or vehicle (VEH). The behavioural tests were carried out 1 day and 4 weeks post-treatment. Anxiety-like behaviour was assessed with the open field (OF) test while depression-like behaviour was examined with the forced swim test (FST).

Results:

The major results of this study can be summarized as follows: LPS affected anxiety-like behaviour and locomotion 1 day post-treatment in a strain related manner. LPS failed to cause a prolonged depression, and its effects on depression-like behaviour were variable. Group housing (social context) was able to inhibit the adverse effects of intervention on anxiety-like behaviour and the activation of the HPA axis measured by the plasma level of corticosterone.

Conclusion:

The data show that

a) a social context (group housing) has a protective effect against the increase of anxiety-like behaviour following an acute intervention;
b) this effect depends on the mouse strain;
c) acute immune stimulation with LPS has variable effects on anxiety- and depression-like behaviour in the short and long term;
d) systemic LPS does not seem to be the most appropriate model to study the influence of immune stimulation on anxiety and depression.

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I) Introduction

1.1) General background

Severe acute and chronic diseases are often accompanied by sickness behaviour, major depressive disorders and/or chronic fatigue syndrome. Due to the inflammation process, people feel depressed, get fever, feel nervous, powerless, lose interest in their daily life and social community and develop mild cognitive disorders (Dantzer et al. 2008). The reason for this sickness complex lies in the response of the activated innate immune system to an infection and is mediated by proinflammatory cytokines, mainly interleukin 1 (IL-1α and IL-1β) , IL-6 and tumour necrosis factor α (TNF-α) (Dantzer 2006). The current understanding of pro-inflammatory cytokine induced sickness behaviour holds that, on the one hand, cytokines provoke sickness and on the other hand, can induce true major depressive disorders in people with no preceding history of mental diseases (Dantzer et al. 2008). But how can cytokines - most are built at the site of infection and act in the periphery in a paracrine or autocrine manner - reach the brain to provoke sickness behaviour (Dantzer 2006)?

1.2) The transmission of information

When infectious microorganisms invade the body, their first contact with the immune system is via monocytes and macrophages who have TLR (Toll Like Receptors) on their surface to recognize the invaders through their PAMP (Pathogen Associated Molecular Pattern) expression.

There are two major pathways whereby the local immune system communicates with the brain:

- The fast neural transmission:

  The vagus nerve contains macrophages and dendritic cells in its perineural sheaths, which produce IL-1 after stimulation by LPS (lipopolysaccharide - endotoxin of Escherichia coli bacteria). The sensory neurons of the vagus

nerve express IL-1 receptors which are activated by the IL-1 produced in response to LPS. The role of the vagus nerve is less important for cytokine induced fever and activation of the hypothalamic–pituitary–adrenal (HPA) axis than it is for the induction of depressive disorders in response to peripheral immune activation (Konsman et al. 2002).

- The slow humoral pathway:

Macrophage – like cells settled in the circumventricular organs and the choroid plexus express TLR and produce cytokines when they get stimulated by circulating PAMPs (Quan et al. 1998). Other pathways include c) special cytokine transporters through the blood brain barrier for proinflammatory cytokines (Banks 2006) and d) IL-1 receptors that are resident in the perivascular macrophages and endothelial cells of brain venules (Konsman et al. 2004).

Clinical studies showed an important time effect in endotoxin induced changes in emotional behaviour: early elevation of anxiety levels, followed by depressed mood which is later reduced but still present (Reichenberg et al. 2001). Although the similarity of sickness behaviour and depression is remarkable (pain, malaise, anhedonia), animal data point out that sickness is an adaptive response to infection and fully reversible when the infectious cause is eliminated. Contrary to sickness behaviour, depression-like behaviour represents a cytokine-induced sickness due to over-activation of the innate immune system in its intensity and/or duration. As shown in Fig. 1 another differentiation is revealed in mouse experiments, in which peripheral immune stimulation with LPS leads to a sickness behaviour peak 2 – 6 hours post-treatment, whereas depression-like behaviour emerges peaks 24 h (Dantzer et al. 2008).

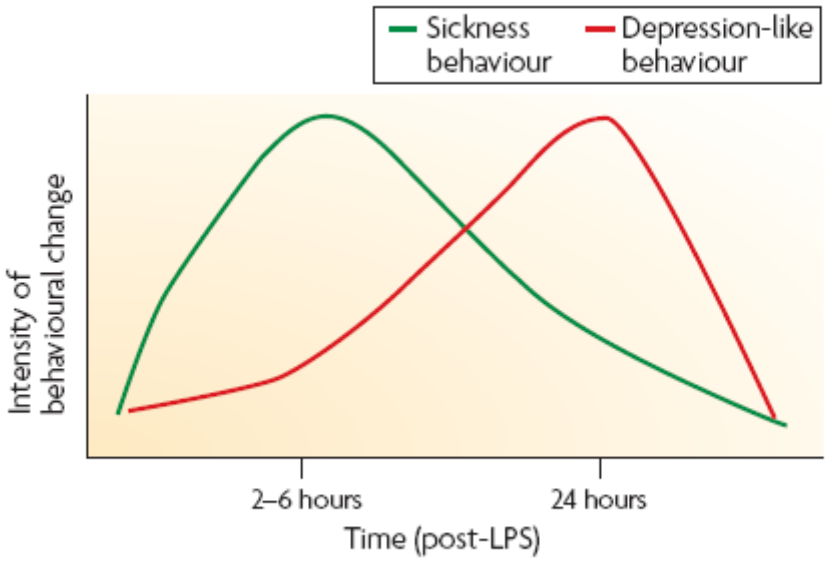

Fig. 1: LPS induced sickness and depression-like behaviour after peripheral lipopolysaccharide (LPS) administration (Dantzer et al. 2008).

1.3) The role of cytokines in sickness behaviour and depression

Cytokines play a role in the cascades of inflammation, intensifying or diluting the immunological process and keeping it in a type of balance. Clinically, certain types of cytokines are used to boost the immune system and to eliminate diseases, e.g. hepatitis C treatment with IL-2 and interferon γ (INF-γ).

There is evidence that this type of hepatitis C treatment and some cancer treatments with IL-2 cause depression like mood disorders (Krabbe et al. 2005). Furthermore there is a significant relationship between chronic inflammation processes and depression, e.g. in autoimmune diseases such as multiple sclerosis and rheumatoid arthritis, stroke and in neurodegenerative diseases such as Alzheimer (Reichenberg et al. 2001). IL-1 is a cytokine that induces, administered peripherally or centrally, all kinds of sickness behaviour including acute phase reaction, HPA activation and depression (Anforth et al. 1998). This symptom complex is mediated by proinflammatory cytokines such as IL-1β or TNF-α and can be emulated by intraperitoneal administration of LPS to mice (Painsipp et al. 2008).

1.4) The IDO induced depression theory

Another pathway for the induction of depression-like mood disorders is by the activation of indoleamine 2,3-dioxygenase (IDO) by proinflammatory cytokines like TNF-α and INF-γ (O'Connor et al. 2008). Depressed people have reduced plasma levels of tryptophan, an essential amino acid that is used for the synthesis of serotonin (Dantzer et al. 2008). Furthermore, IDO is an extra-hepatic enzyme that alters tryptophan levels through the kynurenine pathway and is found in macrophages, as well as in brain endothelial cells and perivascular macrophages, astrocytes and microglia (O'Connor et al. 2008). The majority of gastrointestinally absorbed tryptophan, the substrate amino acid for serotonin synthesis, is metabolised by the liver through the kynurenine pathway and only a small amount is transported into the brain actively and used for serotonin synthesis via the 5-hydroxytryptophan pathway (Dantzer et al. 2008). Although the metabolism of tryptophan by IDO in the periphery is dispensable, it is not in the brain. Because IDO is highly inducible by proinflammatory cytokines like INF-γ and TNF-α (Fig. 2), it is thought to induce depression-like behaviour by activation of inflammatory cells degrading the limited amount of available tryptophan in the brain not via the serotonin producing 5- hydroxytryptophan but through the kynurenine pathway and producing metabolites that may cause an imbalance of kynurenine, a N-methyl-D-aspartic acid (NMDA) receptor antagonist, and quinolinic acid, a NMDA receptor agonist. This imbalance of NMDA receptor activity is one possible explanation why certain conditions may initiate the development of depressive disorders (Fig. 2). (Dantzer et al. 2008; O'Connor et al. 2008).

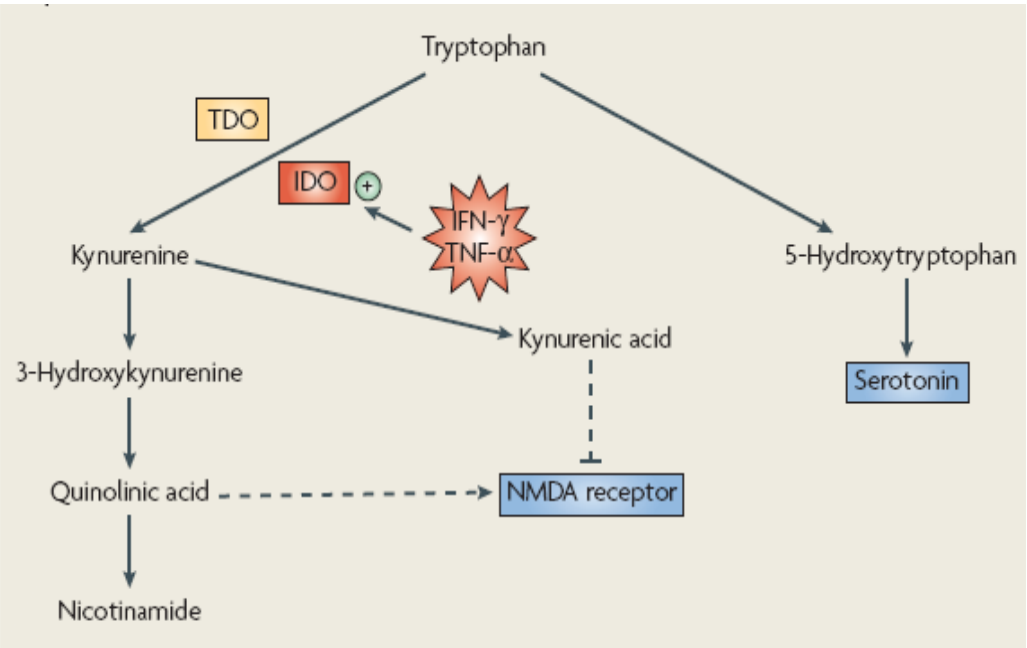

Fig. 2: Tryptophan degradation, catalyzed by tryptophan dioxygenase (TDO). Due to immune stimulation indoleamine 2,3-dioxygenase (IDO) starts catalyzing tryptophan via the kynurenine pathway producing compounds that affect the N-methyl-D-aspartic acid (NMDA) receptor (Dantzer et al. 2008).

This is affirmed by tests with mice given LPS peripherally. The animals showed increased depression-like behaviour, a sickness syndrome and an increase of IDO activity 2-6 hours post-LPS, effects that were induced by proinflammatory cytokines producing more kynurenine and its metabolites (Dantzer et al. 2008; O'Connor et al. 2008). The administration of minocycline, a tetracycline with antiinflammatory effects in the periphery and the brain, before the LPS treatment blocked the LPS-induced sickness and depression-like behaviour (Dantzer et al. 2008). Minocycline operates in the very beginning of immune signal transmission and its therapeutic benefits are used in many CNS diseases such as Parkinson disease, amyotrophic lateral sclerosis and multiple sclerosis (O'Connor et al. 2008). Pretreatment with the competitive IDO inhibitor 1-methyltryptophan (1-MT) blocked the LPS-induced reduction of tryptophan levels and the increase of kynurenine levels as well as the subsequent depression-like behaviour, but had no effect on sickness behaviour (Fig. 3) (Dantzer et al. 2008).

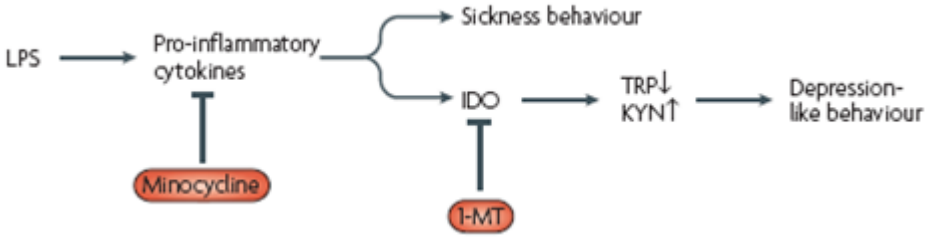

Fig. 3: LPS-induced depression-like behaviour and sickness behaviour can be modified by minocycline and 1-methyltryptophan (1-MT) (Dantzer et al. 2008).

1.5) The hyperactive hypothalamus–pituitary–adrenal (HPA) axis

Another hypothesis holds that depression results from a hyperactive HPA axis as reflected by increased production of corticotropin releasing hormone (CRH) and vasopressin (Dantzer et al. 2008). As it has been observed in recent years, there is a significant relation between hyperactive HPA axis, increased CRH levels and depression (Holsboer 2003).

