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RESEARCH

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Electroconvulsive seizures (ECS) do not prevent LPS-induced behavioral alterations and microglial activation

E. M. van Buel^{1,3} , F. J. Bosker², J. van Drunen¹, J. Strijker¹, W. Douwenga¹, H. C. Klein^{2,3} and U. L. M. Eisel^{1,2*}

Abstract

Background: Long-term neuroimmune activation is a common finding in major depressive disorder (MDD). Literature suggests a dual effect of electroconvulsive therapy (ECT), a highly effective treatment strategy for MDD, on neuroimmune parameters: while ECT acutely increases inflammatory parameters, such as serum levels of pro-inflammatory cytokines, there is evidence to suggest that repeated ECT sessions eventually result in downregulation of the inflammatory response. We hypothesized that this might be due to ECT-induced attenuation of microglial activity upon inflammatory stimuli in the brain.

Methods: Adult male C57Bl/6J mice received a series of ten electroconvulsive seizures (ECS) or sham shocks, followed by an intracerebroventricular (i.c.v.) lipopolysaccharide (LPS) or phosphate-buffered saline (PBS) injection. Brains were extracted and immunohistochemically stained for the microglial marker ionized calcium-binding adaptor molecule 1 (Iba1). In addition, a sucrose preference test and an open-field test were performed to quantify behavioral alterations.

Results: LPS induced a short-term reduction in sucrose preference, which normalized within 3 days. In addition, LPS reduced the distance walked in the open field and induced alterations in grooming and rearing behavior. ECS did not affect any of these parameters. Phenotypical analysis of microglia demonstrated an LPS-induced increase in microglial activity ranging from 84 to 213 % in different hippocampal regions (CA3 213 %; CA1 84 %; dentate gyrus 131 %; and hilus 123 %). ECS-induced alterations in microglial activity were insignificant, ranging from -2.6 to 14.3 % in PBS-injected mice and from -20.2 to 6.6 % in LPS-injected mice.

Conclusions: We were unable to demonstrate an effect of ECS on LPS-induced microglial activity or behavioral alterations.

Keywords: ECS, Depression-like behavior, LPS, Inflammation, Microglia

Background

Electroconvulsive therapy (ECT) is a highly effective clinical treatment strategy for major depressive disorder (MDD). Multiple studies have shown superior efficacy for ECT compared to pharmaceutical treatment strategies [1]. Over the past decades, numerous studies have been published that have attempted to unravel the neurobiological

mechanisms behind the antidepressant effects of ECT. These studies have resulted in a rapid increase in the understanding of these mechanisms, with one of the most consistent findings being a strong effect on neurogenesis accompanied by increased levels of neurotrophic factors [2–16]. Nevertheless, many questions remain unanswered.

Long-term neuroimmune activation is a common finding in MDD. MDD is associated with elevated levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines [17]. In addition, there is evidence for altered functionality of immune cells, including increased activity of microglia, the resident immune cells of the brain [18]. Literature suggests that antidepressant

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treatment may, at least in part, normalize neuroimmune changes associated with MDD [19–21].

Although literature on the effects of ECT on neuroimmune parameters is relatively scarce, the available literature points towards a dual effect: while ECT acutely increases inflammatory parameters, repeated ECT sessions eventually result in a downregulation of the inflammatory response [22–25]. For example, although acute ECT upregulates serum levels of pro-inflammatory cytokines such as TNF α , IL-1 β , and IL-6 in MDD patients [22–24], serum TNF α levels gradually decrease over the course of multiple ECT sessions until they eventually reach levels within the range of healthy control subjects [22]. In addition, animal studies have provided evidence for acute transient microglia activation upon electroconvulsive seizures (ECS; an animal model for ECT) [26].

Based on these findings, we hypothesized that repeated ECT sessions suppress the activation of resident immune cells upon inflammatory stimuli in the brain. This might explain the downregulation of inflammatory parameters and clinical improvement as observed in successfully treated MDD patients. To test this hypothesis, we exposed mice that had received a series of ECS to a pro-inflammatory stimulus (an intracerebroventricular lipopolysaccharide injection) inducing depressive-like behavior. We assessed the extent of microglia activation in the hippocampus upon this inflammatory stimulus with an ionized calcium-binding adaptor molecule 1 (Iba1) immunohistochemical staining. In addition, behavioral tests were performed to investigate whether ECS could prevent behavioral alterations associated with inflammation.

Methods

Animals

Three-month-old male C57Bl/6J mice were obtained from Janvier. Animals were housed in individual cages under standardized conditions (temperature 21 \pm 2 $^{\circ}$ C, humidity 50–60 %, 12:12 h light/dark cycle). Food and water were available ad libitum. Animals were handled for at least 1 week prior to the start of the experiment. Throughout the experiment, body weight was measured on a daily basis. The Ethical Committee for the Use of

Experimental Animals of the University of Groningen had approved all experiments (DEC6229G).

Experimental design

Figure 1 shows the experimental design that was followed during this study. Mice ($n = 40$) were equally divided across four groups (PBS + sham, PBS + ECS, LPS + sham, and LPS + ECS). A sucrose preference test was started 6 days prior to the first ECS treatment and was continued throughout the experiment. ECS or sham ECS was performed daily for 10 days. Lipopolysaccharide (LPS) or phosphate-buffered saline (PBS) injections were delivered 1 day after the last ECS/sham treatment. Three days after the LPS/PBS injections, an open-field test was performed and mice were sacrificed. In previous experiments, this timepoint was associated with maximal neuroinflammation [27].

