

Differences in fatigue-like behavior in the lipopolysaccharide and poly I:C inflammatory animal models

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ABSTRACT

Central fatigue is a condition associated with impairment of the central nervous system often leading to the manifestation of a range of debilitating symptoms. Fatigue can be a consequence of systemic inflammation following an infection. Administration of lipopolysaccharide (LPS) and polyriboinosinic:polyribocytidic (poly I:C) to animals can induce systemic inflammation by mimicking a bacterial or viral infection respectively and therefore have been used as models of fatigue. We evaluated a range of phenotypic behaviors exhibited in the LPS and poly I:C animal models to assess whether they adequately replicate fatigue symptomology in humans. In addition to standard observation- and intervention-based behavioral assessments, we used powerful in-cage monitoring technology to quantify rodent behavior without external interference. LPS and poly I:C treated Sprague Dawley rats displayed 'sickness behaviors' of elevated temperature, weight loss and reduced activity in the open field test and with in-cage monitoring within 24 h post-treatment, but only LPS-treated rats displayed these behaviors beyond these acute timepoints. Once sickness behavior diminished, LPS-treated rats exhibited an increase in reward-seeking and motivation behaviors. Overall, these results suggest that the LPS animal model produces an extensive and sustained fatigue-like phenotype, whereas the poly I:C model only produced acute effects. Our results suggest that the LPS animal model is a more suitable candidate for further studies on central fatigue-like behavior.

1. Introduction

Central fatigue, referred to as fatigue throughout the rest of this article, is often viewed as a symptom of an underlying condition, or a side effect of therapeutic treatments. Fatigue is associated with impairment of the central nervous system (CNS) and can be characterised by headaches, muscle weakness, slowed reflexes, chronic tiredness, impaired coordination, impaired cognitive ability and reductions in motivation and attention, and the majority of these symptoms do not resolve following rest [1-3]. The ability to adequately model all fatigue-induced behaviors is the first critical step to identifying the mechanism(s) of fatigue, developing potential therapies, and ultimately diagnosing and treating patients suffering from this debilitating condition.

The precise causes of fatigue are unknown, but one suggested mechanism is prolonged activation of the immune system, by illness or disease [4-6]. Infections are often associated with acute sickness behavior, which in some cases causes prolonged systemic inflammation, resulting in chronic fatigue [7]. High concentrations of circulating

pro-inflammatory cytokines such as interleukin-1 β (IL-1 β), interferon- α (IFN- α) and tumor necrosis factor- α (TNF- α) have been observed in chronic fatigue syndrome (CFS) patients [8,9], and are associated with low-grade fevers that sporadically occur during bouts of chronic fatigue [10]. Current evidence suggests that fatigue may be induced by these pro-inflammatory molecules entering the brain, which can disrupt the blood brain barrier, injure serotonergic neurons, and impair the hypothalamic-pituitary-adrenal axis and basal ganglia [11-14], leading to the behavioral symptoms associated with fatigue.

Given that fatigue is postulated to be driven by an aberrant immune response, current fatigue models attempt to replicate this immune response to induce fatigue-like behaviors. Two widely used animal models to produce a fatigue-like phenotype employ the administration of lipopolysaccharide (LPS) and polyriboinosinic:polyribocytidic acid (poly I:C). The poly I:C model involves injection of synthetic double-stranded RNA (dsRNA), that mimics a viral infection in the periphery, resulting in an immune-mediated inflammatory response cascading into the production of pro-inflammatory cytokines such as IFN- α , IL-1 β and TNF- α [15-17]. Reports indicate that rodents and hamsters exhibit