Usually, the HPA axis work flow starts in the paraventricular nucleus of the hypothalamus where neuroendocrine neurons produce vasopressin and CRH. CRH stimulates the pituitary gland to release adrenocorticotropic-hormone (ACTH), which stimulates the adrenal cortices to produce and release cortisol. Cortisol in turn inhibits the hypothalamus and pituitary through a negative feedback cycle (Silbernagl 1998).

Due to the chronic inflammation process, proinflammatory cytokines can cause glucocorticoid receptor resistance in immune cells and the production of an inactive glucocorticoid receptor that binds its ligand witout inducing a biological effect (Dantzer et al. 2008). Because of the absent down-regulation of the CRH production and the missing inhibitory effect of glucocorticoids on activated pro-inflammatory immune cells, there is an overproduction of proinflammatory cytokines and a rising inflammatory reply in the brain that results in an overproduction of CRH (Raison et al. 2003).

All things considered, it is clear that a couple of ways can lead to depression and sickness behaviour through induction of proinflammatory cytokines and activation of IDO. Moreover IDO activation metabolises the limited amount of tryptophan through the kynurenine pathway and produces neuroactive metabolites and glucocorticoid receptor resistance. This abrogates the CRH feedback mechanism and escalates the inflammatory response, which leads to an inflated production of CRH (Dantzer et al. 2008).

1.6) Neuropeptide Y (NPY) and its behavioural impact

NPY is a neurotransmitter found in the autonomic nervous system and the brain, plays a role in food intake, anorexia, obesity, immunological, emotional and behavioural processes, learning, memory and epilepsy (Colmers et al. 2003; Painsipp et al. 2008). Further on it stimulates the corticotrophic axis, inhibits the gonadotrophic and somatotrophic axis, can block neuronal excitability and has anti-convulsant effects (Kask et al. 2002).

There are several NPY receptors, five of which are known to transduce via the Gi/o signalling pathway (Y1,Y2,Y4,Y5,Y6).

To elucidate the functional implications of NPY, genetically modified mice were bred and used in behavioural tests, e.g. forced swim test (FST), to dissect the roles of NPY receptors. Recent data showed that, on the one hand, Y1 receptor agonists mediate anti – stress effects and in this way induce anti-depressive and anxiolytic actions whereas Y1 receptor antagonists block this anti-stress action (in some cases this effect may be compensated for by the Y5 receptor). On the other hand, Y2 receptor antagonists cause a similar effect as Y1 receptor agonists (Heilig 2004). Furthermore, both the Y2 and Y4 receptors play a role in depression and anxiety, as Y2 and Y4 receptor knock-out mice display less depression-like behaviour and anxiety-like behaviour than control mice. In addition knock-out of the Y2 receptor enhances immune stress-induced changes in anxiety-like, locomotor and social behaviour (Painsipp et al. 2008).

1.7) The leaky gut, chronic fatigue syndrome and depression

Recent studies showed that major depression and chronic fatigue syndrome, which share many behavioural similarities, are often accompanied by increased serum levels of IgA and IgM against the LPS of gram-negative enterobacteria (Maes et al. 2008).

This increase of IgA and IgM against LPS is assumed to be caused by an increase in gut permeability, allowing bacteria and toxins like LPS to cross the mucosal barrier. Within the lamina propria, they start a local inflammation which, damages the mucosa, and induce an IgM- and IgG-mediated immune response (Maes et al. 2007; Maes et al. 2008). As a result of the leaky gut an inflammation process triggers a local and systemic increase of proinflammatory cytokines. It is known that systemic administration of LPS leads to a chronic neuronal inflammation in the mouse brain caused by TNF-α which is still elevated 10 months after LPS administration. TNF-α activates brain microglia cells to produce chronically proinflammatory cytokines which trigger a sickness syndrome and depressive behaviour (Maes et al. 2007).

All aspects considered, chronic fatigue syndrome is not only triggered by viral and bacterial infections, physical and emotional stress, type III and IV allergies against certain type of food, but also by a leaky gut – starting an inflammatory process that has behavioural impact (Maes et al. 2007).

1.8) Inflammation, oxidative and nitrosative stress (IO&NS) and depression

Inflammation is often induced and/or attended by oxidative and nitrosative stress which damages DNA, lipid membranes and cellular structures. Because of this lipid membrane damage, the membrane protein appears to the innate immune system as something exogenous. The result is an immune response to this IO&NS damage which can be measured via increased serum levels of IgM against cellular components and increased levels of malondialdehyde and arachidonic acid, both

of which are a side product when DNA is injured and decomposed. Some of these damages may have impact on intracellular signal processing, e.g. anti-Pi antibodies may modify the production of the second messenger inositol 1,4,5-triphosphate (IP3), the signal transmission phospholipids phosphatidylinositol-4,5-bisphosphate (PIP2) and diacylglycerol and phosphatidylinositol-3,4,5-triphosphate (PIP3) (Maes 2008)

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Moreover, high serum levels of IgM against damaged structures are often found in depressive patients and in patients with autoimmune diseases, like acute multiple sclerosis (Maes 2008).

1.9 Inflammation – the neglected link to depression?

As previously described, many different pathways can lead to depressive behaviour, sickness syndrome and anxiety-like behaviour. All different theories have one thing in common: inflammation. In recent years clinical data have pointed out that there is a significant relationship between depression and inflammatory processes in physically ill people and in conditions that are known to activate the innate immune system, like ageing and adiposity. Coronary heart disease, a disease in which inflammation is now thought to be an important conducing factor, is often accompanied by depression (the prevalence of depression in patients with coronary heart disease is up to three times higher than in the general population). Because depression is often a co-morbid risk factor of coronary heart disease, cardiac events and mortality, it has been hypothesized that depression aggravates the disease process. However, this association must be reconsidered in view of emerging evidence that inflammation can trigger depression in vulnerable patients (Dantzer et al. 2008).

1.10 Questions under study

As discussed before, preclinical data and clinical experience indicate that immune stimulation, for instance by cytokine therapy, elicits an acute sickness response and may give rise to long-term depression as has been observed in patients under IFN therapy. The hypothesis has been put forward that systemic inflammation associated with elevated levels of proinflammatory cytokines plays a role in the pathophysiology of depression. The investigation of the pathophysiological mechanisms behind this immune-mood connection would greatly benefit from the availability of an animal model reflecting this relationship. Against this background, the current experimental project set out to investigate the short- and long-term effects of a systemic LPS injection on anxiety- and depression-related behaviour in mice and to address some factors that may be of relevance to the behavioural changes evoked by LPS. Four specific questions were addressed:

(1) Are any short-term changes in anxiety- and depression-related behaviour, assessed within 1 day post-LPS, followed by any long-term changes in emotional-affective behaviour, as assessed 28 days post-LPS?

(2) Do the short- and long-term effects of LPS depend on the social context of the animals under study? There is evidence that single housing represents a stressor for female, but not male, mice (Palanza et al. 2001). It was therefore investigated whether the emotional-affective behaviour of female mice differs if they are housed singly or in a group and whether these different housing conditions modify the behavioural effects of LPS.

(3) The emotional-affective behaviour differs substantially between different strains of mice (Crawley et al. 1997; Griebel et al. 2000; David et al. 2003). In addition, C57BL/6J mice differ from other mouse strains by particular polymorphisms in the serotonin transporter which is known to be of relevance to the regulation of depression-like behaviour (Carneiro et al. 2009). For this reason, an inbred mouse strain, C57BL/6J, and an outbred mouse strain, CD1, were compared with each other with regard to the effects of LPS on anxiety- and depression-related behaviour. Most studies on the sickness response of mice to LPS were carried out with CD1 mice (O'Connor et al. 2008).

(4) The anxiety- and depression-related behaviour is under the control of the HPA axis. For this reason, the circulating levels of corticosterone, the final messenger of the HPA axis, were measured and put in relation with the behavioural parameters measured in LPS-treated animals.

II) Methods

2.1) Experimental animals

The study was realised with age matched adult female mice, which were kept at the animal house of the Institute of Experimental and Clinical Pharmacology at the Medical University of Graz. The housing conditions involved controlled temperature (set point 21°C), controlled relative air humidity (set point 50%), a circadian light cycle (lights on at 6:00h, lights off at 18:00h) and maximal light intensity 150 lux. The experimental procedures and number of animals used in this study were approved by an ethical committee at the Federal Ministry of Science and Research of the Republic of Austria and conducted according to the Directive of the European Communities Council of 24 November 1986 (86/609/EEC). The experiments were designed in a way that the number of animals used and their suffering was minimized. For this reason, only female mice were studied. CD1 mice were bred at Charles River (Sulzfeld, Germany) and C57BL/6J mice were bred at the Division of Laboratory Animal Science and Genetics, Department of Biomedical Research, Medical University of Vienna (Himberg, Austria). The experiments were executed at the behavioural laboratory of the Experimental and Clinical Pharmacology Institute.

2.2) Administration of lipopolysaccharide (LPS)

LPS extracted from E.coli 0127:B8 (Sigma, Vienna, Austria) was dissolved in pyrogen-free sterile saline (0.9% NaCl) at a concentration of 1 mg/ml. This stock solution was diluted with pyrogen-free saline to produce injection solution of 0.083 mg/ml LPS, which were injected IP at a volume of 0.01 ml/g, equivalent to a dose of 0.83 mg/kg LPS, respectively. Pyrogen-free sterile saline injected at the same

volume was used as vehicle control. In the acute group study LPS was injected 23 hours before testing. The interval was chosen because there is evidence that after this period of time the LPS-induced depression of motor activity has waned (Frenois et al. 2007)

2.3) Experimental protocols

A total of 64 CD1 and 60 C57BL/6J were used and allocated to different treatment groups as shown in Fig. 4. Each strain of mice was separated into two cohorts: mice tested 1 day after intraperitoneal injection of vehicle (VEH) or LPS (acute group) and mice tested 28 days post-treatment (long term group). These two cohorts of each strain were further subdivided into groups of mice that were housed single or in groups of 2 – 3 mice (Fig. 4). Mice were housed in cages of size II L (length x width x height = 26 cm x 20.5 cm x 14 cm). After injection of LPS or VEH to acute group mice, the animals were subjected to the open field (OF) test, which was performed 23 -25 h post-treatment, followed by the forced swim test (FST), which was performed 25 – 28 h post-treatment. The “long term group” mice were subjected to these behavioural tests 28 days post-treatment. After each test, mice were instantly returned to their home cage, and care was taken not to change cage mates during the tests.

2.4) Behavioural tests

Before the behavioural tests, mice were allowed to adapt to the test room (temperature set point: 22°C, relative air humidity set point: 50%, lights on at 6:00h, lights off at 18:00h, maximal light intensity 100 lux for at least 1 day. The OF test and FST were performed during the period of 08:00h – 13:00h. The open field test was started at 08:00h and followed by the FST at 10:00h.

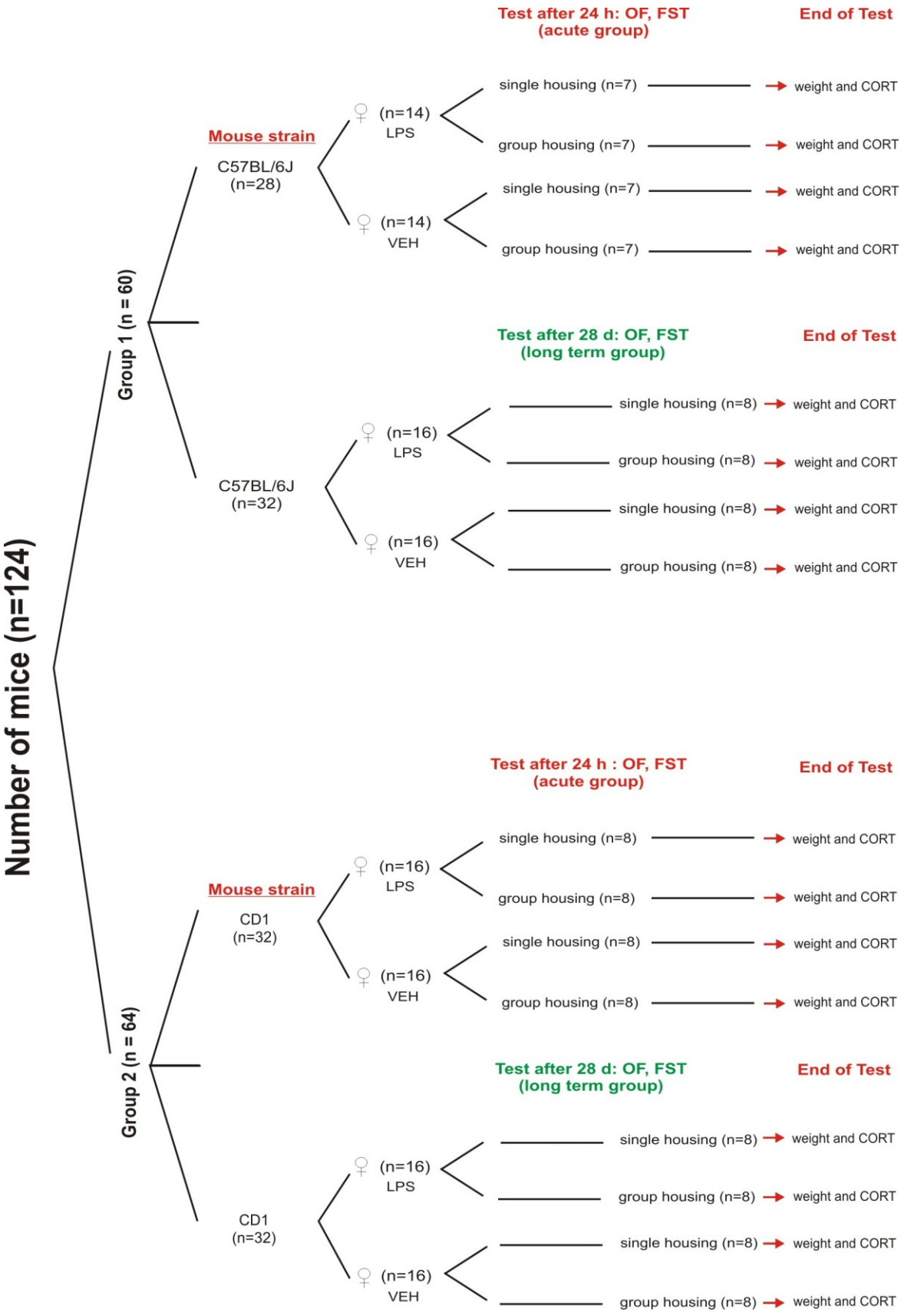

Fig. 4: Study design. Two groups of mice: CD1 and C57BL/6J mice were used

2.5) The open field (OF) test:

