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Brief communication

Interleukin-10 (IL-10) but not Lipopolysaccharide (LPS) produces increased motor activity and abnormal exploratory patterns while impairing spatial learning in Balb/c mice

Deirdre Harvey [a,*], Roisin Smith [a], Karen English [b], Bernard Mahon [b], Sean Commins [a]

[a] *Department of Psychology, National University of Ireland, Maynooth, Co. Kildare, Ireland*
[b] *Institute of Immunology, National University of Ireland, Maynooth, Co. Kildare, Ireland*

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Abstract

Lipopolysaccharide (LPS) is a potent endotoxin, which produces "sickness behaviours" including loss of weight, loss of interest in food and decreased exploration. LPS has also been shown in some studies to cause deficits in various learning and memory abilities, while in others these LPS-induced learning impairments have been attributed to performance-related deficits rather than learning deficits per se. Here, we use the novelty-preference paradigm, a task that minimises performance-related factors such as motivation, in an attempt to extract and examine the effects of LPS on spatial learning. In addition, some studies have indicated that the anti-inflammatory cytokine Interleukin-10 (IL-10) can alleviate some of the symptoms induced by LPS. Here, we also examine the effect of IL-10 on feeding, motor and learning behaviours. We demonstrate that a single injection of LPS does produce a lack of interest in food and weight loss; LPS, however, does not impair habituation in the novelty-preference paradigm. Furthermore, co-injection of IL-10 with LPS does not attenuate the LPS-induced effects of weight loss and lack of food intake. Interestingly, a single injection of IL-10 produces abnormal patterns of exploration, a general increase in activity and abnormal patterns of habituation.

Keywords: Lipopolysaccharide; Interleukin-10; Cytokine; Exploration; Habituation; Balb/c

1. Introduction

Exposure to the endotoxin Lipopolysaccharide (LPS), a non-infectious component of Gram-negative bacterial cell walls, results in a similar pyrogenic response produced by pathogens [1]. This adaptive response, often referred to as "sickness behaviours" produces several symptoms including lethargy, reduced activity and exploration, decreased social interaction, fever, appetite suppression, activation of the hypothalamic–pituitary–adrenal (HPA) axis, as well as decrements in cognitive functioning such as in learning and memory [2,3]. LPS stimulates the synthesis and release of pro-inflammatory cytokines by attaching to Toll-like receptors (TLRs), in particular TLR4, which mediate the expression of genes involved in the inflammatory response [4] leading to the sickness experienced.

The adverse effect of LPS on learning and memory has been demonstrated across a range of behavioural paradigms. Research has found that LPS-treated mice take longer and make more errors when performing a spatial learning task [5]. LPS has also been shown to impair context-dependent fear-conditioning [6], the Morris Water Maze task [7], the novelty-preference paradigm [8], and the passive avoidance learning task [9]. There is however, a growing body of evidence suggesting that the LPS effects observed on learning in many of these behavioural paradigms may actually be due to the performance-related impairments rather than learning deficits per se. Such performance-related impairments may be attributed to deficits in attention, motivation, anxiety and/or other factors, which in turn, may or otherwise contribute to the learning deficits seen with LPS [2]. Research examining the effects of LPS on spatial memory in the Water Maze, for

* Corresponding author. Tel.: +353 1 7086182; fax: +353 1 7084767.
E-mail address: DEIRDRE.R.HARVEY@nuim.ie (D. Harvey).

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example, demonstrates a significant reduction in swim speed but no effect on the learning itself [10]. Other studies involving intracerebroventricular infusion of IL-1β also demonstrate no effect on spatial memory when tested in the Water Maze task, while infusions of the IL-1 receptor antagonist (IL-1Ra) resulted in memory impairment [11], suggesting that some cytokines, such as IL-1β, may in fact enhance memory processes. In addition, other studies examining the effects of LPS and IL-1 on a delayed-match to sample task and the Y-maze task showed motivational effects rather than learning impairments [12], again adding credence to the suggestion that LPS and cytokines, like IL-1, may result in performance-related rather than cognitive deficits.