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decreases in locomotion, weight and exploratory behavior 7–9 days following poly I:C administration [18–20]. The LPS model, mimicking a gram-negative bacterial infection, activates blood monocytes and hepatic macrophages to produce IL-1 β resulting in systemic inflammation [21,22]. The LPS model can lead to weight loss and ‘sickness behaviors’ including fever and lethargy, along with reductions in water, food intake and activity for 3–5 days [4,23]. While both these models have shown common fatigue-like behaviors such as decreased locomotion and sickness behaviors [18–22], other symptoms of fatigue, such as those associated with reward seeking and motivational impairment, have only been explored in the LPS model [24–28]. Impairment in reward seeking and motivation are prominent in human fatigue. Self-reporting of reduced motivation in human fatigue is linked to impairment of the basal ganglia and reductions in neuronal activation towards reward cues [14,29,30]. Therefore, it is important to assess motivation and reward-seeking behavioral changes in fatigue models. Here, we wished to establish whether these behaviors occur in the poly I:C and LPS animal models, and ascertain whether their behavioral phenotypes adequately replicate fatigue symptoms seen in humans. We examined how poly I:C and LPS treated rats responded to tests that assessed exploratory behavior, reward seeking and motivation. In addition to these standard observation- and intervention-based behavioral assessments, we used powerful in-cage monitoring technology to quantify rat behavior without external interference.

2. Methods

2.1. Animals

Seventy two male Sprague Dawley rats from 12–16 weeks of age and weighing 250–300 g were housed in GR1800 double-decker green line cages or 1291 NEXT blue line cages with a 12-h light/dark cycle (light on at 7:00 h) with *ad libitum* access to food and water. All animal experimentation was approved by the Animal Ethics Committee, University of

Tasmania (A0016311) and performed in accordance to the Australian NHMRC Code of Practice for the Care and Use of Animals for Scientific Purposes. All results are reported in accordance with the ARRIVE guidelines.

2.2. Drug administration

Treatments were randomly allocated using a random number generator and the researcher administering the compounds was blinded to the treatment group. Rats were injected once intraperitoneally (IP) with 0.5–1 mL of 4.5 mg/kg poly I:C (Sigma-Aldrich, MO, USA, Cat #p9582–5MG, Lot # 086M4045V), or 3.0 mg/kg of LPS E.coli 0127:B8 (Phenol Extraction) (Sigma-Aldrich, MO, USA, Cat #L3129–10MG, Lot # 037M4067V) dissolved in 0.9% NaCl, or received an equivalent volume injection of 0.9% NaCl alone (control vehicle). Dosages of LPS and poly I:C were chosen based on the published literature where a systemic inflammatory response with fatigue was exhibited [4,20,27,31].

2.3. Experimental design

Three experiments were undertaken: experiment 1 assessed behavioral tests, experiment 2 assessed both behavioral tests and in-cage monitoring, and experiment 3 assessed only in-cage monitoring (Fig. 1). Details of behavioral tests and in-cage monitoring are outlined below. Rats were habituated to researchers and behavior testing equipment and protocols for two weeks prior to baseline recordings. To minimize potential effects induced by uncontrolled factors such as stress and anxiety during testing, animals were habituated to the open field box, where both the open field and sunflower seed tests were conducted, every two days for 2 weeks to ensure stable baseline recordings. Baseline measurements for each behavioral test were taken in the week prior to drug administration (every day for general health assessment and weight; twice for open field and sunflower seed test). After baseline behavioral testing, rats were randomly assigned to either a control, LPS

Experiment 1 n = 10 per group		Baseline	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
	Drug Injection														
	Weight														
	Open Field														
	Sunflower Seed Test														
	Sucrose Preference Test														
Experiment 2 n = 10 per group		Baseline	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
	Drug Injection														
	Weight														
	Open Field														
	Sunflower Seed Test														
Experiment 3 n = 3 per group		Baseline	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
	Drug Injection														
	Weight														
		Baseline	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
	In-Cage Monitoring														

Fig. 1. Experimental Design. Experimental design for each experiment which depicts when behavioral tests and in-cage monitoring were conducted, relative to the day of LPS, poly I:C or vehicle injection (day 0), over a 12-day period. Baseline testing was performed over the two weeks prior to day 0. A white square indicates when an event/test was not completed on that day and gray indicates it was completed.

or poly I:C group and the researcher conducting subsequent behavioral assessments was blinded to group allocation. Following treatment, general health assessment, weight, and behavioral tests (open field and sunflower seed test), sucrose preference and in-cage monitoring were conducted during the 12 days post-treatment period (Fig. 1). At day 12, rats were euthanized by I.P injection of 60 mg/kg pentobarbital.