The OF consisted of a box (50 × 50 × 30 cm) that was made of opaque grey plastic and illuminated with 80 lux at floor level. The OF box was divided in two virtual zones: the 36 x 36 cm centre zone and the surrounding area. The mice were placed in the centre of the OF box, and their behaviour was recorded for 5 minutes. Movements of the mouse were tracked by a video camera positioned above the centre of the OF and recorded with the software VideoMot2 (TSE Systems, Bad Homburg, Germany). This software was used to evaluate the time spent in the central area and the surrounding area, the number of entries into the central area and the total distance travelled in the OF. The more the mouse enters the central area and spends time there, the less it is anxious. When the mouse spends more time in the surrounding area, it is counted as anxious. At the end of this test the number of boli (faecal pellets) each animal produced during the OF test was counted.

2.6) The forced swim test (FST)

Each mouse was placed in a 20.5 cm high cylinder (diameter: 16.5 cm) containing water up to a height of 15 cm kept at 25±1 °C. The water was changed and the cylinder cleaned after every third animal test. Mice had to swim in the water for 6 minutes and were returned to their home cage afterwards. The time the mouse spent swimming, climbing and floating during the FST was recorded with a home-made computer programme. When a mouse stopped trying to climb outside the cylinder or stopped swimming, it was judged as immobile (floating). Immobility in this test is assumed to be an index of depression.

2.7) Circulating corticosterone

Thirty minutes after the FST, mice were deeply anaesthetized with pentobarbital (150mg/kg intraperitoneally) and sacrificed. Heart blood was taken and collected in vials coated with ethylenediamine tetraacetate (EDTA; Greiner, Kremsmünster, Austria) kept on ice. Following centrifugation for 10 min at 4 °C and 1200 x g, blood plasma was collected and stored at −20 °C until assay. The plasma levels of corticosterone were determined with an enzyme immunoassay kit (Assay Designs, Ann Arbor, Michigan, USA). According to the manufacturer's specifications, the sensitivity of the assay is 27 pg/ml, and the intra- and inter-assay coefficient of variation amount to 7.7 and 9.7%, respectively.

III) Statistics

Statistical evaluation of the results was made with SPSS 14.0 (SPSS Inc. Chicago, Illinois, USA). All data were analysed by two-way or three-way analysis of variance (ANOVA). Whenever ANOVA was performed, the homogeneity of variances was assessed with the Levene test. If a significant interaction between the test factors (time, treatment, housing) was found, post-hoc analysis of group differences was made with two-way ANOVA or Student’s t test. In view of the exploratory nature of the study, probability values $P \leq 0.1$ were regarded as statistically significant (Winer 1991; Kirk 1995; Hays 2007). All data are presented as means ±SEM, n referring to the number of mice in each group. Different symbols (#, +, *) are used to describe significant differences: # shows a treatment effect (LPS vs. VEH); + shows a time effect (acute vs. long term); * shows a housing effect (single vs. group housing).

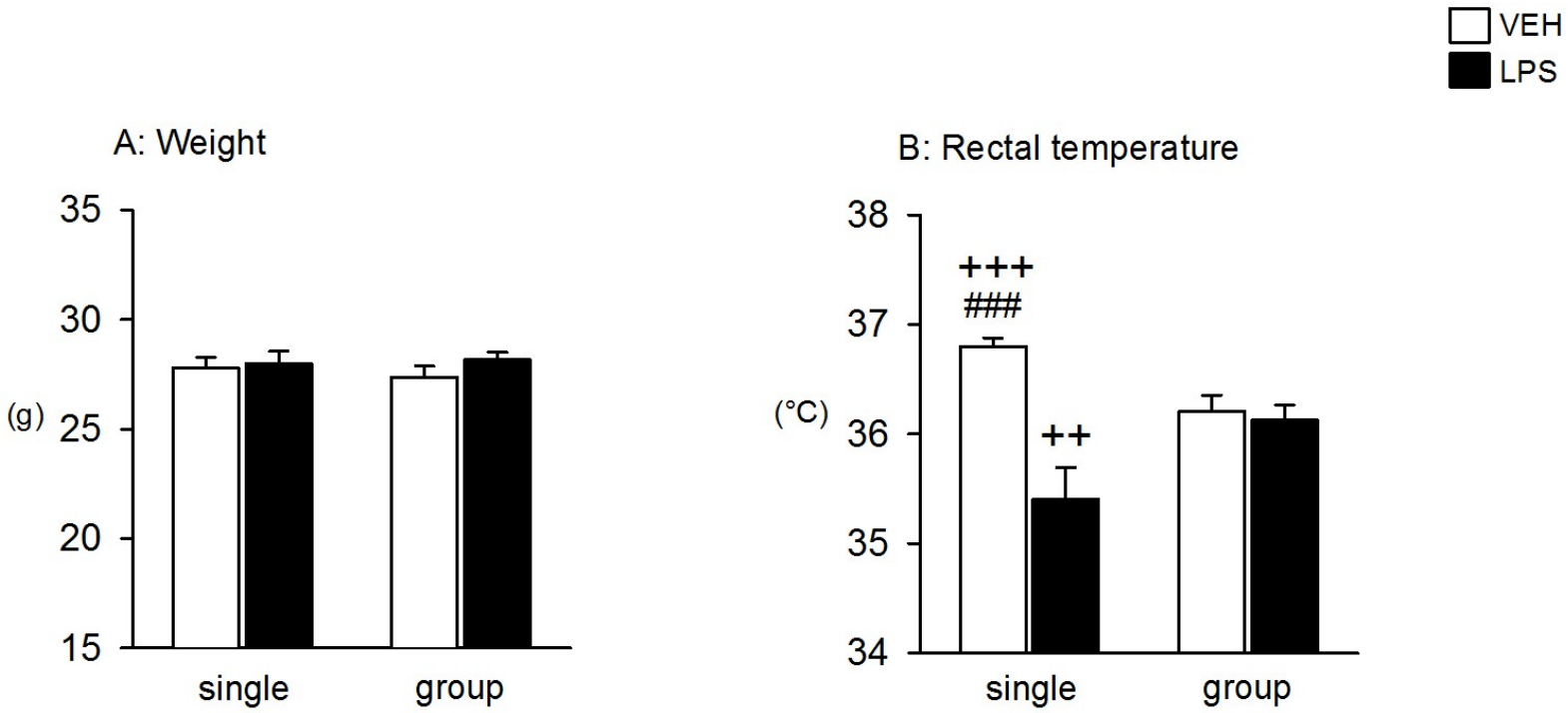

Fig.5: Effect of intraperitoneally administered LPS (0.83 mg/kg), relative to vehicle (VEH), on the body weight (A) and rectal temperature (B) in CD1 mice. The body weight was measured immediately before the injection of VEH or LPS. The rectal temperature was measured 5 hours post-treatment. The values represent means ± SEM, n = 7–8. ### $P < 0.01$ versus group housed LPS-treated mice, ++ $P < 0.05$, +++ $P < 0.01$ versus group housed mice with the same treatment.

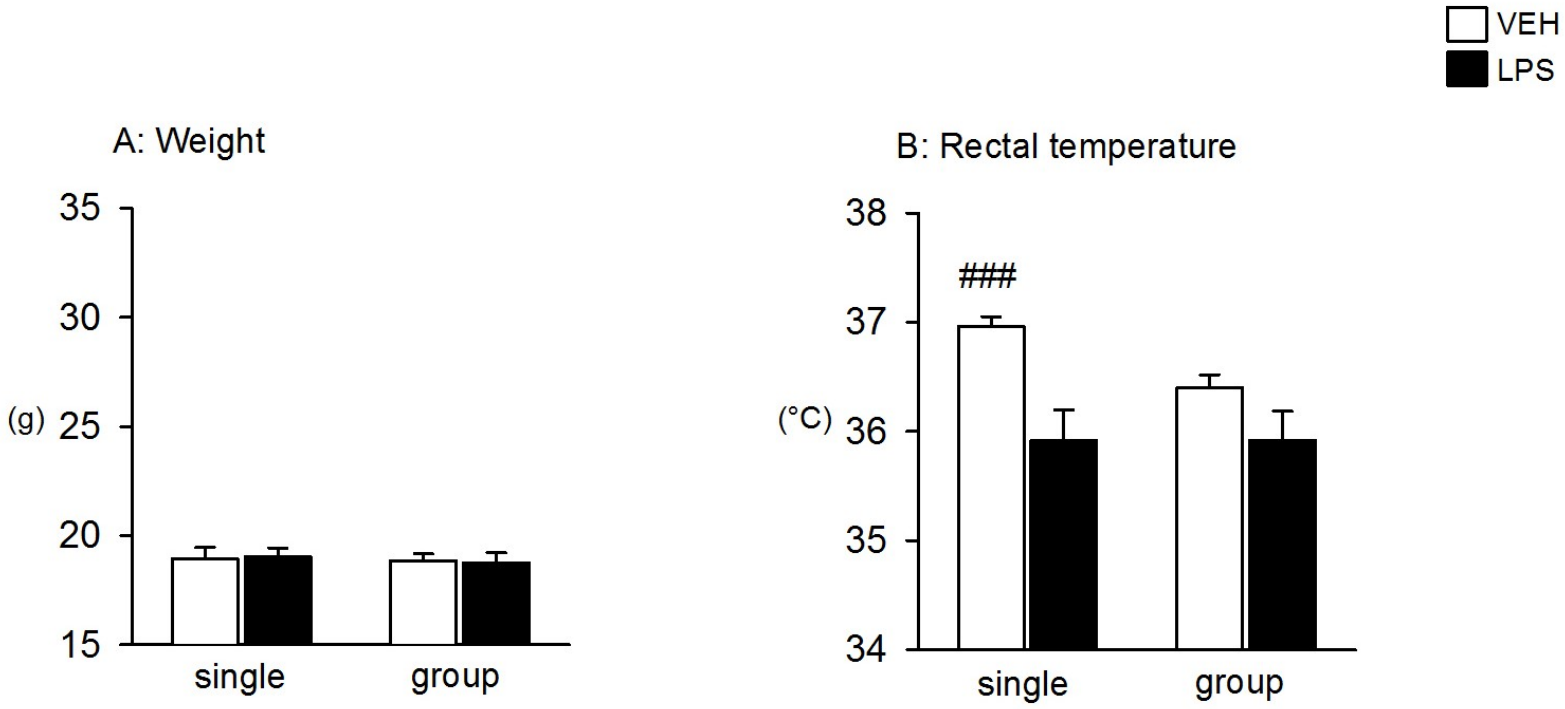

Fig. 6: Effect of intraperitoneally administered LPS (0.83 mg/kg), relative to vehicle (VEH), on the body weight (A) and rectal temperature (B) in C57BL/6J mice. The body weight was measured immediately before the injection of VEH or LPS. The rectal temperature was measured 5 hours post-treatment. The values represent means ± SEM n = 7–8. ### P < 0.01 versus group housed mice with LPS treatment.

IV) Results

4.1) Temperature and weight

a) Rectal temperature and weight in CD1 mice:

Two-way ANOVA showed a significant treatment effect ($F(1.60) = 16.871$; $P = 0.000$) on rectal temperature in CD1 mice (Fig. 5). Post-hoc analysis revealed that single housed VEH-treated mice had a higher rectal temperature than single housed LPS-treated mice ($t(17.21) = -4.693$; $P = 0.000$) and group housed VEH-treated mice ($t(23.005) = 3.605$; $P = 0.001$)(Fig. 5). In addition there was a significant time effect in LPS-treated mice. The rectal temperature in LPS-treated single housed mice was lower than in group housed mice ($t(21.808) = -2.252$; $P = 0.035$) (Fig. 5).

b) Rectal temperature and weight in C57BL/6J mice:

Two way ANOVA revealed a significant treatment effect ($F(1.56) = 13.650$; $P = 0.001$) (Fig. 6). This means that regardless of single or group housing VEH-treated mice exhibited a higher rectal temperature than LPS-treated mice (Fig. 6). The different groups of mice did not differ in their body weight (Figs. 5 and 6).