Interleukin-10 (IL-10) has been used to reduce synthesis and release of several pro-inflammatory cytokines like IL-1, TNFα and IL-6 [13–15]. IL-10 plays a central role in striking a balance between responding to and eliminating an infection while minimising injury to host tissue [16]. IL-10 pre-treatment suppresses inflammation through interference with monocyte functioning and macrophage activation, reducing cytokine synthesis, giving resistance to the fever responses induced by LPS [16]. The inhibitory effects of IL-10 on IL-1 and TNF production are fundamental to its anti-inflammatory activities, as these cytokines intensify the response by initiating secondary mediators [16]. IL-10 has also been shown to attenuate the behavioural effects of LPS [1], reversing the effects upon social exploration and interaction, mobility and depressed body weight while modulating the vascular effects of LPS [17,18]. Further research has found that giving IL-10 prior to LPS administration increases exploratory locomotor activity, as measured by the frequency of rearing and ambulations made, in an open field arena [19]. It has been demonstrated that IL-10 also reduces sleeping patterns, in particular non-rapid eye movement sleep [20].

One simple task used to examine spatial learning and memory is the novelty-preference paradigm. In this task animals are required to passively explore an environment filled with objects. When placed in an unfamiliar arena rodents display exploratory activity towards most aspects of this new environment, particularly towards the objects present [21]. It is believed that an animal's spatial knowledge of its surroundings depend on such exploration. When the animal is placed in the same environment a second time there is a decrease in the locomotor and exploratory activities (habituation), which will continue to occur as long as the environment remains constant [22,23]. Several of the previously outlined paradigms (such as the Morris Water Maze) make use of the working memory and long-term memory, engaging multiple cognitive processes. However, the novelty-preference paradigm involves a lighter cognitive load as it does not actively teach animals any clear relationship between the consequences of their behaviour and the presence of the objects [24]. Furthermore, unlike many paradigms (such as the Water Maze) which require strong motivational and perhaps anxiety-driven behaviour (escaping from relatively cold water), the novelty-preference task does not rely on such factors and therefore may be useful in attempting to separate LPS-induced learning deficits from any performance-related impairments.

The current study examines the effect of LPS on learning in the novelty-preference paradigm and also examines the role played by IL-10 in alleviating fever responses induced by LPS. Specifically, we hypothesize that many "sickness behaviours" induced by LPS such as lack of appetite and weight loss will be attenuated by a co-injection of IL-10 and LPS. In addition, we further hypothesize that the reduced exploration and learning consequences of LPS, in the novelty-preference paradigm, will be reversed by co-administration with IL-10.

2. Methods

Male Balb/c mice ($N=32$, Jackson Laboratory, Bar Harbour, Maine, USA) aged 6–8 weeks and weighing approximately 30 g were used as subjects. Mice were housed 8/large cage in a temperature-controlled room (21±1 °C), maintained on a 12 h:12 h light/dark cycle (lights on at 07:00 hr). Animals had free access to food and water. Animals were randomly assigned to one of four groups ($N=8$/group): *Saline control group*: Saline (0.9%). *LPS group*: LPS at a dose of 300 μg/kg (*Escherichia coli*, serotype 0111:B4; Sigma Chemical, Dorset, UK). LPS was dissolved in sterile phosphate buffered saline (PBS, 0.9%). *LPS and IL-10 group*: IL-10 at a dose of 130 μg/kg and LPS at a dose of 300 μg/kg in 0.3 ml PBS (0.9%) co-administered. *IL-10 only group*: IL-10 at a dose of 130 μg/kg (BD Bioscience, Oxford, UK) in 0.3 ml PBS (0.9%). Each group was injected (i.p.) once 6 h prior to habituation training with their respective solution. A 6 h period allowed sufficient time for the administered dosages to take effect [1,2,5]. The dosage amounts were selected upon these previous findings and the animal bodyweight measurements [2]. Following injections, mice were weighed and returned to an individual cage. The food and water supplied in each cage were recorded. After 6 h food, water, and bodyweight were reweighed.

Following this animals were brought to the testing laboratory. Each animal was brought into the experimental room in individual cages. Each mouse was then individually tested in a separate, cordoned off experimental arena, out of view of the other animals. Once finished experimenting, each animal was then returned to a common group cage. Each animal received one trial in an open field where they were allowed to explore the empty arena for 1 min. This consisted of a small box (40×30×10 cm). The experimenter was not in the experimental area during this phase. EthoVision (Noldus Information Technology, Wageningen, The Netherlands), a computerised digital tracking system measured the distance travelled (cm), velocity (cm/sec), and the total duration (%) spent in predefined sections of the arena, (the outer edge and inner section), that were equal in area during this trial. Upon the completion of this trial, subjects were transferred to the habituation arena and received three trials.