Electroconvulsive seizures

Mice received a series of one electroconvulsive or sham treatment per day for 10 days. Mice were anesthetized with sevoflurane, and electroconvulsive shocks were delivered via ear clip electrodes using a pulse generator (Ugo Basile, Comerio, Italy), set at the following shock parameters: 80 mA, 50 Hz, 1 s duration, and 0.5 ms pulse width. All mice developed tonic-clonic seizures lasting approximately 10–15 s, which is comparable to the seizure duration induced by electroconvulsive seizures (ECS) in other experiments [28–30]. The total duration of anesthesia was approximately 5 min per mouse. Mice in the sham groups underwent the same procedure, including sevoflurane anesthesia and placement of the ear clip electrodes, without delivery of the electroconvulsive shock.

Intracerebroventricular LPS injections

Mice were anesthetized with sevoflurane and fixated in a stereotact. Analgesia was provided via a subcutaneous injection of 2.5 mg/kg finadyne. A skin incision was made to expose the skull, and holes were drilled perpendicularly to the skull. Mice were injected with 1 μ l PBS or 1 μ l 5 mg/ml LPS (l-6529, serotype 055:B5, Sigma-Aldrich) in PBS at the following coordinates: -2.5 mm

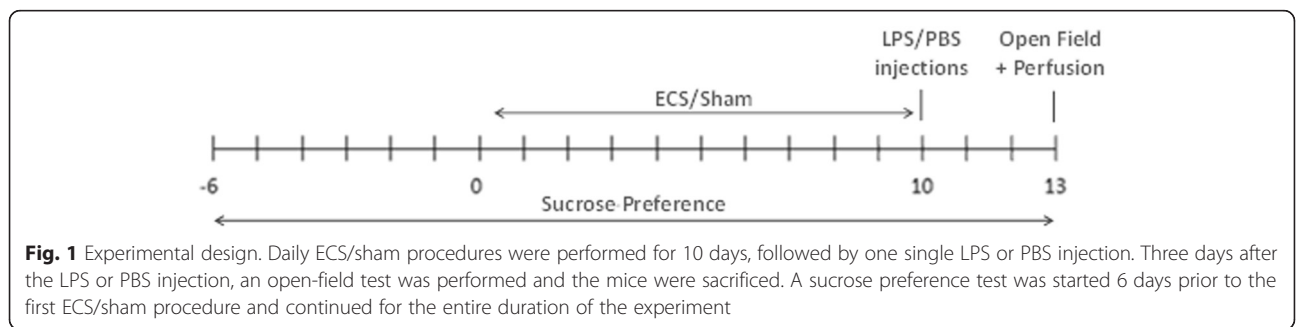


Fig. 1 Experimental design. Daily ECS/sham procedures were performed for 10 days, followed by one single LPS or PBS injection. Three days after the LPS or PBS injection, an open-field test was performed and the mice were sacrificed. A sucrose preference test was started 6 days prior to the first ECS/sham procedure and continued for the entire duration of the experiment

dorsal/ventral, -1.0 mm lateral, and -0.5 mm anterior/posterior from bregma. Injections were performed at a constant rate of 0.3 $\mu\text{l}/\text{min}$ using a syringe pump (TSE Systems, Bad Homburg, Germany) in combination with a 25 - μl Hamilton syringe connected to a 1 - μl Hamilton needle (cat. nr.170431, Omnilabo). The needle was not removed from the brain until 5 min after the injection to prevent any leakage of LPS via the injection canal. The holes were sealed with dental cement, and the incision was closed with sutures.

Sucrose preference test

Mice were given a choice between two drinking pipettes, one containing normal drinking water and the other containing a 0.5 % sucrose solution. Mice had access to both pipettes 24 h per day. Water and sucrose intake were measured on a daily basis. The relative location of both pipettes was switched on a daily basis to avoid place preference. Mice that showed such a place preference and therefore failed to develop a consistent preference for sucrose were excluded from subsequent analysis.

Open-field test

Mice were placed in a round arena with a diameter of 50 cm consisting of two zones: a border zone adjacent to the wall of the arena and an inner zone in the center of the arena. Mice were allowed to explore their surroundings freely for 7 min. All experiments were recorded by an overhead camera. Distance moved and time spent in the different zones were scored using EthoVision software (Noldus Information Technology, Wageningen, Netherlands). An observer blinded to the treatment scored grooming and rearing behavior manually.

Iba1 immunohistochemistry

Mice ($n = 6$ per group) were transcardially perfused with a saline solution containing 0.5 % heparin followed by a 4 % paraformaldehyde (PFA) solution in 0.1 M phosphate buffer. Brains were removed and postfixed for 24 h in 4 % PFA in 0.1 M PBS, followed by 18 h in a 30 % sucrose solution for cryoprotection. Brains were frozen using liquid nitrogen and cut into sections of 20 μm . A DAB staining for the microglial marker Iba1 was performed on free-floating hippocampal and prefrontal cortex (PFC) sections. Sections were incubated for 72 h with primary antibody (rabbit anti-Iba1, Wako Chemicals, Neuss, Germany) diluted $1:2500$ in 0.01 M PBS (pH 7.4) containing 1 % bovine serum albumin (BSA) and 0.1 % triton X-100. Subsequently, sections were incubated with secondary antibody ($1:500$; goat anti-rabbit, Jackson ImmunoResearch, Suffolk, UK) for 2 h, followed by a 1 -h incubation with avidin-biotin complex ($1:500$; Vector Laboratories Burlingame, CA, USA). Finally, 0.075 mg/ml DAB was added and the

DAB reaction was initiated with 100 μl 0.1 % H_2O_2 . Sections were carefully rinsed with 0.01 M PBS between all steps of the protocol, and all antibodies and chemicals were diluted in 0.01 M PBS unless stated otherwise.