Previous literature indicates that animals exhibit 'sickness behaviors' and decreases in exploratory, motivational and reward-seeking behaviors, 3–4 days and 7–9 days following LPS and poly I:C administration respectively [4,18–20,23]. Therefore, 12 days post-administration was chosen for behavioral observations to allow behavioral changes to peak and then subside towards baseline values. These behavioral peaks and troughs were then used to determine when fatigue symptomology for these animal models is present.

Sample sizes for behavioral testing were determined for the primary outcome (activity in the open field arena measured by distance travelled) by a power calculation with power $(1-\beta) = 80\%$, $\alpha = 0.05$, and an effect size (Cohen's d) = 1.5 using means and standard deviations from a previous study using this test [20]. Thus, for all behavioral protocols 10 animals were used per treatment group (Fig. 1). As the in-cage monitoring was a new technique and we had no prior data available for these types of studies, a power calculation was not performed and an n of 3 animals per treatment group was used.

2.4. Behavioral tests protocols

2.4.1. Open field test

The open field test was conducted using the methods adapted from Tatem et al. [32]. Rats were placed in a red translucent plastic box (60 cm x 60 cm x 40 cm) with a black floor and a GoPro camera (GoPro, Inc., CA, USA) was placed 40 cm directly above the center of the box to record animal movement and behavior. The box was used in a normally lit room. The rat was placed in the center of the arena and allowed to explore for 5 min which was video recorded. After 5 min, the rat was placed back into its home cage, the box was cleaned with 70% ethanol and left for at least 5 min before the next trial. For each video recording, open field parameters such as distance travelled, time spent not moving, and time spent in outer zone were extracted using EthoVision XT software (Noldus, Wageningen, Netherlands) based on contrast monitoring of the video of the animal.

2.4.2. Sucrose preference test

The sucrose preference test was conducted using the methods adapted from Yankelevitch-Yahav et al. [33]. Each rat was habituated for 4 days to the presence of two drink bottles in the cage before baseline assessment. For experimental testing, each cage received one pre-weighed bottle filled with 600 mL of water and one pre-weighed bottle filled with 600 mL of 2% sucrose in water. The behavioral researcher was blinded to the content of each bottle. Positions for each bottle were the same across all cages with bottle positions for all cages switched at the end of each day to prevent bias towards a bottle's location. Bottles were weighed each morning to determine the amount from each bottle that had been drunk. If a bottle was empty it was refilled to 600 mL with 2% sucrose or water depending on its allocated grouping, re-weighed and placed back in the cage. Total fluid intake, water consumption and sucrose consumption were calculated each day.

2.4.3. Sunflower seed test

The sunflower seed test was conducted using the methods adapted from Petullo et al. [34]. Rats were habituated to the presence of sunflower seeds in their home cages two weeks prior to baseline testing. For experimental testing and following the open field tests, rats were placed back in the open field box with 10 sunflower seeds of similar size dispersed in the outer zone of the box. The rat's behavior was then video recorded for 10 min. If all ten seeds were eaten before 10 min had completed, the rat was removed from the arena and recording was

stopped. The open field box was cleared of all sunflower seeds and cleaned with 70% ethanol after each trial. The time taken to eat the first seed, number of seeds eaten, and time taken to eat all seeds were calculated.

2.4.4. In-Cage monitoring - actual HCA system

The in-cage monitoring Actual Home Cage Analyser (HCA) (Actual Analytics, Edinburgh, UK) was used to observe animal behavior without external interference. The Actual HCA system uses radio-frequency identification (RFID) tags, implanted into the animal to identify animals and monitor changes in core body temperature, in conjunction with sensors built into the cage base and a video recording system to analyze the animals' activity, interactions, and other behavioral measurements [35]. Home cages were placed into the Actual HCA system overnight to habituate the animals, while for experimental testing home cages were placed into the Actual HCA system at approximately 16:00 on day 0 and recorded for up to 12 days.