The experimental apparatus used for the habituation testing consisted of a white circular arena (diameter 40 cm, height 15 cm) resting on a platform 70 cm off the ground. A black curtain surrounded the entire arena, with a white rectangular

card located on the east side. A 25 W light bulb located in the south east of the arena and a second light bulb directly above the circular arena provided the light. Four objects were placed in the centre of the arena in a square formation, approximately 10 cm apart. The objects consisted of a circular black jar lid (diameter 5 cm, height 0.5 cm); a small rock (approximately 4.2 cm diameter); a glass cylinder (diameter 5 cm, height 7 cm); and a yellow matchbox (diameter 3.5 cm, height 1 cm).

A trial consisted of the mouse being placed into the arena, allowed to explore the environment for one minute, whilst the experimenter, located at the west side of the arena, recorded the nose contacts made with each object. Subjects were removed from the arena after the allotted time and placed in an open-topped box for an inter-trial interval of 15 s. All data were analysed using the standard analysis of variance (ANOVA) procedures followed by post-hoc comparisons (Tukey HSD).

3. Results

The amount of food consumed by each group was measured 6 h post-injection. A significant difference was found between the groups' overall food consumption ($F(3,28)=33.04$, $p<0.001$). Post-hoc tests (Tukey HSD, $p<0.05$) indicated that the LPS group ate significantly less food than the Saline control group and the IL-10 only group over the 6 h period. Furthermore, the LPS and IL-10 group also consumed significantly less food overall compared to the Saline control group and the IL-10 only group (see Fig. 1a).

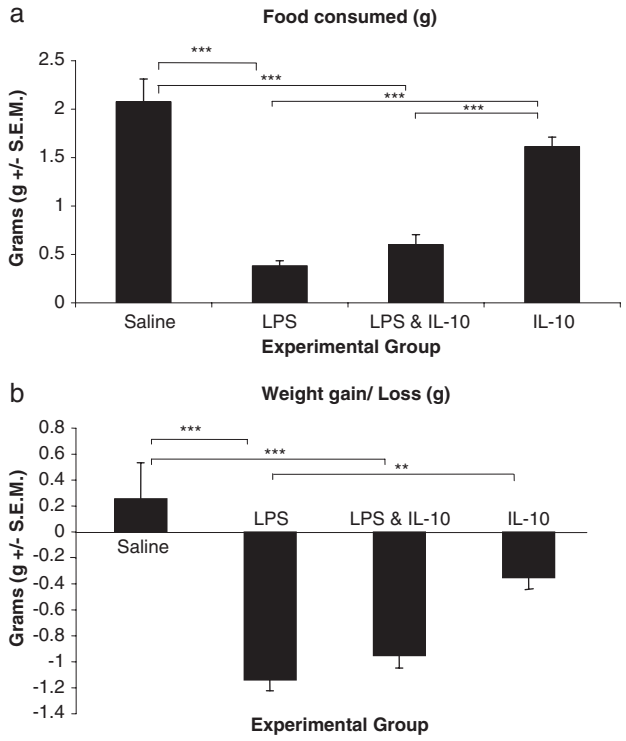

Fig. 1. (a) The mean amount of food consumed and (b) the mean weight gain/loss at 6 h post-injection. $*p<0.05$, $**$ $p<0.01$, $***$ $p<0.001$ levels of significance.

All mice were weighed twice, once immediately post-injection and once 6 h post-injection. Overall significant differences were found between the groups ($F(3,28)=15.68$, $p<0.001$). Post-hoc comparisons revealed that the LPS group lost significantly more weight compared to the Saline control group and the IL-10 only group during this time (see Fig. 1b). The LPS and IL-10 group also lost significantly more weight than the Saline controls six hours post-injection. No further differences were found.

Prior to the habituation task all mice were allowed one minute in the open field. Fig. 2a shows the mean distance travelled by all groups during this trial. A significant difference was found between the groups ($F(3,28)=2.896$, $p=0.05$). Post-hoc comparisons revealed that the LPS only group travelled a significantly shorter distance than the IL-10 only group. No further differences were found. Analysis of the mean velocity per day for each group again showed an overall difference between the groups' scores ($F(3,28)=4.51$, $p<0.05$). Subsequent post-hoc comparisons indicated that the LPS only group was significantly slower than both the Saline control group and the IL-10 only group, illustrating the LPS group's diminished rate of activity (see Fig. 2b).