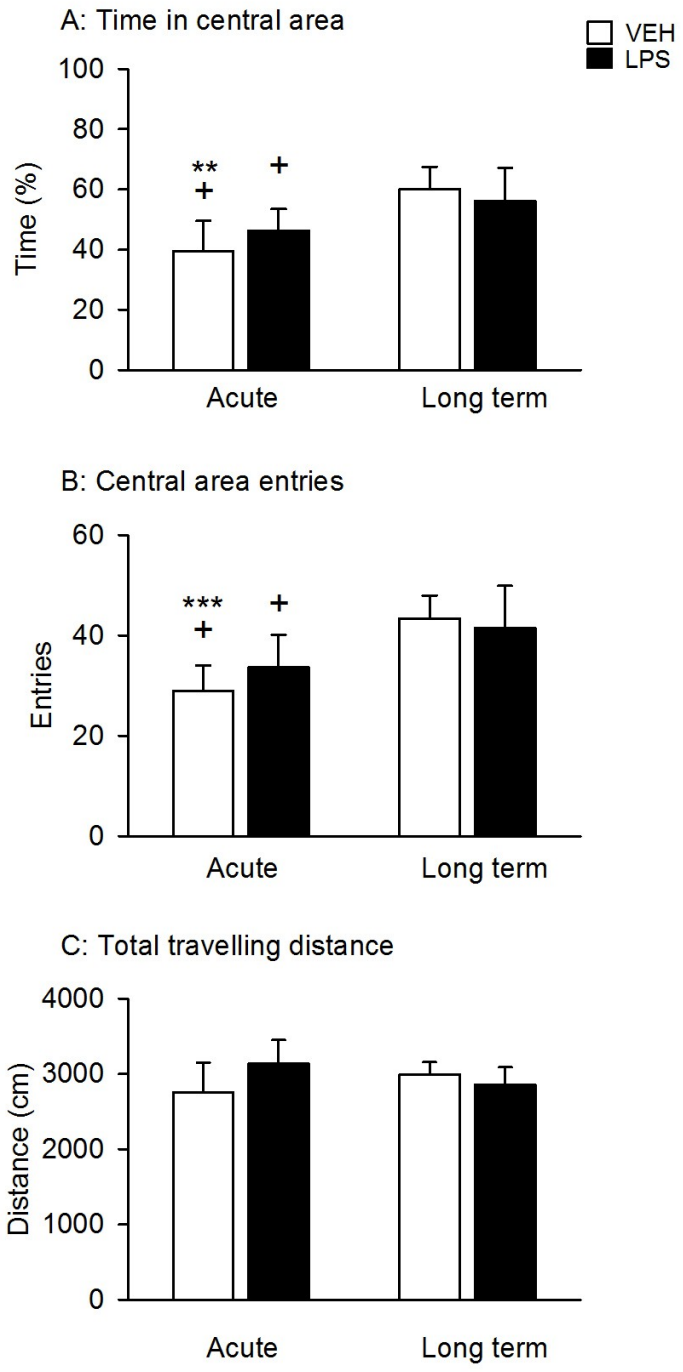

Fig. 7: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the behaviour of single housed CD1 mice in the open field test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. A: Time the animal spent in the central area, expressed as a percentage of the duration of the test (5 min). B: Number of entries into the central area. C: Total travelling distance. The values represent means ± SEM, n = 7–8. **P < 0.05, ***P < 0.01 versus group housing, same treatment (Fig. 8), +P ≤ 0.1 versus long term, single housing, same treatment.

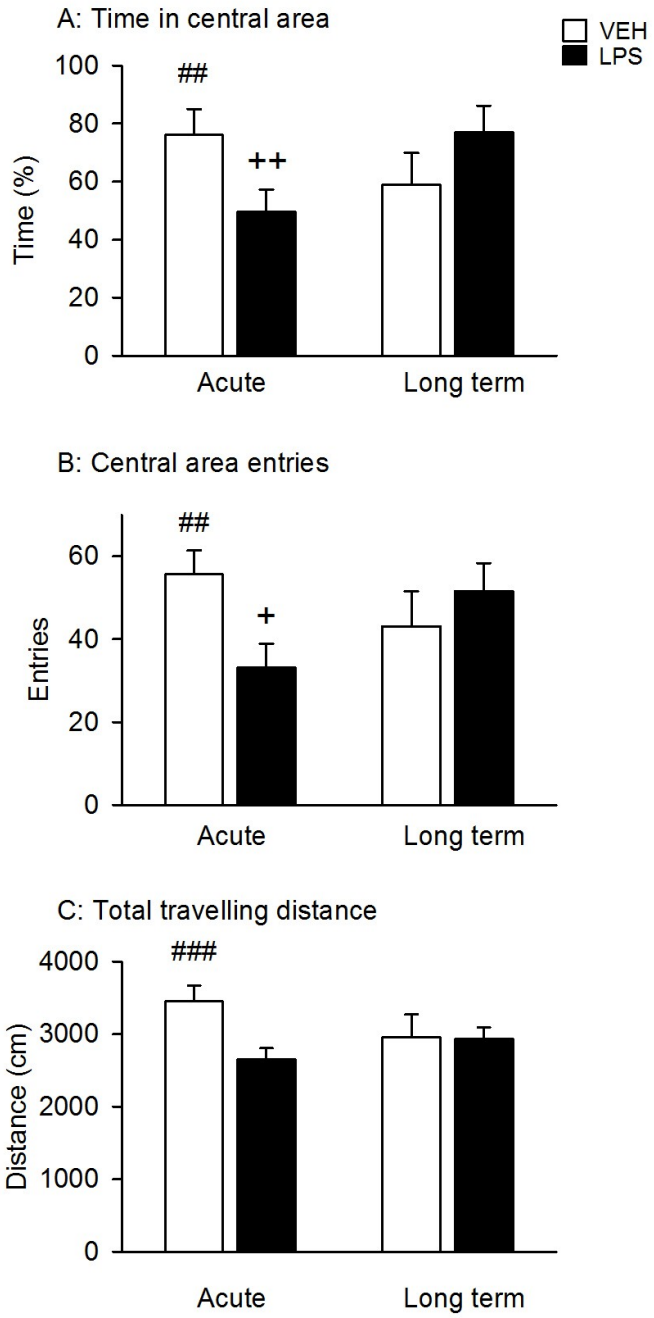

Fig. 8: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the behaviour of group housed CD1 mice in the open field test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. A: Time the animal spent in the central area, expressed as a percentage of the duration of the test (5 min). B: Number of entries into the central area. C: Total travelling distance. The values represent means ± SEM, n = 7–8. ##P < 0.05, ###P < 0.01 versus acute, LPS treatment, +P ≤ 0.1, ++P < 0.05 versus long term, same treatment.

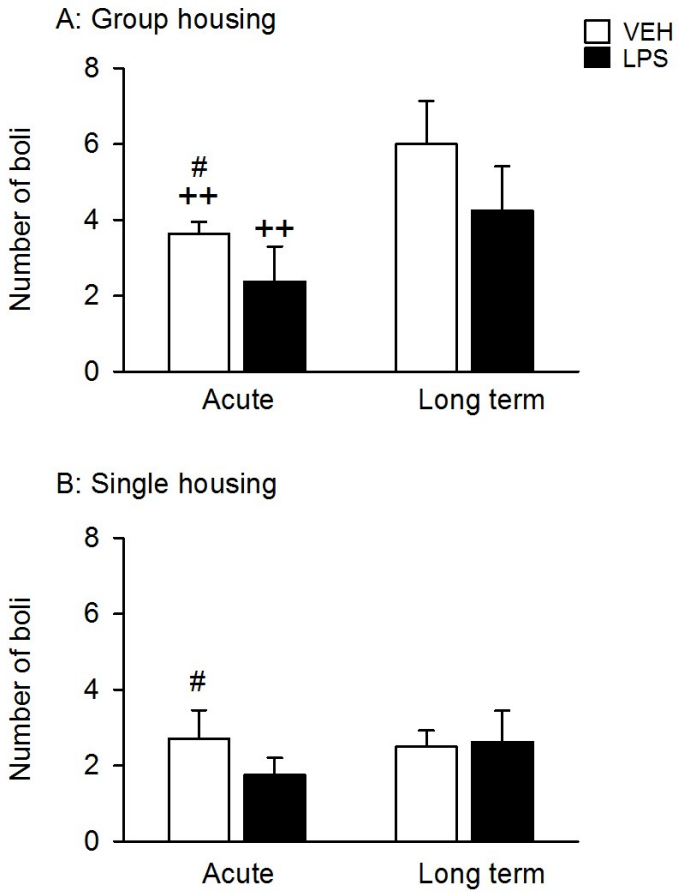

Fig. 9: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the expulsion of faecal pellets (boli) by CD1 mice during the open field test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. The values represent means ± SEM, n = 7–8. #P ≤ 0.1, versus acute, LPS treatment, ++P < 0.05 versus long term, same treatment.

4.2) Open field (OF) test in CD1 mice

Three way ANOVA revealed a significant time*treatment*housing effect on the time spent in the central area ($F(1.55) = 4.553$; $P = 0.037$), the entries into the central area ($F(1.55) = 4.038$; $P = 0.049$) and the total travelling distance ($F(1.55) = 3.250$; $P = 0.077$). Further analysis by two-way ANOVA showed that regardless of treatment single housed CD1 mice spent more time in the central area when tested 28 days post-treatment than when tested 1 day post-treatment (compare Fig. 7 and Fig. 8). Two-way ANOVA also disclosed that the factor time (long term

vs. acute group) was significant (F(1.27) = 3.015; P = 0.094) with regard to the dependent variable centre entries. Furthermore, ANOVA revealed that the factor time (acute versus long term) is significant to the duration of time the animals spent in the centre of OF (F(1.27) = 2.839; P = 0.104). Regardless of treatment single housed CD1 mice spent more time in the central area when tested in the long-term paradigm than when tested in the acute paradigm (Fig. 7).

In the acute paradigm a significant housing (single vs. group housing) effect on the time spent in the central area (F(1.27) = 5.483; P = 0.027) was observed, with single housed mice spending less time in the central area than group housed animals (Figs. 7 and 8). Further on, there was a significant treatment*time effect on the time spent by single housed mice in the central area (F(1.27) = 3.886; P = 0.59). Post hoc analysis showed that in the acute paradigm single housed VEH-treated mice spent less time in the central area than group housed VEH-treated mice. Both a significant housing (t(13) = 2.716; P = 0.018) and treatment (t(16) = -2.242; P = 0.042) effect were disclosed in the acute paradigm (Figs. 7 and 8).

Similarly, in the acute paradigm a significant housing (single vs. group) effect on the centre entries (F(1.27) = 4.976; P = 0.034) was revealed, as single housed mice entered the central area less often than group housed mice (Figs. 7 and 8). Furthermore, there was a combined treatment*housing effect on the centre entries (F(1.27) = 5.365; P = 0.028). Post-hoc analysis showed that single housed VEH-treated mice entered the central area less often than group housed VEH-treated mice. A significant housing (t(13) = 3.431; P = 0.004) and treatment (t(14) = -2.757; P = 0.015) effect was also disclosed in the acute paradigm (Figs. 7 and 8).

In the next step of analysis of the acute paradigm, a treatment effect on the time spent in the central area, the number of entries into the central area and travelling distance was shown in the group housed CD1 mice. Group housed LPS-treated mice spent less time in the central area than the respective VEH-treated group (F(1.28) = 5.736; P = 0.024) (Fig. 8). In addition, there was a significant treatment*time effect on the centre entries by LPS-treated group housed mice (F(1.28) = 5.247; P = 0.030) (Fig. 8). A significant treatment effect on the travelling distance in group housed mice was also found, as LPS-treated mice moved less

than VEH-treated mice (F(1.28) = 3.468; P = 0.73) (Fig. 8). There was likewise a significant treatment*time effect on the travelling distance in group housed mice (F(1.28) = 3.067; P = 0.091). Post-hoc analysis showed that VEH-treated group housed mice moved more than LPS-treated group housed mice (t(14) = -2.991; P = 0.010) (Fig. 8).

Further post-hoc analysis of the acute paradigm revealed a significant treatment effect on the time spent in the central area (t(14) = -2.242; P = 0.042) and the centre entries (t(14) = -2.757; P = 0.015) in group housed mice (Fig. 8). This means that VEH-treated mice spent more time in the central area and entered the central area more often than LPS-treated mice.

When the acute and long term-term paradigmata were compared with each other, a time effect on LPS-treated group housed mice was disclosed (Fig. 8). Post-hoc analysis showed that 1 day post-treatment LPS-treated group housed mice spent less time in the central area (t(14) = -2.274; P = 0.039) and entered the central area less often (t(14) = -2.084; P = 0.056) than 28 days post-treatment (Fig. 8).