Microglial activation was measured with Image-Pro software (Image-Pro Plus 6.0.0.26, Media Cybernetics, Inc. Rockville, USA) using a method described by Hovens et al. [31]. In short, photographs were taken at $\times 100$ magnification. The software first automatically determined the total coverage of the microglia cells (cell bodies and processes). Subsequently, intensity and size thresholds were changed in such a way that only the coverage of the cell bodies was measured. The cell bodies to total coverage ratio was calculated. This ratio was shown to accurately reflect activation state in previous experiments [31]. In addition, the number of cells was counted manually.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Most data was statistically analyzed using a two-way ANOVA to calculate p values for the main effects of group (LPS/PBS) and treatment (ECS/sham) and the group \times treatment interaction effect. Body weight was analyzed via a repeated measures ANOVA with post hoc Bonferroni test. Data are presented as mean \pm standard error.

Results

LPS decreases body weight

Figure 2 shows body weight as a percentage of baseline body weight (measured on day 0 prior to the first ECS/sham session; see also Additional file 1). Body weight was not significantly influenced by ECS treatment. However, intracerebroventricular (i.c.v.) LPS injections resulted in a marked decrease in body weight in both the ECS and sham groups. Body weight partly normalized 3 days after LPS injection. This is in line with previous experiments that showed that body weight is decreased up to 3 days after LPS injection [27]. I.c.v. PBS injection did not result in significant alterations in body weight.

LPS acutely decreases sucrose preference

Figure 3a shows sucrose preference over the entire duration of the experiment (see also Additional file 2). During the habituation phase prior to the start of the ECS sessions, sucrose preference gradually increased to around 80 %. Sucrose preference was not influenced by electroconvulsive seizures. I.c.v. LPS injections caused a temporary decrease in sucrose preference in both the ECS and sham groups, which fully normalized 3 days after injection. An area under the curve (Fig. 3b, Additional file 2) was calculated for the time between LPS injection and the end of the experiment (days 10 – 13 ; the gray area in a).

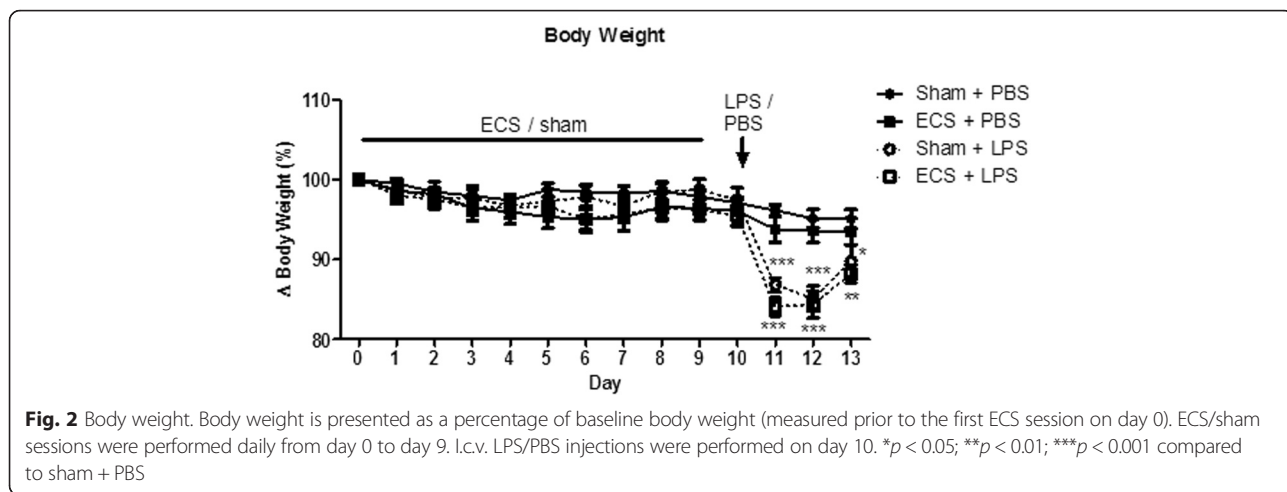


Fig. 2 Body weight. Body weight is presented as a percentage of baseline body weight (measured prior to the first ECS session on day 0). ECS/sham sessions were performed daily from day 0 to day 9. I.c.v. LPS/PBS injections were performed on day 10. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to sham + PBS