The implantation of the RFID tag occurred 2 weeks prior to habituation in the Actual HCA system. Rats were anesthetized with isoflurane (5% induction, 3% maintenance) balanced in oxygen, placed on their back and their abdomen swabbed with ethanol and betadine. A sterilized Biotherm13 RFID tag (Biomark, ID, USA) was implanted subcutaneously along the ventral midline of the abdomen with a sterile 12 G needle and the wound closed with vet-grade superglue, as described previously [36]. Animals were closely monitored during recovery post-surgery for signs of pain and distress. As the implantation of RFID tags is a minor surgical procedure taking less than one minute and did not induce any detectable adverse effects, post-operative analgesia was not administered. Moreover, the procedure was performed 2 weeks prior to baseline testing to minimize any subsequent influence on tested behavior.

2.5. Statistics

All data, except for temperature (in-cage monitoring) and number of seeds eaten (sunflower seed test), were normalized to the individual and then represented as a percentage of baseline. To detect differences between groups, two-way ANOVA with repeated measures was performed using Prism 7.02 (GraphPad Software, CA, USA). A repeated measure multiple comparisons (Sidak) post hoc test comparing treatments against control was also performed for each time point. Data of more than two standard deviations from the mean were excluded as outliers. Between 0 and 2 outliers were excluded for each behavioral test parameter based on this criterion. Within the excluded outliers we saw no indication of a pattern or systematic loss of animals from any experimental cohort. A mixed-effects model was used for data analysis where such outliers were excluded. A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Body weight

Administration of LPS induced significant weight loss, compared to baseline, from day 1 to day 4 compared to controls ($p < 0.0001$), while between days 7 and 11, LPS-treated animals gained significantly less weight than controls ($p < 0.05$) (Fig. 2A). By day 12, there was no difference in relative weight between LPS and controls. Administration of poly I:C did not influence the relative weight of animals compared to controls at any timepoint (Fig. 2B).

3.2. Open field: activity, stress and locomotion

Open field tests were used to assess activity, stress and locomotion following LPS and poly I:C treatments (Figs. 3, S1 and S2). Representative open field heat maps (Fig. 3A), show similar activity in the open