Two-way ANOVA showed that the number of faecal boli expelled during the OF test differed with housing and time (acute vs. long term) (F(1.28) = 5.026; P = 0.033) (Fig. 9). This means that 28 days post-treatment mice produced more boli than 1 day post-treatment independently of treatment (Fig. 9). In the acute paradigm VEH-treated mice produced more boli than LPS-treated mice (F(1.27) = 2.883; P = 0.101) (Fig. 9).

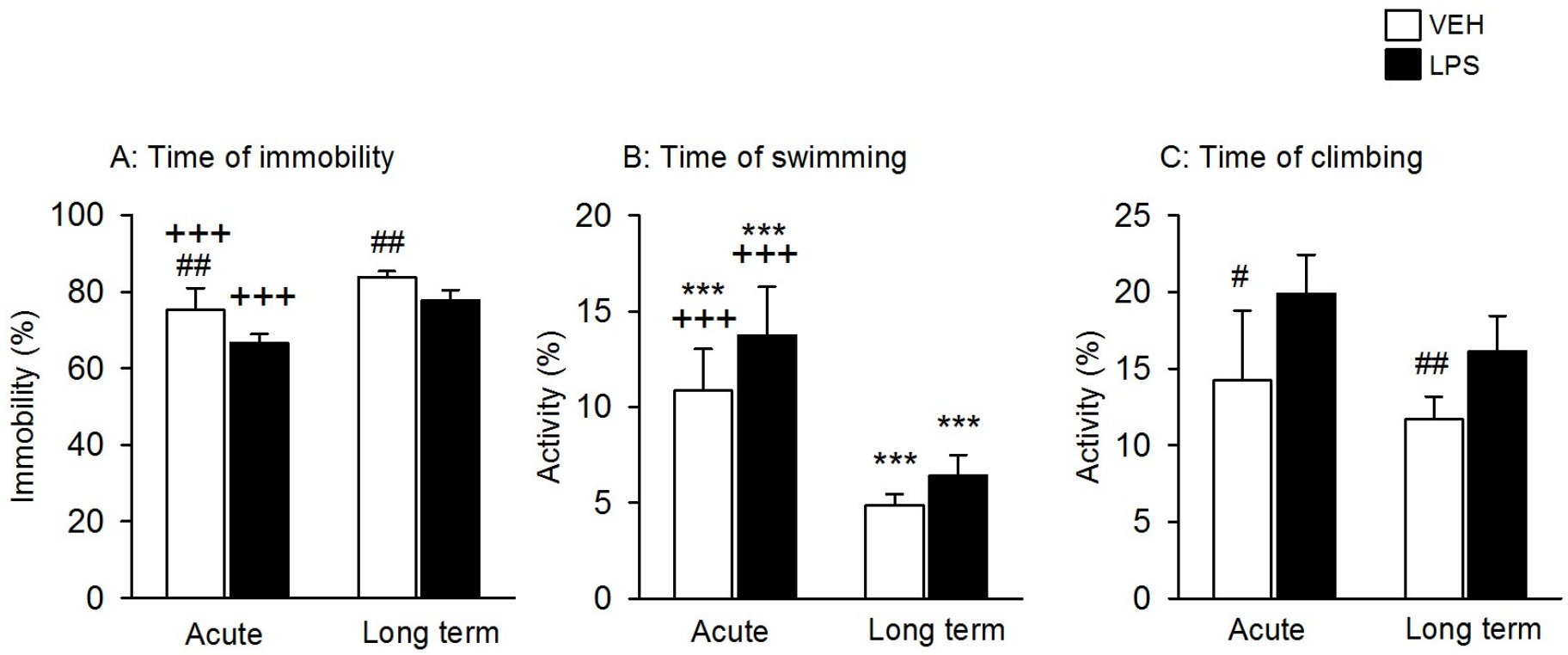

Fig. 10: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the behaviour of single housed CD1 mice in the forced swim test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. A: Time the animal was immobile. B: Duration of swimming activity. C: Duration of climbing activity. The different activities are expressed as a percentage of the total test duration (6 min). The values represent means ± SEM, n = 7–8. #P ≤ 0.1, ##P < 0.05 versus single housing, LPS treatment, same post-treatment interval; +++P < 0.01 versus long term, single housing, same treatment; ***P < 0.01 versus group housing, same treatment (Fig.11).

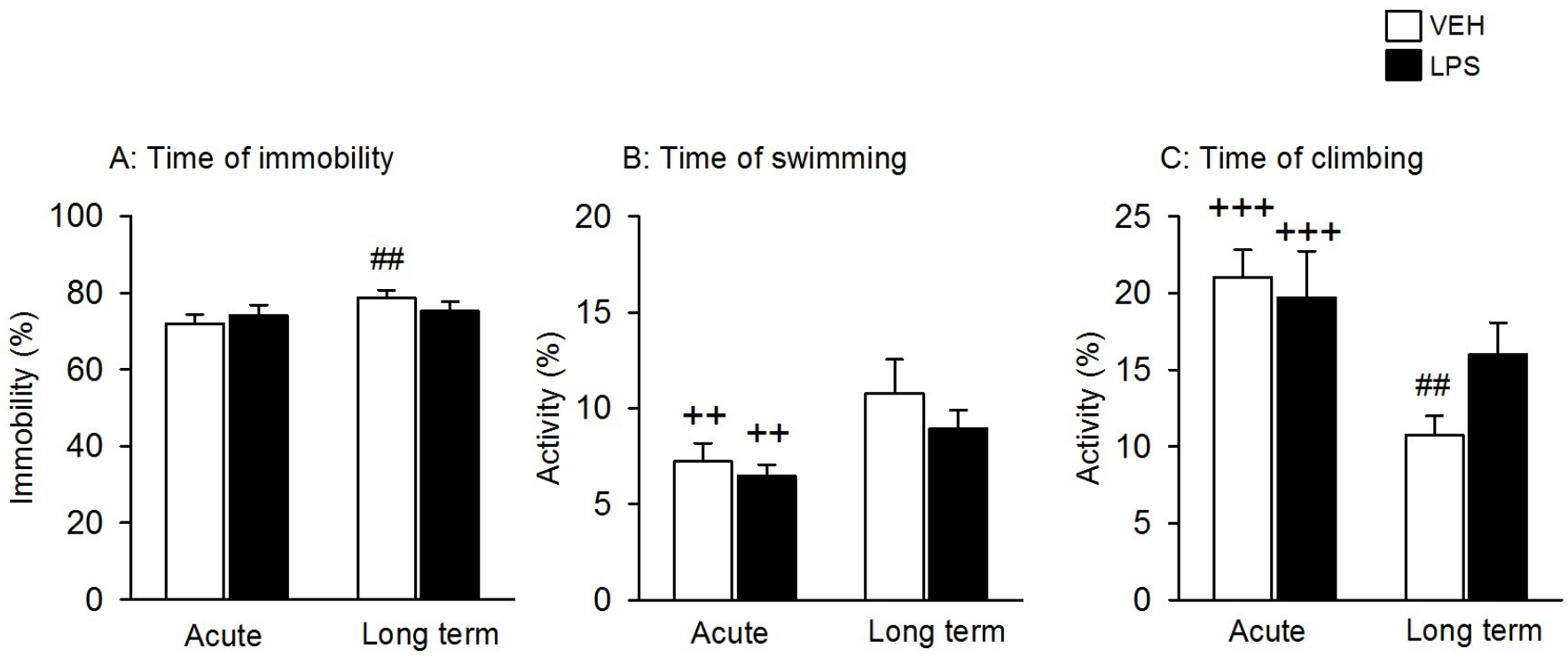

Fig. 11: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the behaviour of group housed CD1 mice in the forced swim test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. A: Time the animal was immobile. B: Duration of swimming activity. C: Duration of climbing activity. The different activities are expressed as a percentage of the total test duration (6 min). The values represent means ± SEM, n = 7–8. ##P < 0.05 versus single housing, LPS treatment, same post-treatment interval; ++P < 0.05, +++P < 0.01 versus long term, group housing, same treatment

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4.3) Forced swim test (FST) in CD1 mice

Three-way ANOVA revealed a significant combined effect of time ($F(1.56) = 2.936$; $P = 0.092$), time*housing ($F(1.56) = 20.760$; $P = 0.000$) and treatment*housing ($F(1.56) = 2.732$; $P = 0.104$) on swimming behaviour. This result was further analyzed by two-way ANOVA. In the acute paradigm single housed mice showed more swimming activity than group housed mice ($F(1.28) = 9.471$; $P = 0.005$) regardless of treatment (Figs. 10 and 11). To the contrary, in the long term paradigm, group housed mice spent more time swimming than single housed mice ($F(1.28) = 12.973$; $P = 0.001$) (Figs.10 and11). Regardless of housing (single or group housing), there was a significant treatment effect on the climbing activity of LPS-treated mice in the long term paradigm, as LPS-treated mice climbed longer than VEH-treated mice ($F(1.28) = 6.950$; $P = 0.014$) (Figs. 10 and 11). In addition, VEH-treated mice spent more time immobile 28 days post-treatment than LPS-treated mice ($F(1.28) = 4.477$; $P = 0.043$) (Figs.10 and 11).

Furthermore, a time effect (acute vs. long term) on swimming and climbing activity was revealed in group housed mice. One day post-treatment, group housed mice swam less than 28 days post-treatment ($F(1.28) = 6.945$; $P = 0.014$) but climbed more than in the long-term paradigm ($F(1.28) = 10.749$; $P = 0.003$) (Fig. 11). In contrast, single housed mice swam more 1 day post-treatment than 28 days post-treatment ($F(1.28) = 13.860$; $P = 0.001$) (Fig. 10). In addition, there was a treatment effect in single housed mice, in which LPS treatment prolonged the time of climbing activity, relative to vehicle treatment ($F(1.28) = 2.907$; $P = 0.099$) (Fig. 10). Independently of treatment, single housed mice floated for a longer time 28 days post-treatment than one day post-treatment ($F(1.28) = 7.997$; $P = 0.009$) (Fig. 10). Independently of time (acute vs. long term) there was also a treatment effect on single housed mice: LPS-treated mice spent a longer time floating than VEH-treated mice ($F(1.28) = 4.368$; $P = 0.046$) (Fig. 10).

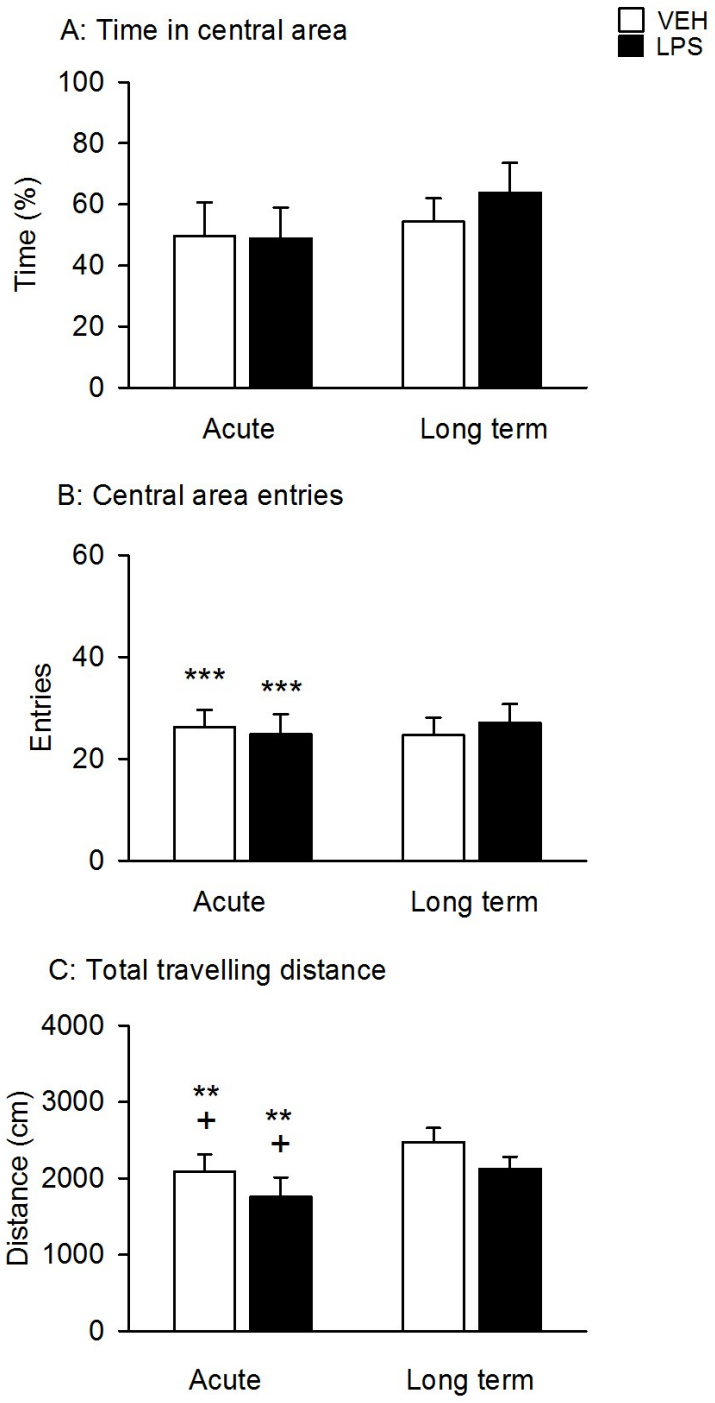

Fig. 12: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the behaviour of single housed C57BL/6J mice in the open field test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. A: Time the animal spent in the central area, expressed as a percentage of the duration of the test (5 min). B: Number of entries into the central area. C: Total travelling distance. The values represent means ± SEM, n = 7–8. **P < 0.05, ***P < 0.01 versus group housing, same treatment (Fig. 13), +P ≤ 0.1 versus long term, single housing, same treatment.