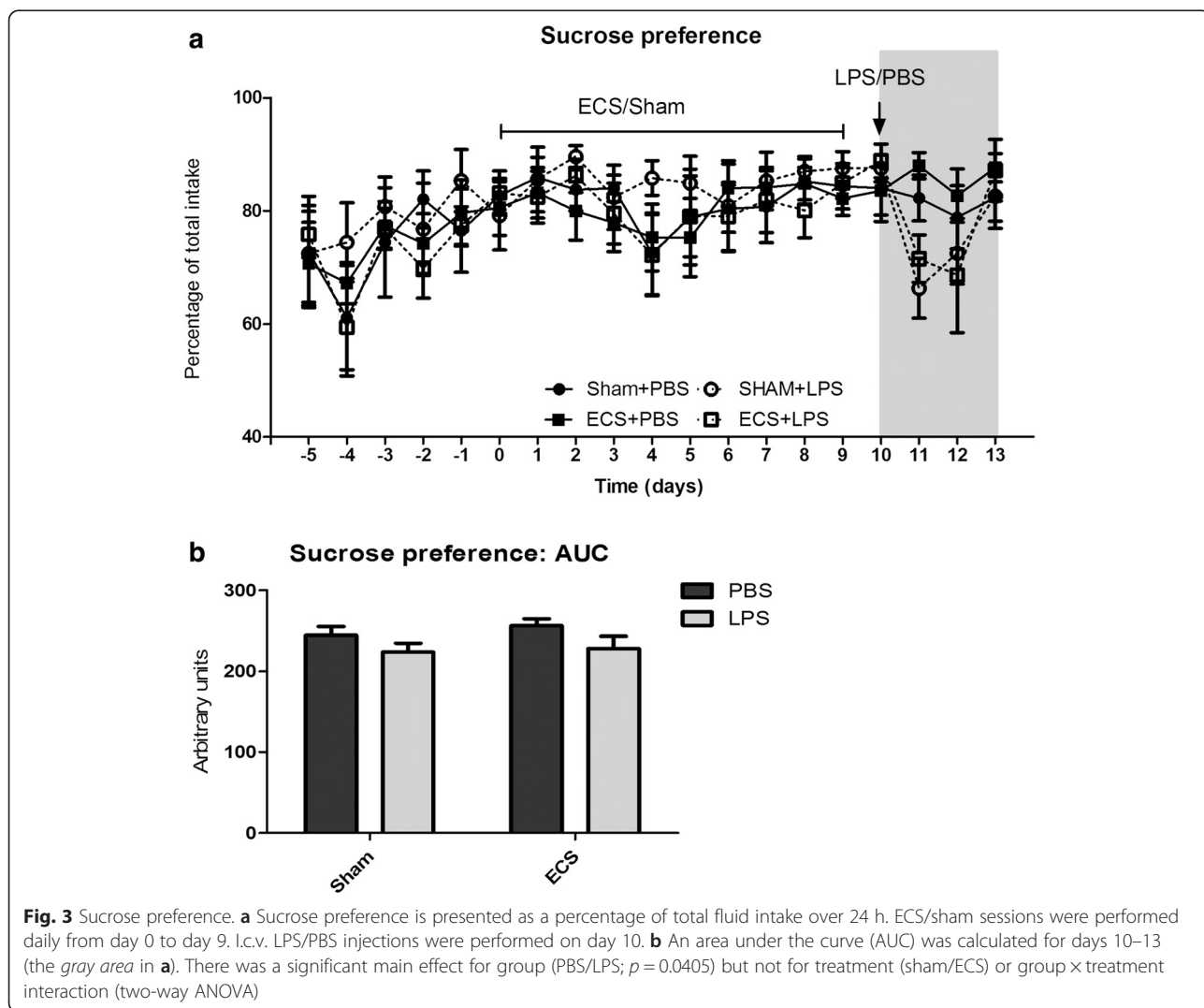


Fig. 3 Sucrose preference. **a** Sucrose preference is presented as a percentage of total fluid intake over 24 h. ECS/sham sessions were performed daily from day 0 to day 9. I.c.v. LPS/PBS injections were performed on day 10. **b** An area under the curve (AUC) was calculated for days 10–13 (the gray area in **a**). There was a significant main effect for group (PBS/LPS; $p = 0.0405$) but not for treatment (sham/ECS) or group \times treatment interaction (two-way ANOVA)

The area under the curve (AUC) was analyzed via a two-way ANOVA, demonstrating a significant main effect for group (LPS/PBS; $p = 0.0405$). No significant effects were found for treatment or treatment \times group interaction ($p = 0.7372$ and $p = 0.4808$, respectively).

Both LPS and ECS influence behavior in the open-field test

Distance moved and time spent in the border zone of the arena (Fig. 4a, b; Additional file 3) were scored automatically. LPS decreased the total distance moved during the test regardless of electroconvulsive seizures (main effect for group: $p = 0.0361$). No significant effects were observed for sham/ECS treatment and treatment \times group interaction ($p = 0.4525$ and $p = 0.9956$, respectively).

The time spent in the border zone was significantly increased in the ECS groups (main effect for sham/ECS treatment: $p = 0.005$). No significant differences were observed for the PBS/LPS group effect and the treatment \times group interaction effect ($p = 0.6323$ and $p = 0.4739$, respectively).

Grooming and rearing behavior (Fig. 4c, d; Additional file 3) were scored manually. In both the ECS and sham groups, LPS increased grooming behavior (main effect for group: $p = 0.0334$) but decreased rearing behavior (main effect for group: $p = 0.0159$). The main effect for sham/ECS treatment and the treatment \times group interaction effect were not significant for either behavior (grooming: $p = 0.3702$ and $p = 0.2830$, respectively; rearing: $p = 0.1126$ and $p = 0.9521$, respectively).

ECS does not prevent LPS-induced microglial activity

The depression-like behavioral effects of LPS are believed to be related to their inflammatory effects. Here, we were interested in the effect of ECS on LPS-induced inflammatory changes in the brain. Therefore, we measured the ratio of the microglia cell bodies to the total coverage of cell bodies and processes. As activated microglia undergo a conformational change towards shorter and thicker processes and increased size of the cell bodies, this ratio reflects the level of microglial activity [31].