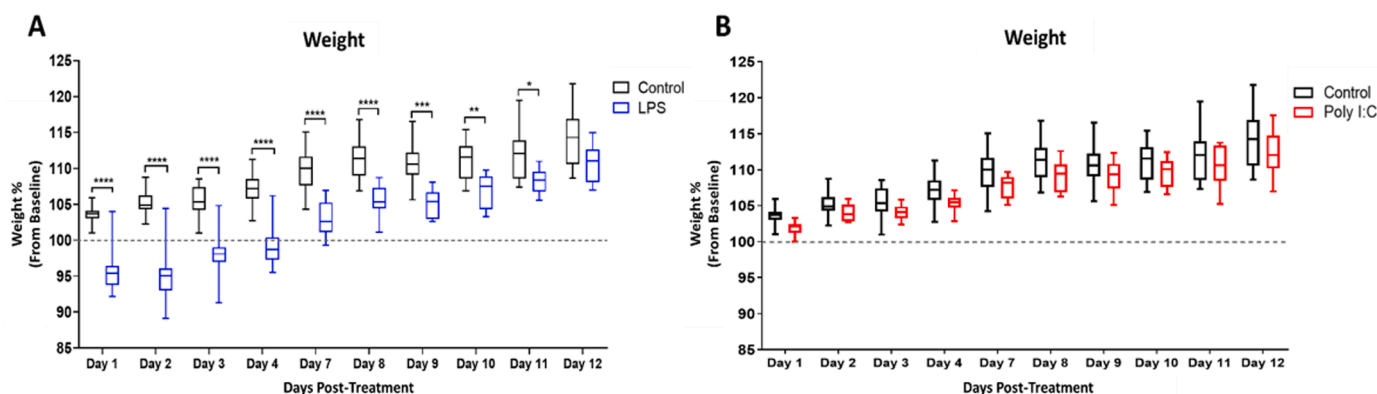


Fig. 2. Weight Changes Post-Treatment with LPS or Poly I:C. Box and whisker plots of percentage weight compared to baseline (dotted line), normalized to individual, following administration of: (A) 3 mg/kg of LPS or vehicle or (B) 4.5 mg/kg of poly I:C or vehicle. The mean is represented as the line within the box, with the box representing the upper and lower quartiles, and the minimum and maximum percentages represented by the whiskers. Comparisons showing significance are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. $N = 10$ animals per group.

field for the control and poly I:C groups, with the majority of the time spent either in all four corners of the field or moving between corners. However, in the LPS group, the majority of time was spent in only one corner of the open field over the 5 min. When quantified, the LPS group showed significant reductions in activity on day 3, as assessed by distance covered ($p = 0.0463$) and increased time spent not moving ($p = 0.0456$), compared to controls, but these changes were not apparent on other days tested (Fig. 3B–C). Rats treated with LPS showed no difference in time spent in the outer zone compared to controls (Figure. S1A). The Poly I:C group displayed no significant overall changes in distance or time spent not moving, compared to controls (Fig. 3D–E). However, poly I:C showed a reduction for time spent in the outer zone on day 1 ($p < 0.001$), day 3 ($p < 0.01$) and day 10 ($p < 0.001$), compared to controls (Figure. S2A).

3.3. Sunflower seed and sucrose preference: reward seeking & motivation

The sunflower seed test was conducted to assess reward seeking and motivation behaviors (Figs. 4, S1 and S2). LPS treatment in rats led to a reduction in the time to eat the first seed ($p < 0.01$; Fig. 4A) and an increase in the number of seeds eaten on day 7 compared to controls ($p = 0.0442$; Fig. 4B), although there was no significant difference on other days. Poly I:C-treated rats also displayed a significant reduction in time to eat first seed on day 10 compared to controls ($p = 0.0401$; Fig. 4C) but no change in the number of seeds eaten on any day (Fig. 4D). Both LPS and poly I:C displayed no changes in total time to eat all sunflower seeds compared to controls (Figure. S1B, S2B). To further assess reward seeking and motivation behaviors, we also performed the sucrose preference test. Treatment with LPS or poly I:C had no effect on total fluid intake, water consumption or sucrose consumption compared to controls (Figure. S1C–E, S2C–E).

3.4. In-cage monitoring

3.4.1. Core body temperature

Given that both LPS and poly I:C can mimic infection, and fever is a response to infection, body temperature was assessed. During the first 24 h after treatment, rats in both LPS and poly I:C groups displayed an elevated core body temperature compared to vehicle-treated controls (Fig. 5). Following LPS treatment, rats had an increase in body temperature at 2.5–8 h ($p < 0.05$) and 16–24 h ($p < 0.01$) post-injection compared to controls (Fig. 5A), while poly I:C treated rats had an increased temperature only at 4.5–6.5 h ($p < 0.05$) post-injection compared to untreated controls (Fig. 5B). Beyond 24 h, the LPS-treated group displayed elevated body temperatures out to 5 days after treatment ($p < 0.05$, Fig. 5C), whereas poly I:C-treated animals had

a similar body temperature to control animals (Fig. 5D).

3.4.2. Activity, stress and locomotion

Due to the potential influence that researchers may have on behavior in traditional behavioral testing, in-cage monitoring was used to quantify activity and locomotion for each rat within their home cage environment. Distance travelled over 12 hour periods was reduced in the LPS group on days 1–3 ($p < 0.05$) compared to controls (Fig. 6A) whereas poly I:C did not affect distance travelled (Fig. 6B). Time spent isolated was increased in the LPS group on day 3 ($p = 0.022$) compared to controls (Fig. 6C) whereas there was no change in the poly I:C group (Fig. 6D). Interestingly, for both the LPS (Fig. 6E) and poly I:C (Fig. 6F) groups, there was a reduction in time spent drinking during days 3 and 4 ($p < 0.001$), while on day 1 the poly I:C group actually had a small increase in time spent drinking ($p < 0.05$) compared to controls (Fig. 6F). Time spent mobile, rearing, and in the center zone of the cage were unchanged for LPS and poly I:C treated animals compared to controls for all timepoints (Figure. S3).