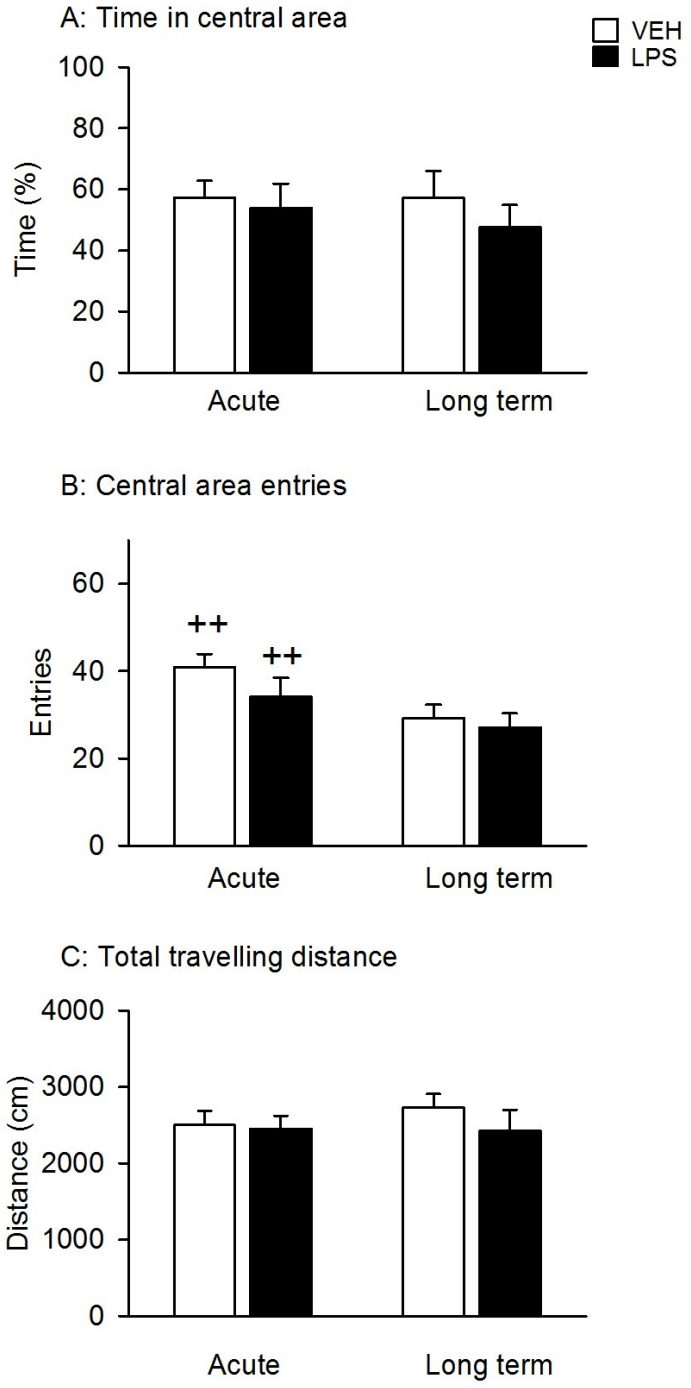

Fig. 13: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the behaviour of group housed C57BL/6J mice in the open field test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. A: Time the animal spent in the central area, expressed as a percentage of the duration of the test (5 min). B: Number of entries into the central area. C: Total travelling distance. The values represent means ± SEM, n = 7–8. ++P < 0.05 versus long term, group housing, same treatment.

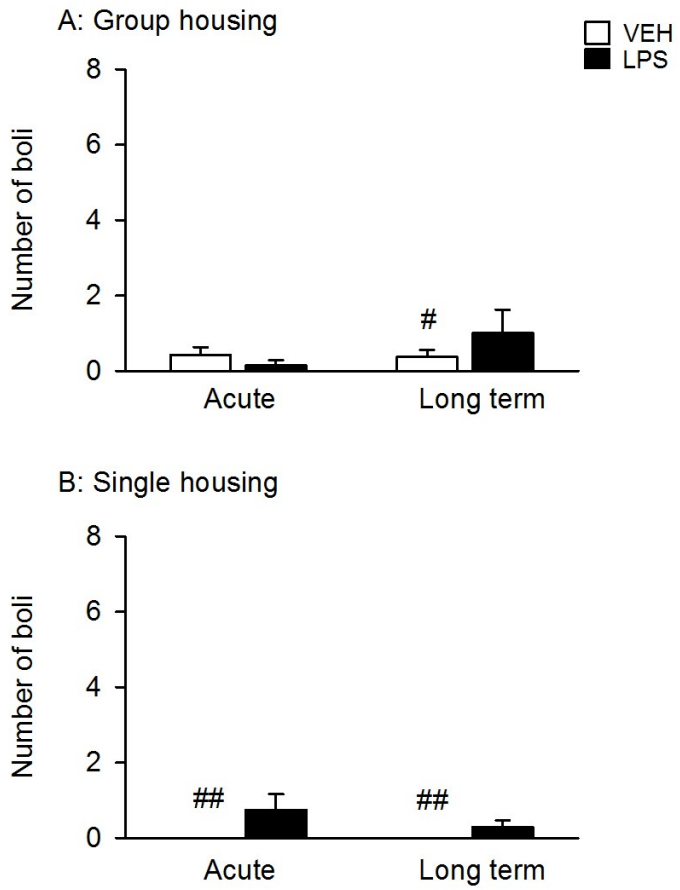

Fig. 14: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the expulsion of faecal pellets (boli) by C57BL/6J mice during the open field test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. The values represent means ± SEM, n = 7–8. #P ≤ 0.1, ##P < 0.05 versus LPS treatment, same housing, same post-treatment interval.

4.4.) Open field (OF) test in C57BL/6J mice

Three-way ANOVA displayed a significant time*housing effect ($F(1.52) = 3.755$; $P = 0.058$) on central area entries, and two-way ANOVA showed that in the acute paradigm there was a significant housing effect. Thus independently of treatment, group housed mice entered the central area more often than the single housed mice ($F(1.24) = 10.503$; $P = 0.003$) (Figs. 12 and 13). Similarly, there was a significant effect of housing on the total travelling distance in the acute paradigm, as single housed mice moved less than group housed mice ($F(1.24) = 6.793$; $P =$

0.015) (Figs. 12 and 13). The number of faecal pellets shed during the OF test were subject to a treatment effect in the long term paradigm: LPS-treated group housed mice expelled more boli than VEH-treated mice ($F(1.28) = 3.127$; $P = 0.086$) (Fig. 14). There was also a treatment effect in single housed mice, as LPS-treated mice shed more boli than VEH-treated mice ($F(1.26) = 4.764$; $P = 0.038$) (Fig. 14).

A significant time effect (acute term vs. long term) on the total travelling distance was found in single housed mice: independently of treatment, mice moved more in the long-term than in the acute paradigm ($F(1.26) = 3.446$; $P = 0.075$) (Fig. 12).

Further analysis by ANOVA revealed a time effect (acute vs. long term) on central area entries in group housed animals. Independent of treatment, 1 day post-treatment mice entered the central area more often than 28 days post-treatment mice ($F(1.26) = 7.489$; $P = 0.011$) (Fig. 13).

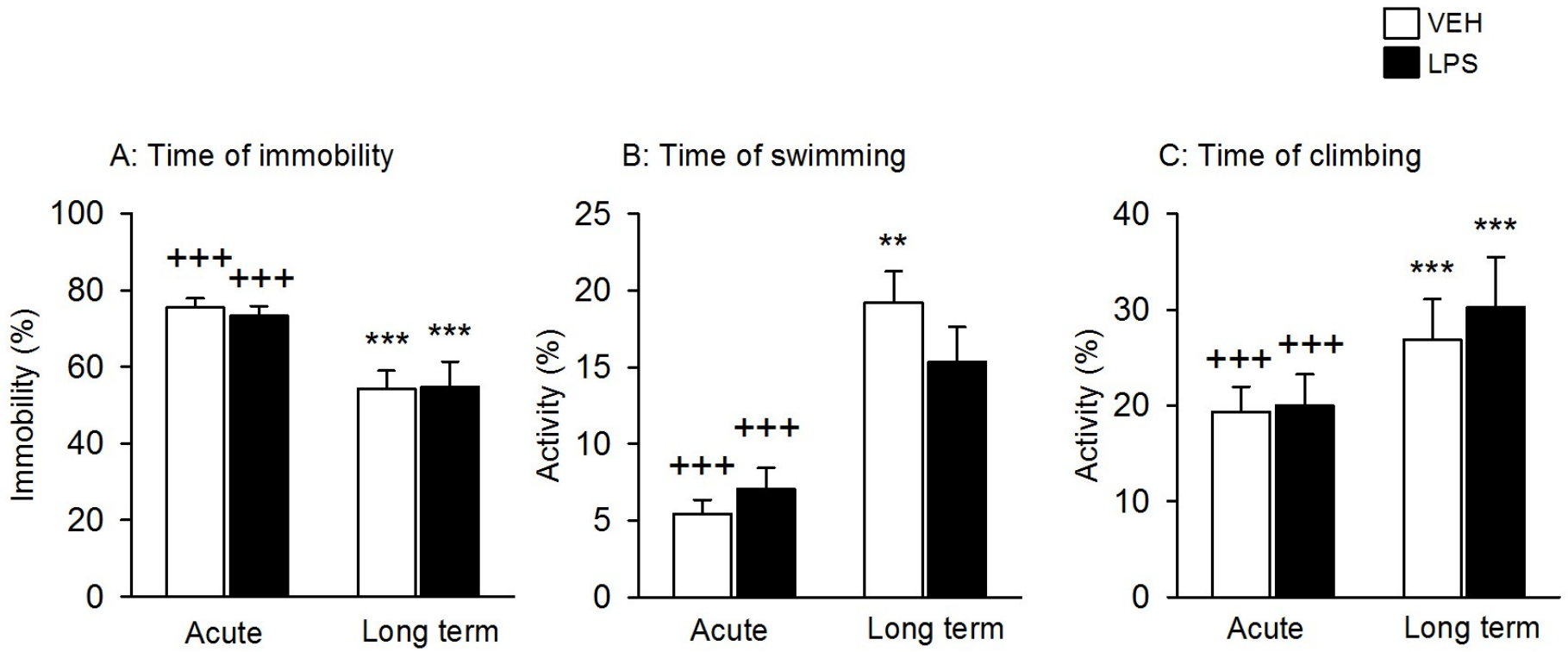

Fig. 15: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the behaviour of single housed C57BL/6J mice in the forced swim test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. A: Time the animal was immobile. B: Duration of swimming activity. C: Duration of climbing activity. The different activities are expressed as a percentage of the total test duration (6 min). The values represent means ± SEM, n = 7–8. +++P < 0.01 versus long term, single housing, same treatment; **P < 0.05, ***P < 0.01 versus group housing, same treatment (Fig. 16).