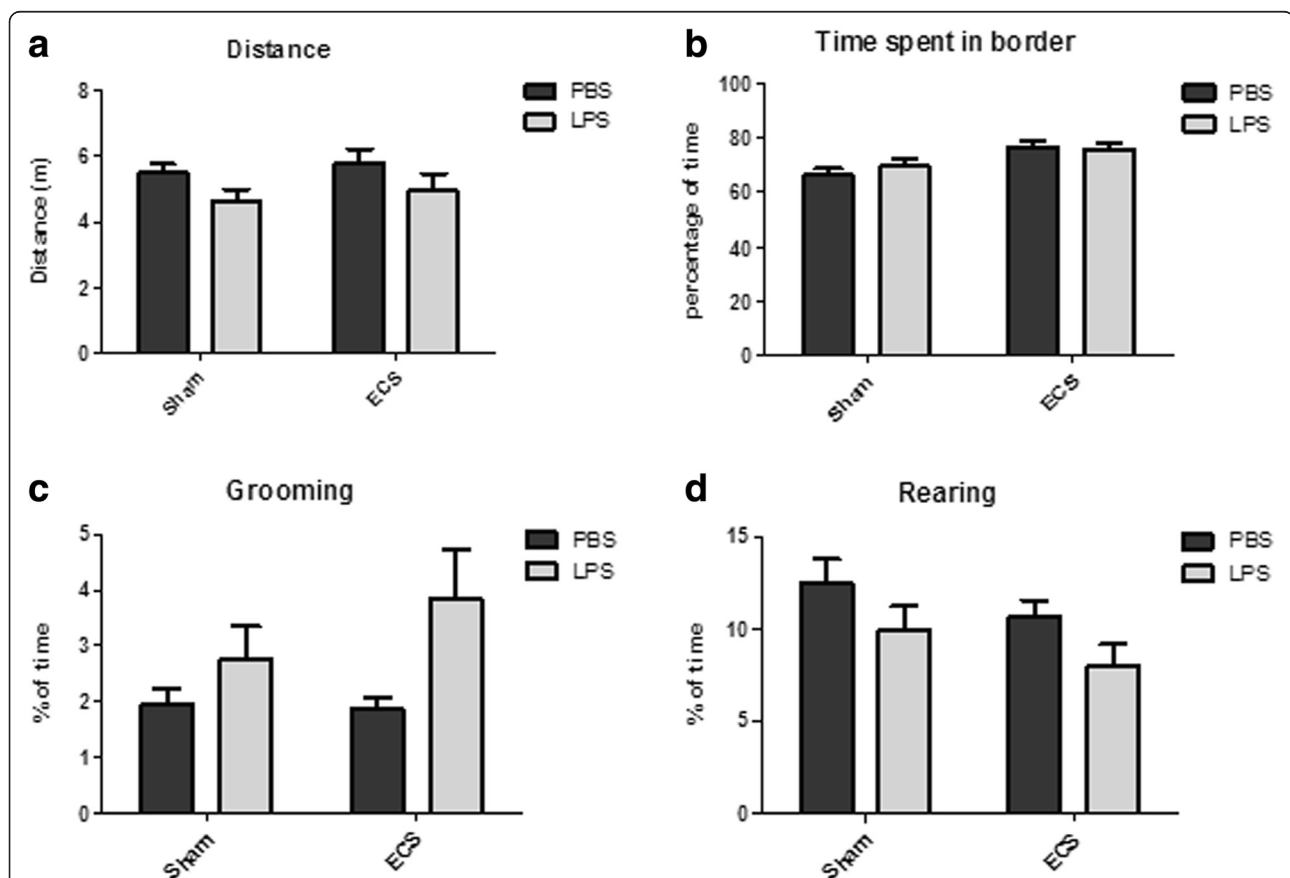


Fig. 4 Open-field test. Three days after PBS/LPS injection, an open-field test was performed. The graphs represent **a** distance moved during the test, **b** percentage of time spent in the border zone of the arena, **c** percentage of time spent grooming, and **d** percentage of time spent rearing. There was a significant main effect for group (PBS/LPS) on the distance moved during the test (**a**; $p = 0.0361$), percentage of time spent grooming (**b**; $p = 0.0334$), and percentage of time spent rearing (**d**; $p = 0.0159$). In addition, a significant main effect for treatment (sham/ECS) was found for the percentage of time spent in the border zone (**b**; $p = 0.005$). Other main effects did not reach significance, and interaction effects were not significant for any of these parameters

Representative pictures of Iba1-stained microglia in the hilus, CA1, and CA3 regions of the hippocampus are shown in Fig. 5. Figure 6 presents microglial activity in the different hippocampal areas (see also Additional file 4). In the sham groups, LPS increased microglial activity by 84 to 213 % in different hippocampal areas (CA3 213 %; CA1 84 %; dentate gyrus 131 %; and hilus 123 %). ECS altered microglial activity by -2.6 to 14.3 % in PBS-injected mice (CA3 1.6 %; CA1 -2.6 %; dentate gyrus 5.4 %; and hilus 14.3 %) and by -20.2 to 6.6 % in LPS-injected mice (CA3 -20.2 %; CA1 6.6 %; dentate gyrus 2.6 %; hilus -8.8 %; compared to the sham + LPS group). In all areas that were analyzed (CA1, CA3, hilus, and dentate gyrus), there was a highly significant main effect for group ($p < 0.0001$ for all areas) but not for treatment (CA3: $p = 0.2708$; CA1: $p = 0.6038$; dentate gyrus: $p = 0.6610$; hilus: $p = 0.8269$). The group \times treatment interaction effect was not significant for any of the hippocampal regions (CA3: $p = 0.2468$; CA1: $p = 0.4246$; dentate gyrus: $p = 0.9815$; hilus: $p = 0.2318$).

In addition to microglial activity, the number of microglia was counted in each hippocampal area (Fig. 7, Additional file 4). LPS induced an increase in the number of microglia in all hippocampal areas (main effect for LPS/PBS: $p < 0.0001$ for the CA3 and the hilus, $p = 0.0336$ for

the CA1, and $p = 0.0002$ for the dentate gyrus). Again, ECS did not affect the number of microglia and there was no significant interaction effect (CA3: $p = 0.7831$ and $p = 0.5466$, respectively; CA1: $p = 0.3027$ and $p = 0.3606$, respectively; dentate gyrus: $p = 0.3508$ and $p = 0.3046$, respectively; hilus: $p = 0.6494$ and $p = 0.8480$, respectively).