The arena was divided into two equal areas, an outer edge and an inner section (see inset Fig. 2c), to examine the exploration patterns of each experimental group. The total duration (%) spent in both of these predefined sections was then assessed using a 4×2 repeated-measures ANOVA. Although there was no main effect for the experimental group, a significant effect for the arena section was revealed ($F(1,14)=21.04$, $p<0.001$). An interaction (Group X Section) effect was also demonstrated ($F(3,42)=13.079$, $p<0.001$). Subsequent *t*-tests revealed that the LPS and IL-10 group and the IL-10 only group spent significantly more time in the outer edge of the arena when compared to the inner section ($t(7)=2.761$, $p<0.05$; $t(7)=9.237$, $p<0.001$). A further one-way ANOVA revealed a significant difference between the four groups' total duration (%) time spent in the outer edge ($F(3,28)=4.644$, $p<0.01$). Post-hoc comparisons showed that the IL-10 only group spent significantly more time in this section of the arena compared to the Saline control group and the LPS only group (see Fig. 2c). No further differences were noted between the groups.

Learning among the four experimental groups was assessed using a 3×4 repeated measures ANOVA. A main effect for the group ($F(3,28)=3.576$, $p<0.05$) was revealed. Post-hoc tests (Tukey HSD) showed that the LPS group made significantly less nose contacts overall when compared to the IL-10 only group. An overall main effect for the trial was also found ($F(2,56)=45.08$, $p<0.001$). Each individual group showed significant decreases in the nose contacts made during the habituation trials. The Saline control group showed a significant decrease in the nose contacts made ($F(2,23)=8.313$, $p<0.01$) during the task with significantly less made in trial 3 than in trial 1. The LPS and IL-10 group also showed habituation ($F(2,23)=13.226$, $p<0.001$) with significantly less nose contacts made on trials 2 and 3 than on trial 1. Interestingly, the LPS only group also demonstrated habituation ($F(2,23)=5.296$, $p<0.05$)