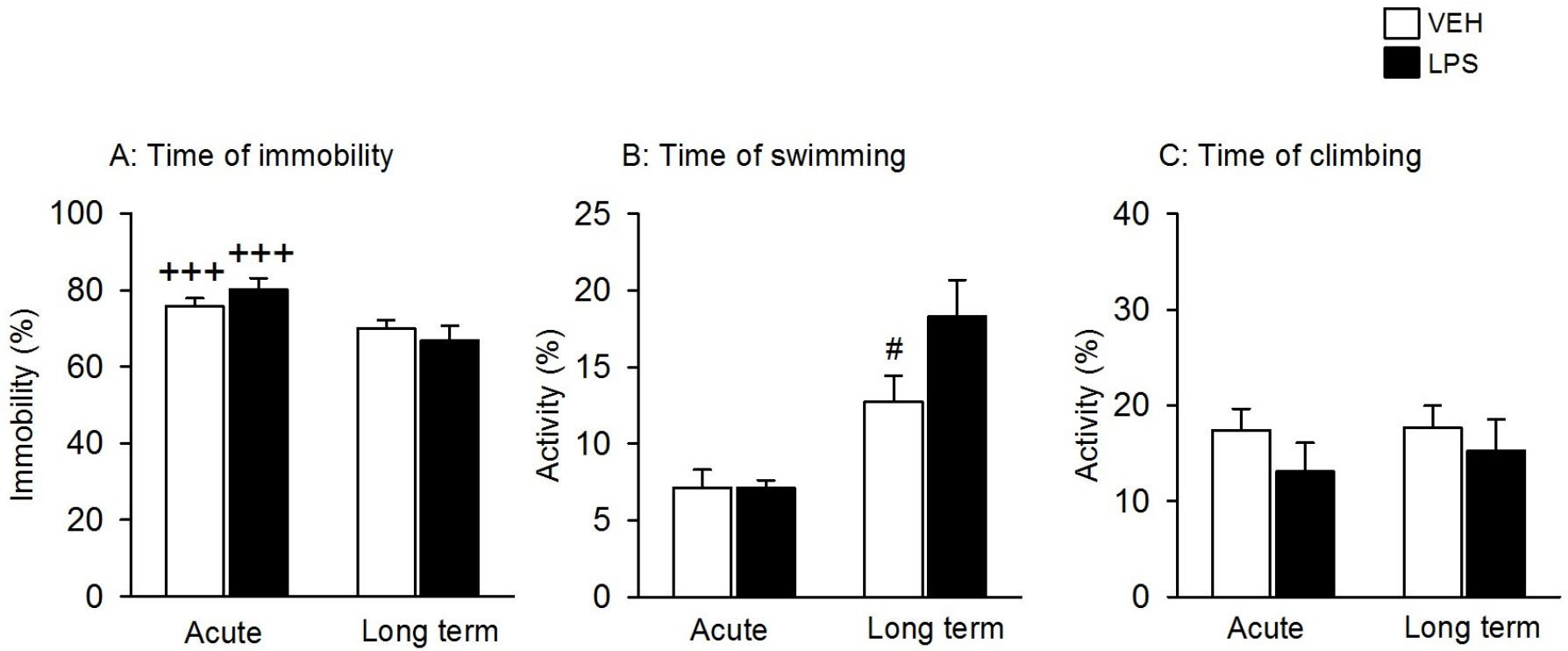

Fig. 16: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the behaviour of group housed C57BL/6J mice in the forced swim test. Acute: test performed 23 – 25 h post-treatment. Long term: test performed 28 days post-treatment. A: Time the animal was immobile. B: Duration of swimming activity. C: Duration of climbing activity. The different activities are expressed as a percentage of the total test duration (6 min). The values represent means ± SEM, n = 7–8. #P ≤ 0.1 versus single housing LPS treatment, same post treatment interval; +++P < 0.01 versus long term, group housing, same treatment.

4.5) Forced swim test (FST) in C57BL/6J mice

Three-way ANOVA showed a significant combined time*treatment*housing effect on swimming behaviour ($F(1.52) = 5.029$; $P = 0.029$) and a combined time*housing effect on floating ($F(1.52) = 3.404$; $P = 0.071$). Two-way ANOVA revealed a significant housing effect on the time of immobility in the long term paradigm: independently of treatment, group housed mice spent more time immobile than single housed mice ($F(1.28) = 8.672$; $P = 0.006$) (Figs. 15 and 16). There was also a significant housing effect on the climbing activity 28 days post-treatment as, independently of treatment single housed mice spent more time climbing than group housed mice ($F(1.28) = 9.614$; $P = 0.004$) (Figs. 15 and 16). Next a significant time effect (acute vs. long term) in the group housed mice was disclosed: group housed mice spent more time immobile 1 day post-treatment than 28 days post-treatment ($F(1.26) = 10.384$; $P = 0.003$) (Fig. 16).

In the long term paradigm a combined treatment*housing effect was found ($F(1.28) = 4.990$; $P = 0.034$). Post-hoc analysis showed a significant treatment effect inasmuch as group housed mice treated with LPS swam a longer time than VEH-treated mice ($t(14) = 1.904$; $P = 0.078$) (Fig. 16). In addition, VEH-treated single housed mice spent more time swimming than VEH-treated group housed mice ($t(14) = 2.436$; $P = 0.029$) (Fig. 15).

A significant time effect was observed in single housed mice which, independently of treatment, spent more time immobile ($F(1.26) = 17.880$; $P = 0.000$), but swam less ($F(1.26) = 36.955$; $P = 0.000$) and climbed less ($F(1.26) = 4.750$; $P = 0.039$) in the acute than in the long term paradigm (Fig. 15).

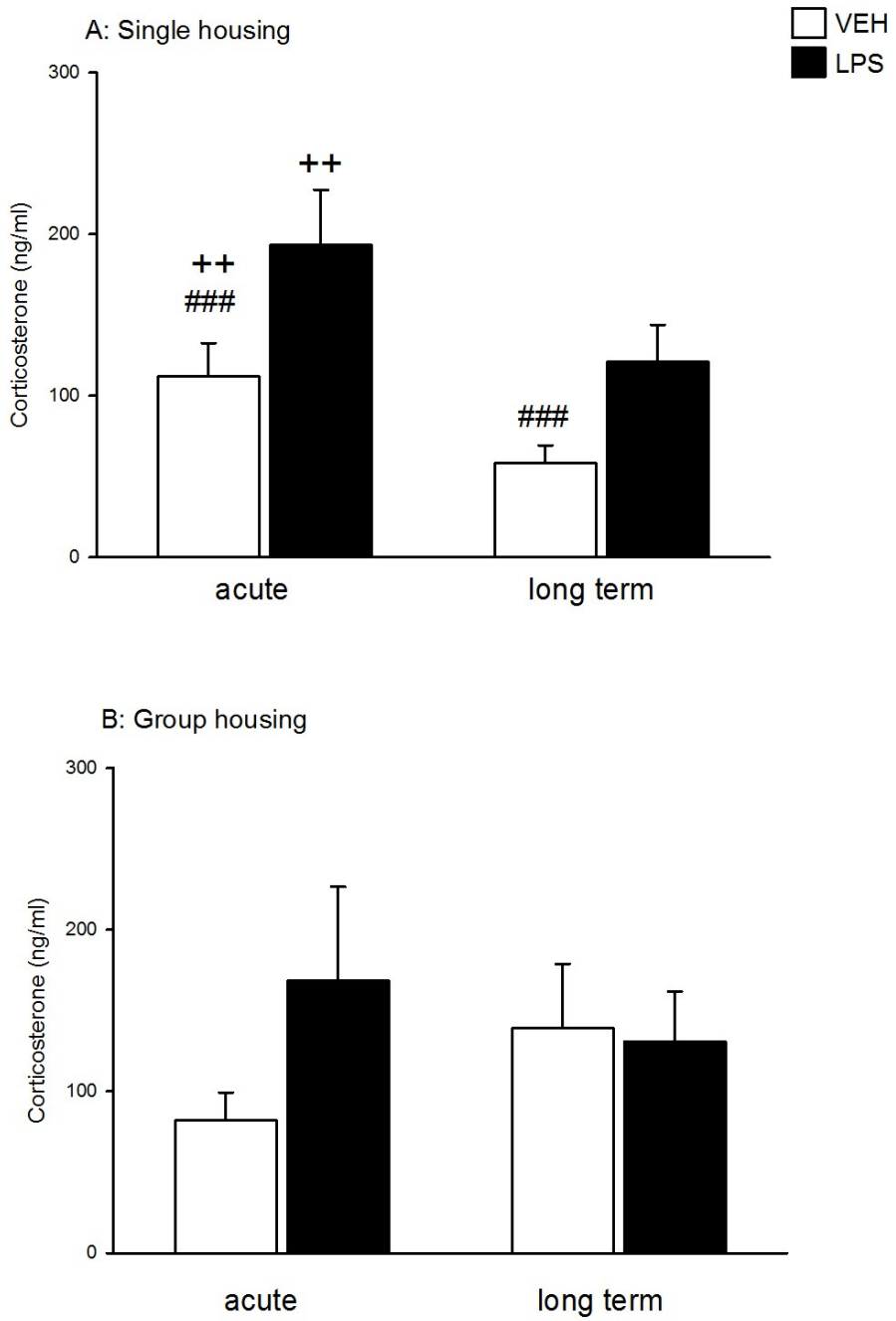

Fig. 17: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH), on the plasma levels of corticosterone in CD1 mice. Corticosterone was determined 30 minutes after the FST. A: Single housing; B: Group housing. The values represent means ± SEM, n = 7–8. ###P < 0.01 versus single housing, LPS treatment, same post-treatment interval; ++P < 0.05 versus long term, same housing, same treatment.

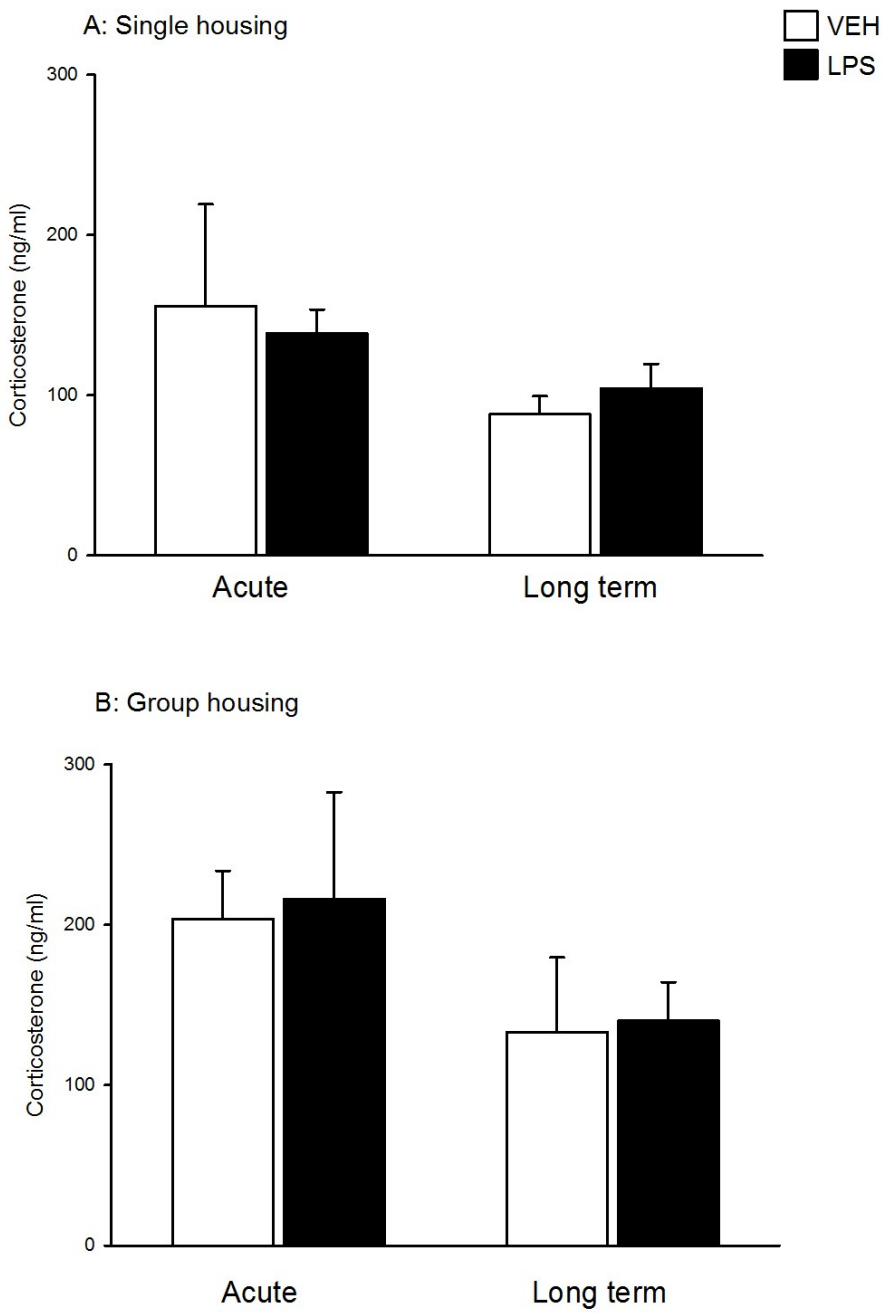

Fig. 18: Acute and long term effects of intraperitoneally injected LPS (0.83 mg/kg), relative to vehicle (VEH) on the plasma levels of corticosterone in C57BL/6J mice. Corticosterone was determined 30 minutes after the FST. A: Single housing; B: Group housing. The values represent means ± SEM, n = 7–8.

4.6) Circulating corticosterone in CD1 mice

Three-way ANOVA revealed a significant treatment effect ($F(1.55) = 6.211$; $P = 0.016$) and time*housing effect ($F(1.55) = 2.643$; $P = 0.100$) on the plasma level of corticosterone. Further analysis with two-way ANOVA showed a significant treatment effect ($F(1.28) = 9.263$; $P = 0.005$) and significant time effect ($F(1.28) = 7.041$; $P = 0.013$) in single housed mice (Fig. 17). This means that LPS-treated mice had higher plasma levels of corticosterone 30 minutes after the FST than VEH-treated mice (Fig. 17). In addition, regardless of treatment, single housed mice exhibited higher levels of circulating corticosterone in the acute than in the long term paradigm (Fig. 17).