4. Discussion

To determine if LPS and poly I:C treated animals display a behavioral phenotype similar to fatigue seen in human patients, we evaluated a number of behaviors in both the LPS and poly I:C animal models. In this study, we have analysed multiple behavioral tests for activity, anxiety, reward seeking and motivation. In addition to these observation- and intervention-based behavioral assessments, we used powerful in-cage monitoring technology to quantify rodent behavior without external interference. We have demonstrated that the LPS model produced much stronger and more sustained effects on behavior than the poly I:C model, resembling some of the features presented by humans with fatigue.

4.1. Systemic inflammation & sickness behavior

We show that both the LPS (3 mg/kg) and poly I:C (4.5 mg/kg) models produce acute systemic inflammation, shown with increased core temperature within 12 h post-treatment. LPS-induced elevated temperature is consistent with other studies where a 3 mg/kg or higher dose of LPS was administered [21,37]. The acute increase in temperature for a short-period post-treatment in the poly I:C group is also consistent with previous literature [19,38]. While both LPS and poly I:C exhibited signs of systemic inflammation within 12 h post-treatment, LPS treatment led to elevated temperature for several days compared to poly I:C treatment. The elevation in temperature in LPS treated animals was also shown not to follow diurnal temperature fluctuations, compared to poly I:C and control animals. This reflects 'sickness