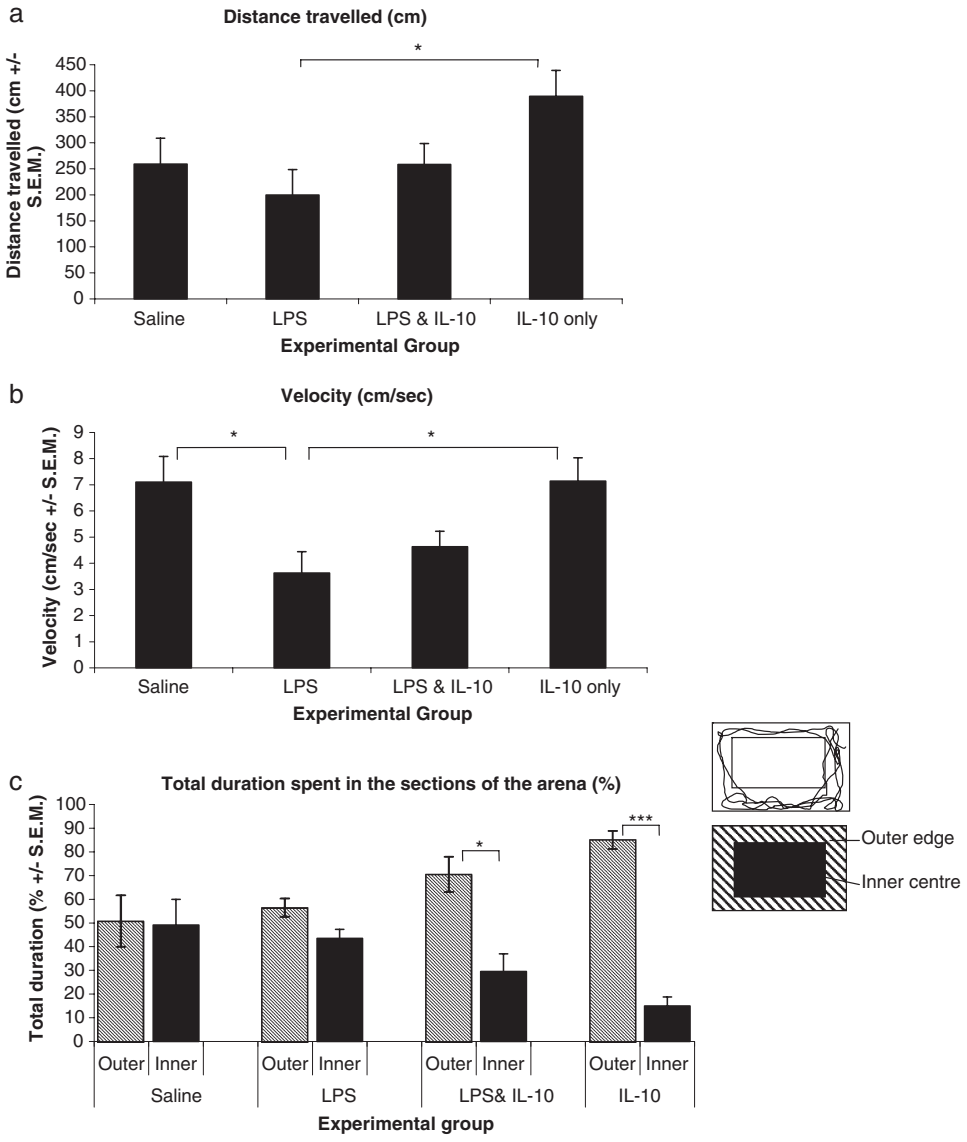

Fig. 2. (a) The mean distance travelled (cm) and (b) the mean velocity (cm/sec) and (c) the total duration (%) spent in the inner and outer sections of the arena during the open field task (arena sections outlined and a typical IL-10 only group trial inset). $*p<0.05$, $**p<0.01$, $***p<0.001$ levels of significance.

with this group making significantly less nose contacts on trial 3 compared to trial 1. The IL-10 only group showed an overall significant difference in nose contacts made with the objects throughout the habituation task ($F(2,23)=5.133$, $p<0.05$). Significantly less nose contacts were made with the objects on trial 2 compared to trial 1. However, no differences were noted between trial 1 and trial 3 due to the irregular increase in nose contacts shown between trial 2 and 3. A Group X trial interaction effect ($F(6,56)=2.172$, $p=0.05$) was also found. A significant difference between the four groups on trial 1 ($F(3,28)=4.978$, $p<0.01$) and trial 3 ($F(3,28)=4.524$, $p<0.05$) was found. No differences were observed between the groups on trial 2. Post-hoc comparisons indicated that the IL-10 only group made significantly more nose contacts with the objects compared to both the Saline control group ($p<0.05$) and the LPS only group ($p<0.01$) on trial 1. On trial 3 the IL-10 only group made significantly more nose contacts than the LPS

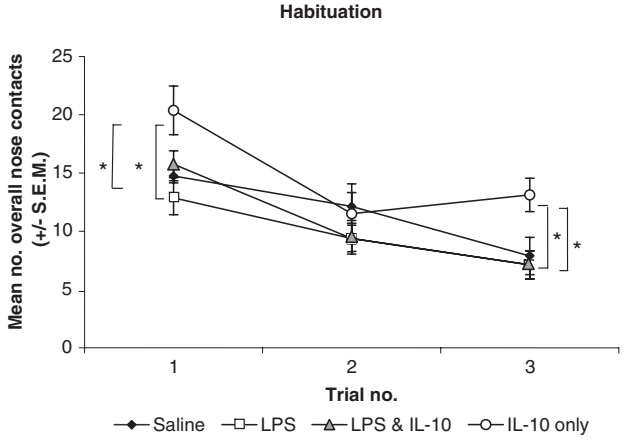

Fig. 3. Line chart demonstrating the mean number of nose contacts made with each of the four objects for each group during the three habituation trials of the novelty preference task. $*p<0.05$ level of significance.

group ($p<0.05$) and the LPS and IL-10 group ($p<0.05$) (see Fig. 3).