4.7) Circulating corticosterone in C57BL/6J mice

Statistical analysis failed to reveal any differences in the circulating corticosterone levels due to treatment, housing or post-treatment interval (Fig. 18).

V) Discussion

5.1) Major objectives of the study

Preclinical data and clinical experience indicate that systemic immune stimulation elicits an acute sickness response and may evoke long-term depression in vulnerable individuals. The present investigation focussed on the short and acute sickness response and long-term effects of systemic immune stimulation by LPS on the emotional-affective behaviour in two different mouse strains (CD1 and C57BL/6J). In addition, the effect of social context (group vs. single housing) was also addressed

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Anxiety-related and locomotor behaviour was explored with the OF test where a reduction of central area entries and central area time is considered to reflect enhanced anxiety-related behaviour and the total travelling distance reflects locomotion (Belzung et al. 2001). Depression-like behaviour was explored with the FST, where activity was evaluated by the time the animal spent swimming, climbing and floating (immobility). In this test immobility is counted as depression-like behaviour. The major results of the study are: a) the social context has an effect on anxiety-like behaviour: group housing has a protective function on anxiety due to an intervention; b) this effect is depends on the mouse strain; c) acute immune stimulation with LPS has variable effects on anxiety- and depression-like behaviour; d) systemic LPS does not seem to be the most appropriate model to study the influence of immune stimulation on anxiety and depression.

5.2) Observations in CD1 mice

With regard to the acute effects of LPS, the present data indicate that the group housing has a protective function. As it is shown in Fig. 5B, LPS influenced body temperature in CD1 single housed mice but not in CD1 group housed mice. Moreover, the results show that single housed mice reacted more clearly to the different treatments (VEH or LPS) than group housed mice. For instance, LPS-treated single housed mice showed a treatment effect in the reduction of rectal temperature

whereas this effect was not seen in group housed mice. Importantly, LPS reduced the rectal temperature only in single-housed but not in group housed CD1 mice, which indicates that it had a systemic effect at the dose tested.

The OF revealed distinct differences in the anxiety-related and locomotor activity of the mice (Figs. 7 and 8): While there was no effect in the long term, in the acute paradigm single housed VEH-treated mice displayed a more pronounced anxiety-like behaviour than group housed mice. Further on, in the acute setting single housed mice appeared more anxious than in the long term setting, whereas locomotion remained unaltered. LPS affected the OF behaviour in group housed mice in the acute setting, inasmuch as it enhanced anxiety-like behaviour and reduced locomotion. In addition, defaecation was also influenced, inasmuch as group-housed mice expelled less faecal pellets than single housed mice, and LPS reduced defaecation further (Fig. 9). This result indicates that the dose of LPS was too low to cause diarrhoea, but sufficient to influence defaecation as an index of the autonomic output of emotional-affective behaviour.

In the FST, treatment-, time- and housing-related differences in depression-like behaviour (stress-coping behaviour) were observed. In single housed CD1 mice, LPS prolonged the time of climbing and shortened the time of immobility both in the acute and long-term paradigm, while in group housed mice such an effect of LPS was encountered in the long-term setting only (Figs. 10 and 11). In addition, depression-like behaviour was more pronounced 28 days than 1 day post-treatment (single housed mice). Importantly, housing and time interacted with each other in affecting the behaviour in the FST. Thus, in the acute setting, single housed mice exhibited a more active stress-coping behaviour (swimming) than in the long-term setting, whereas in group housed mice the opposite was the case (compare Figs. 10 and 11).

5.3) Role of social context in time-related behavioural alterations following an intervention in CD1 mice

All things considered, the present data point out that social community plays a role in the establishment of short-term sickness behaviour and delayed depression-like behaviour following an intervention.

1) Rectal temperature: Group housed mice did not respond to LPS with a change in rectal temperature whereas in single housed mice LPS reduced rectal temperature relative to VEH. As it is known that mice react, contrary to other rodents, to an acute LPS infection with reduced body temperature (Fraifeld et al. 1998) (as CD1 single housed mice did), these data suggest that social community has a protective effect against acute LPS immune stimulation.

2) Anxiety-like behaviour and locomotion in the OF test: While there was no housing and treatment effect in the long term, anxiety-like behaviour in the acute phase after intervention depended on the housing conditions. Thus, VEH-treated group housed mice were less anxious than single housed mice. LPS enhanced anxiety like behaviour only in group-housed mice but did not significantly affect locomotion. Provided that the intraperitoneal administration of LPS was effective as deduced from its effect on rectal temperature in single-housed mice, it has to be assumed that housing affects anxiety-like behaviour. Group housed mice were settled into a social community which helped them calming down after the intraperitoneal VEH administration, so they were able to cope with this situation and were less anxious in the OF test. Single housed VEH treated mice were not able to calm down in the absence of a social community after the stressful event of VEH administration and showed decreased activity in the OF central area, which can be interpreted that single housed mice are more anxious than group housed mice. This interpretation is supported by the time effect, given that in single housed CD1 mice central area activity was reduced in the

acute but not long term paradigm. Since there was no effect on locomotion, the change in central area activity most likely reflects a change in anxiety-related behaviour. A change in locomotor activity is often a confounding factor in the interpretation of OF test results, which in the present study was not the case. The protective effect of social context against the behavioural disturbances of an acute intervention is further supported by the finding that there was no treatment and housing effect in the long term group. The present experiments reveal that in terms of rectal temperature, anxiety-like behaviour and locomotion, an acute intervention with VEH or LPS fails to have a long-term effect.

3) Depression-like behaviour in the FST: It has been shown that depression-like behaviour can be induced by systemic administration of LPS, as deduced from an increase of the duration of immobility during the FST (Frenois et al. 2007). Unexpectedly, in the present study, LPS had the opposite effect. VEH-treated single housed mice spent more time immobile than LPS-treated single housed mice. Interestingly, in both, single and group housed animals the effect of LPS was seen even 28 days after treatment.

Furthermore, there was an interaction between the housing and time effect. Whereas single housed mice displayed more active stress-coping behaviour (swimming) in the acute than in the long term setting, group housed mice exhibited less swimming activity in the acute than in the long term paradigm.

As mentioned in the Introduction, several mechanisms may lead to major depressive disorders and anxiety-like behaviour. In this experiment I focussed on the impact of a social (single vs. group housing) and a time (acute vs. long term) component in response to a peripheral intervention stimulating the immune system. LPS influences depression and anxiety-like behaviour through activating innate immune cells which produce pro-inflammatory cytokines (Dantzer et al. 2008). The results of this study do not fully conform to the literature. On the one hand they confirm that acute LPS

treatment affects anxiety behaviour in the OF test (Konsman et al. 2002; O'Connor et al. 2008) but, on the other hand, they disagree with reported findings that LPS promotes depression-like behaviour (Frenois et al. 2007; Dantzer et al. 2008; O'Connor et al. 2008).

In the present study, LPS appeared to promote an active stress-coping strategy. Possible reasons for this different outcome in the FST may be:
a) the dose of LPS which depending on many experimental conditions may display variable immune-stimulating activity, although the dose used here (0.83mg/kg) has previously been shown to cause depression-like behaviour (Frenois et al. 2007); b) the type of LPS which may have been insufficiently active in stimulating the immune system because its effect to stimulate cytokine production was not controlled; c) the FST which may have been inadequate in assessing the depression-like behaviour, although this test has previously been used to demonstrate the acute depressogenic effect of LPS (Frenois et al. 2007; O'Connor et al. 2008); d) personal bias that may have distorted the outcome of the FST, the parameters of which were subjectively estimated; e) a shift in observer's criteria to judge immobility, swimming and climbing over the course of the study; and f) factors that affected the breeding, housing and transportation of the animals and had a long-term influence on their emotional-affective behaviour. Despite consideration of these limiting factors it may also be concluded that systemic LPS is an inappropriate stimulus to examine the effect of immune stimulation on emotional-affective behaviour.

4) Corticosterone**:** The pertinent results show that single housing results in higher levels of circulating corticosterone and that LPS increased this parameter only in single housed but not in group housed mice. This finding underlines the concept that a social environment has a preventive effect on the neuroendocrine aspects of stress and immune activation.

5.4) Observations in C57BL/6J mice

With regard to the acute effects of LPS, the present data indicate that the group housing has a protective function. As shown in Fig. 6B, LPS influenced body temperature in single housed C57BL/6J mice but not in group housed C57BL/6J mice. Moreover, the results show that single housed mice reacted more clearly to the different treatments (VEH or LPS) than group housed mice. For instance, single housed mice reacted to LPS with a reduction of rectal temperature whereas this effect was not seen in group housed mice (Fig. 6). Importantly, LPS reduced the rectal temperature only in single-housed but not in group housed C57BL/6J mice, which indicates that it had a systemic effect at the dose tested.

While there was no effect in the long term, in the acute paradigm single housed VEH-treated mice displayed a more pronounced anxiety-like behaviour than group housed mice. Further on, in the acute setting single housed mice appeared more anxious than in the long term setting, as deduced from reduced central area entries and locomotion.

Unlike in CD1 mice, LPS did not have any influence on the OF behaviour in C57BL/6J mice in any setting (single vs. group, acute vs. long term). In contrast, defaecation was influenced by LPS in a manner inverse to the LPS effect in CD1 mice. In the single housed setting, C57BL/6J LPS-treated mice expelled more faecal pellets than its VEH-treated mates.

In the FST, time- and housing related differences in depression-like behaviour were observed. Contrary to CD1 mice, depression-like behaviour was more pronounced 1 day than 28 days post-treatment. In addition, housing and time interacted with each other in the FST: in the long term setting, single housed mice exhibited a more active stress coping behaviour (swimming) than in the acute setting, whereas there was no significant effect in group housed mice.

Furthermore, single housed mice of the long term setting showed decreased signs of depression relative to their group housed mates. These FST data obtained in

C57BL/6J mice differ from those obtained in CD1 (compare Figs. 10; 11; 15 and 16).

5.5) Role of social context in time-related behavioural alterations following an intervention in C57BL/6J mice

1) Rectal temperature: Group housed mice did not respond to LPS, relative to VEH, whereas single housed mice showed a sickness response as demonstrated by a decrease of rectal temperature. As it is known that mice react to an acute LPS infection with reduced body temperature, these data suggest that social community has a protective function against acute LPS immune stimulation (Fraifeld et al. 1998), an effect that was seen here both in CD1 and C57BL/6J mice.

2) Anxiety-related and locomotion in the OF test: The only difference seen in this test was that single housed mice were more anxious than group housed mice in the acute setting, whereas no treatment effect was observed.

3) Depression-like behaviour in the FST: In the FST, single housed mice exhibited reduced depression-like behaviour in the long term paradigm relative to their group housed mates. Unlike in CD1 mice, however, the effect of social housing in C57BL/6J mice is only seen in the long term. Possible reasons for this effect may be analogous to those discussed above for CD1 mice. In addition, C57BL/6J mice differ genetically from other mouse strains with regard to the serotonin 5-hydroxytryptamine (5-HT) transporter (SERT). Recombinant inbred mouse lines show multiple different serotonin receptor associated phenotypes, and the commonly used mouse strain C57BL/6J features a SERT haplotype defined by two nonsynonymous coding variants: Gly-39 and Lys-152(GK). The GK variant is thought to have a reduced synaptic 5-HT uptake (Carneiro et al. 2009). This could mean that the C57 mouse strain may be inappropriate to study behavioural processes involving 5-HT signalling.

5.6) Conclusions

The major conclusions that can be drawn from this study are:
a) social context has a positive impact on anxiety and depression-like behaviour; b) this impact is mouse-strain-dependent; c) acute intervention with LPS has time-related effects on anxiety and depression-like behaviour but the behavioural alterations are variable; d) systemic LPS does not seem to be the most appropriate model to study the influence of immune stimulation on anxiety and depression.

Although the present study attempted to model the impact of immune stimulation by LPS on anxiety and depression, the results do not directly prove such an association, for which there is increasing clinical evidence. For instance there is a case report on “Normalization of the increased translocation of endotoxin from gram negative enterobacteria (leaky gut) is accompanied by a remission of chronic fatigue syndrome (CFS)” (Maes et al. 2007), where a 13 year old girl with CFS had high values of serum IgM against LPS of enterobacteria. She received a combined treatment with antioxidants, leaky gut diet and intravenous immune globulin, which helped her to recover from the syndrome. This outcome was interpreted to indicate that translocation of bacteria through the leaky gut can evoke a local and systemic immune response which contributes to the manifestation of CFS, which shows many similarities with depressive disorders. It is also known that people with major depressive disorders show higher blood levels of IgM and IgA against LPS of enterobacteria due to damage of the gut mucosa (Maes et al. 2007). Further experiments are required to understand the mechanisms that link gut mucosal damage with immune activation and depressive disorders. In order to study this relationship experimentally it is necessary to establish new models that reflect the clinical situation as closely as possible. Possible approaches towards this goal could be: a) treatment with small doses of substances found in human food that damage the gut mucosal barrier; b) treatment with substances that damage the gut mucosal barrier should be continued for some time to mimic the situation as it occurs in patients. Experiments along these lines will comply with the clinical reality more closely than systemic administration of a single LPS dose.

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