Discussion

The goal of this study was to investigate whether repeated ECS sessions suppress the activation of microglia after an inflammatory challenge of the brain. We showed that ECS does not significantly reduce behavioral effects nor microglial activation after an LPS challenge. In contrast, literature indicates that effective repeated ECT treatment in patients may result in normalization of serum levels of cytokines such as TNF-alpha [22, 32]. The dose of LPS was chosen based on its ability to induce depression-like behavior in previous experiments [27]. However, the extreme microglial activation observed in the LPS groups suggests that the inflammatory effects of LPS in this study may not be comparable to the inflammatory alterations in MDD patients, which are possibly relatively mild and could have a more prolonged nature [33]. Therefore, we cannot exclude that the inflammatory and behavioral effect of LPS injection

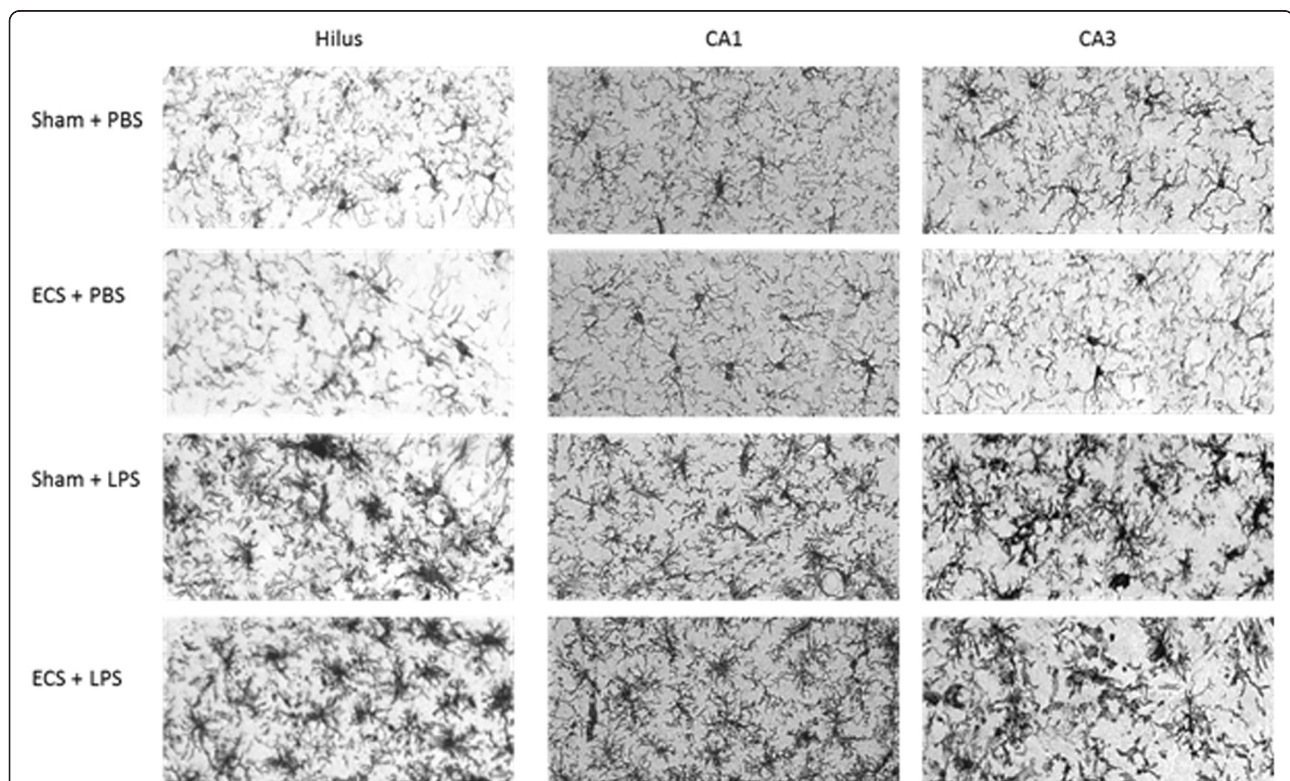
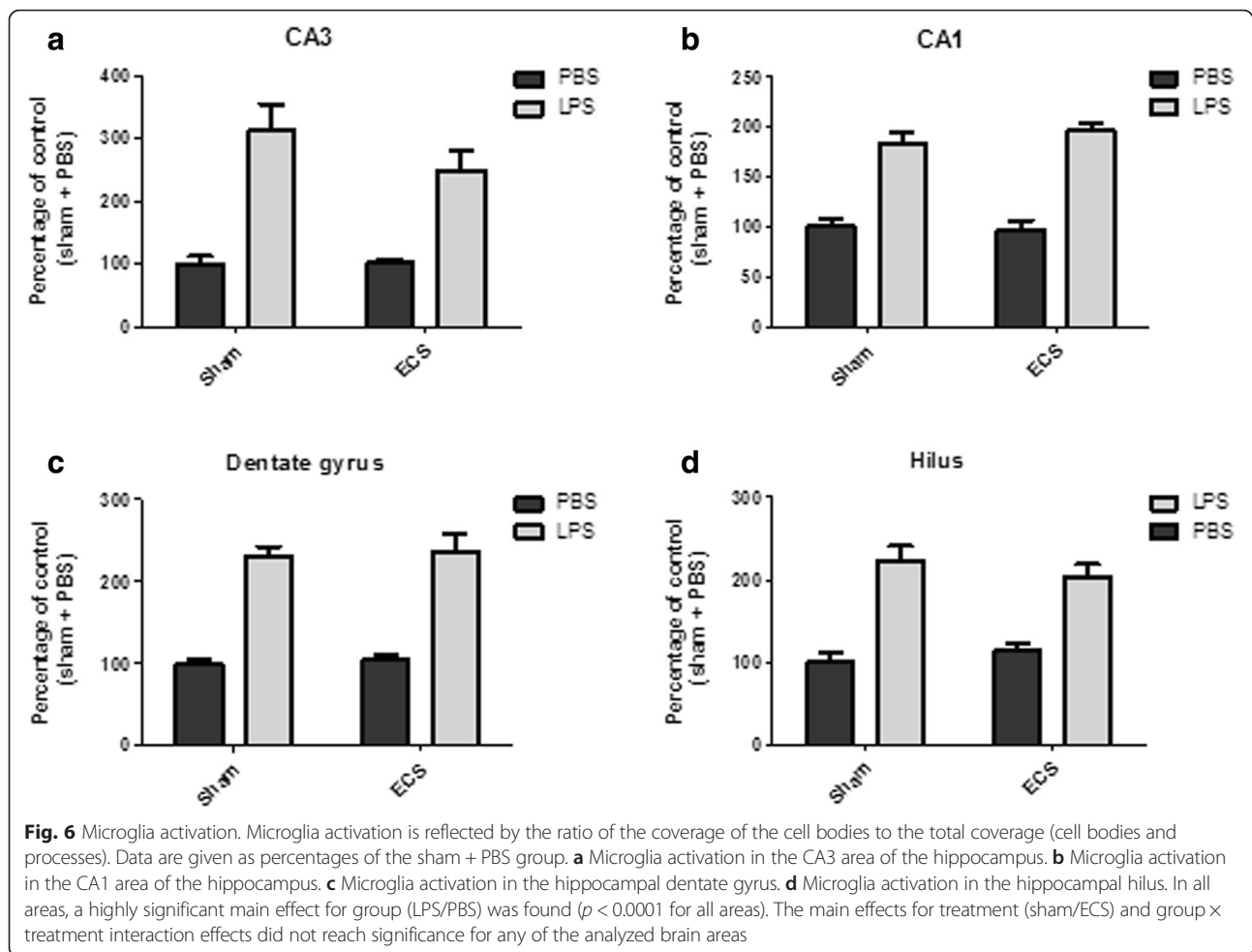