4. Discussion

We demonstrated that LPS does have the predicted effects on some measures of "sickness behaviours", as outlined by the previous research [3,25]. We showed, for example, that the LPS only group consumed significantly less food and water and lost significantly more bodyweight when compared to the Saline control group. Injections of LPS only also reduced the overall velocity in the open field when compared to the Saline control group, in line with the previous research [2,10]. Injections of LPS did not however impair habituation in the novelty-preference paradigm, as we had hypothesized in the introduction. Instead, the LPS only group habituated readily to the task, reducing their interest in the objects significantly by the third trial. In addition, no significant differences in the mean number of nose contacts made with the objects were noted between the LPS and the Saline control group on any of the three trials. This result would suggest to us, in common with many of the previously reported studies [2,10,12], that LPS may not have a strong and clear adverse effect on learning. As suggested by Sparkman et al. [2,10] studies that attribute learning impairments to LPS may in fact be due to the other performance-related impairments, such as motivational or anxiety-related factors. By using the novelty-preference paradigm we had hoped to eliminate some of these motivational issues and we have demonstrated, at least in our set-up, that learning does not seem to be impaired in the LPS only group. However, other methodological possibilities that may explain the lack of learning impairments seen with the LPS group cannot be ruled out. The 6 h timeframe used in this experiment, although used by the previous studies [1] may have been too long and the behavioural effects of LPS may have already reached its peak. In addition, the one-minute trial duration may not have been sufficient to reduce the potential stress of being placed into a novel environment.

It is documented that IL-10 is a potent anti-inflammatory cytokine modulating and regulating the effects of LPS [1,17] through the antagonism of IL-12 [18]. We hypothesize that the co-injection of IL-10 and LPS (i.e. IL-10 and LPS group) would attenuate some of the "behavioural sickness" effects imposed by LPS. However, our results did not confirm this. The LPS and IL-10 group (similar to the LPS only group) ate significantly less and lost significantly more weight when compared to the Saline control group. In addition, the habituation patterns observed with the LPS and IL-10 group did not differ significantly from either the LPS only or the Saline groups. Previous research has shown that IL-10 can attenuate the effects of LPS in certain behavioural conditions, this effect, for example, has been observed in social exploration [1]. Furthermore it is noted that in the current experiment IL-10 and LPS were co-administered, whereas in the previous outlined studies [1,16] pre-treatment was used. This may account for some of the differences in results when directly comparing studies. Future research should take this into consideration. Consequently, we suggest that IL-10 at the concentration administered in the current experiment does not attenuate the effects of LPS.

An unexpected finding to the current study showed that exclusive administration of IL-10 produced a general increase in behavioural activity and abnormal behavioural and learning patterns compared to the other experimental groups. It was observed that the IL-10 only group had higher distance and velocity scores, spent a significantly shorter duration of time in the inner section of the arena, and made more overall nose contacts during the habituation compared to the other groups. The IL-10 only group spent a significantly longer time (approximately 80%) in the outer edge of the arena compared to the Saline controls and the LPS only group, who only spent approximately 50% in each of these sections, highlighting the IL-10 only groups' abnormal behaviour. It was observed that this group would run around the side of the arena demonstrating a thigmotactic-like behaviour [26] (see Fig. 2c inset). One particular interpretation of this behaviour is that IL-10 may be affecting emotionality or anxiety levels of the animals. It has been suggested that thigmotaxis is thought to belong to the category of phylogenetically prepared fear reactions [27]. Indeed, thigmotactic or wall-seeking behaviour has been observed in several species [28–30] and it is reported to be the likely result of small rodents trying to avoid open, unknown and potentially dangerous areas [29,30]. To emphasise the interpretation of thigmotaxis as a sign of anxiety, Treit [27] strongly recommends that this wall-seeking behaviour be used when testing anxiolytic drugs. We would also suggest that the injection of IL-10 had the effect of increasing anxiety in our animals, which manifested in them spending more time at the periphery of the open field rather than any other area. Our results also show that this group was particularly active during habituation trials, making more nose contacts with the objects compared to the other groups (especially trials 1 and 3). Overall they displayed an irregular pattern of habituation with a decrease in the number of nose contacts made between trial 1 and trial 2 as expected. This is then followed by a renewal of exploration, increasing nose contacts between trial 2 and trial 3 (revealed by no significant differences between trial 1 and trial 3). This is again inconsistent with normal habituation patterns, when the number of nose contacts made by trial three should be much reduced. Again, this abnormal pattern may be a direct result from increased anxiety induced by IL-10.

In summary, LPS-treated animals did show certain sickness-type behaviours. They showed a decrease in food consumption, loss in bodyweight and a reduction in velocity in the open field. This group did not however, show any learning impairments thereby adding to the ongoing debate of the exact role of LPS in higher-order cognition. Interestingly, exclusive administration of IL-10 produced abnormal patterns of exploration, by spending much of their time at the outer edge of the arena. In addition, this group were generally more active and made significantly more nose contacts with the objects in the novelty-preference paradigm, as well as an abnormal pattern of habituation to this task.

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