Fig. 5 Iba1 immunohistochemistry. Three days after PBS/LPS injection, mice were sacrificed, and brain sections were immunohistochemically stained for the microglial marker Iba1. This figure shows representative pictures of Iba1 stained microglia for three hippocampal areas (hilus, CA1, and CA3) and four treatment groups (sham + PBS, ECS + PBS, sham + LPS, and ECS + LPS)

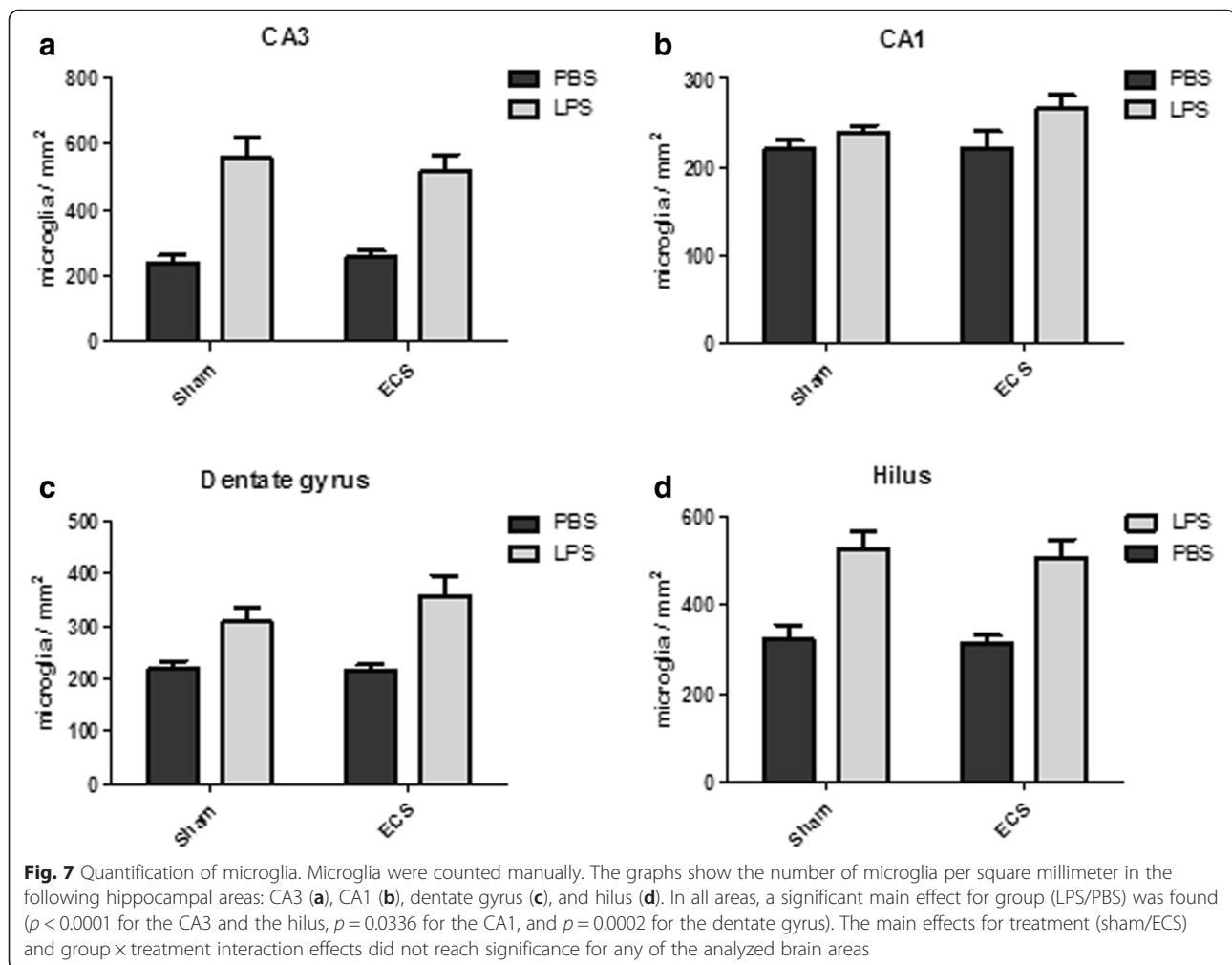


might be too strong to be significantly reduced by ECS treatment.

One should also keep in mind that the mechanisms involved in the relatively short-term inflammatory response induced by LPS might differ substantially from those involved in the more long-term, low-grade inflammation associated with MDD. Therefore, the nature of the inflammatory response in this study may be quite different from the nature of inflammation associated with clinical depression. Indeed, although there is a substantial overlap in the physiological parameters associated with MDD and LPS-induced depression-like behavior, there are also several differences, for example in the relative contribution of certain types of cytokines [34]. Although previous experiments in the same department had demonstrated depression-like behavior up to 4 days after i.c.v. LPS injection [27], the sucrose preference data presented in this study suggest that depression-like behavior normalizes within 3 days after LPS injection. This inconsistency might be due to methodological differences. While Dobos et al. [27] assessed depression-like behavior via a forced

swim test; we have chosen to perform a sucrose preference test as this test allows for repeated measurements.

Most LPS studies have focused on the short-term effects of LPS on behavior (several hours to 2 days after injection); several studies have investigated longer-term effects of LPS, although these studies have employed peripheral intraperitoneal LPS injections instead of central injections. The majority of these studies indicate that the effects of LPS on depressive-like behavior in the sucrose preference test [35], forced swim test [36, 37], or tail suspension test [38] are transient in healthy adult rodents, normalizing within 24–72 h. These studies are in line with the results of this study, demonstrating decreased sucrose preference that normalizes within 72 h. Painsipp et al. [39], however, have found increased immobility in the forced swim test and decreased sucrose preference up to 4 weeks after LPS injection in C57Bl/6 mice but not CD-1 mice. This shows that the effects of LPS may be dependent upon the animal and strain used in the experiment. In addition, differences in the serotype and concentration of LPS used for injection may



have contributed to the discrepancies between these studies.

Several attempts have been made to induce a more chronic type of inflammation in rodents. For example, recent studies demonstrate that repeated peripheral LPS injections in rodents can induce microglial activation, prolonged depression-like behavior, and expression of a specific pattern of cytokines [40–42]. Although determination of the optimal dose and frequency of LPS injection requires further research, this method of inducing inflammation may be more comparable to the pathophysiology of depression. In addition, stress-related depression models, such as the chronic unpredictable mild stress model and the chronic social defeat stress model, are well known to increase the levels of certain inflammatory parameters, including pro-inflammatory cytokines and microglial activity [43–45]. It would thus be interesting to study the effects of ECS on inflammatory parameters in such models.

It is important to note that in this study, we have investigated the ability of ECS to suppress the reactivity of

microglial cells towards inflammatory stimuli. Thus, we have given ECS prior to LPS injection. Therefore, we cannot draw conclusions regarding the ability of ECS to reduce microglial activity in case of pre-existing neuroimmune activation. Thus, it is possible ECS may reduce pre-existing neuroimmune activation without affecting microglial reactivity to inflammatory stimuli administered post-ECS.

The involvement of microglia in MDD pathophysiology is confirmed by a recent PET study investigating translocator protein (TSPO) binding as a biomarker for microglial activity in MDD patients with an active major depressive episode [46]. Microglial activity was found to be increased in various brain areas, including the hippocampus. Moreover, this increase correlated with the severity of depression.

Conclusions

With the read out parameters chosen here, this study suggests that ECS does not have a suppressive effect on microglia activation in an inflammatory depression model,

which is possibly due to the rather powerful challenge with intracerebral LPS injection. This does not rule out that inflammatory states in milder depression models might be effectively reduced by ECS.

Additional files

Additional file 1: Table S1. Presents body weight data per day over the course of the experiment. (PDF 169 kb)

Additional file 2: Table S2. Shows sucrose preference data per day, presented as a ratio of sucrose intake compared to total fluid intake. In addition, data are shown for the AUC for day 10-13 of the experiment. (PDF 174 kb)

Additional file 3: Table S3. Summarizes the distance moved in the open field, as well as the percentage of time spent in each zone and the percentage of time spent rearing and grooming. (PDF 168 kb)

Additional file 4: Table S4. Presents data of microglial activity measurements (ratio cell bodies : total coverage) and microglia cell counts in different hippocampal areas (hilus, dentate gyrus, CA3, and CA1). (PDF 181 kb)

Abbreviations

AUC: area under the curve; BSA: bovine serum albumin; ECS: electroconvulsive seizures; ECT: electroconvulsive therapy; Iba1: ionized calcium-binding adaptor molecule 1; LPS: lipopolysaccharide; MDD: major depressive disorder; PBS: phosphate-buffered saline; PFA: paraformaldehyde; PFC: prefrontal cortex; TSP0: translocator protein.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EMB coordinated the study, carried out the animal experiments and immunohistochemical analysis, and drafted the manuscript. JD and JS were involved in the behavioral testing and immunohistochemical analysis. WD participated in the ECS procedures, the i.c.v. injections, and the collection of brain material for immunohistochemical analysis. FJB contributed to the conception and design of the study. ULME and HCK contributed to the study design, were involved in data interpretation, and helped to draft the manuscript. All authors read and approved the final manuscript.

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