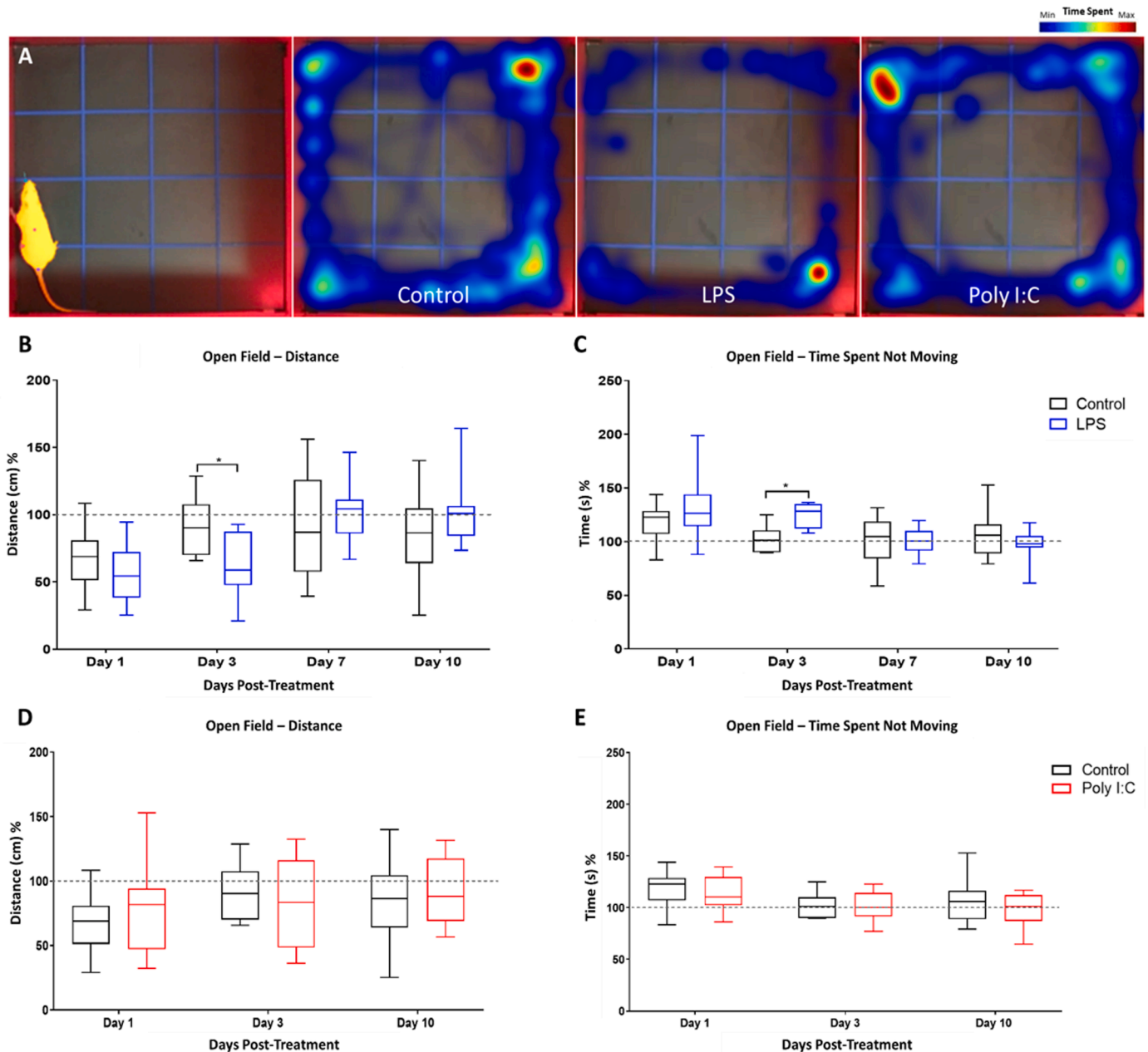


Fig. 3. Heat Maps and Behavioral Testing of Open Field Post-Treatment with LPS and Poly I:C. (A) Ethovision software tracking uses the contrast of a white rat on a black background in an open field box to determine the center point, nose point and tail point to measure multiple open field parameters. Representative images of heat maps depicting the time spent at different locations within the open field box over a 5-minute period on day 3 post-injection of control, 3 mg/kg LPS or 4.5 mg/kg poly I:C. (B-E) Box and whisker plots of the open field test, normalized to individual, with baseline (dotted line), for (B, D) distance and (C, E) time spent not moving for: (B, C) 3 mg/kg of LPS and (D, E) 4.5 mg/kg of Poly I:C treatment groups compared to controls. The mean is represented as the line within the box, with the box representing the upper and lower quartiles, and the minimum and maximum percentages represented by the whiskers. Comparisons showing significance are denoted by $*p < 0.05$. $N = 10$ animals per group. In panel C, an outlier was excluded from the LPS group on day 3. In panel D, an outlier was excluded on day 3 and day 10 (not the same animal) in the poly I:C group.

behavior' for several days post-treatment with LPS as a result of substantial systemic inflammation before recovery typically after 4 days [22,39]. This result is also consistent with fatigue experienced by humans, with CFS patients often suffering low-grade fevers during bouts of fatigue [10].

In our study, we also showed significant weight loss in the LPS animal model. This is a common characteristic of systemic inflammation and fatigue in both experimental animals and human fatigue. However, it should be noted that other studies have shown weight gain and/or obesity to be associated with chronic fatigue syndrome [40,41]. Other studies administering LPS treatment produced significant weight loss

over several days compared to controls, which is consistent with a previous study [42], although many studies only assessed LPS behavioral changes up to five days post-treatment [4]. Therefore, the significantly lower gain in weight in the LPS group compared to controls, from days 7–11, suggests animals may have an impaired ability to gain weight and may be suffering from a fatigue phenotype, in the absence of systemic inflammation at this later timepoint. Studies have highlighted that a characteristic of poly I:C-induced fatigue behavior is significant weight loss compared to healthy animals [19,20]. However, we did not observe any weight loss in the poly I:C group indicating that poly I:C treatment may only cause a short-term acute systemic inflammation, instead of a

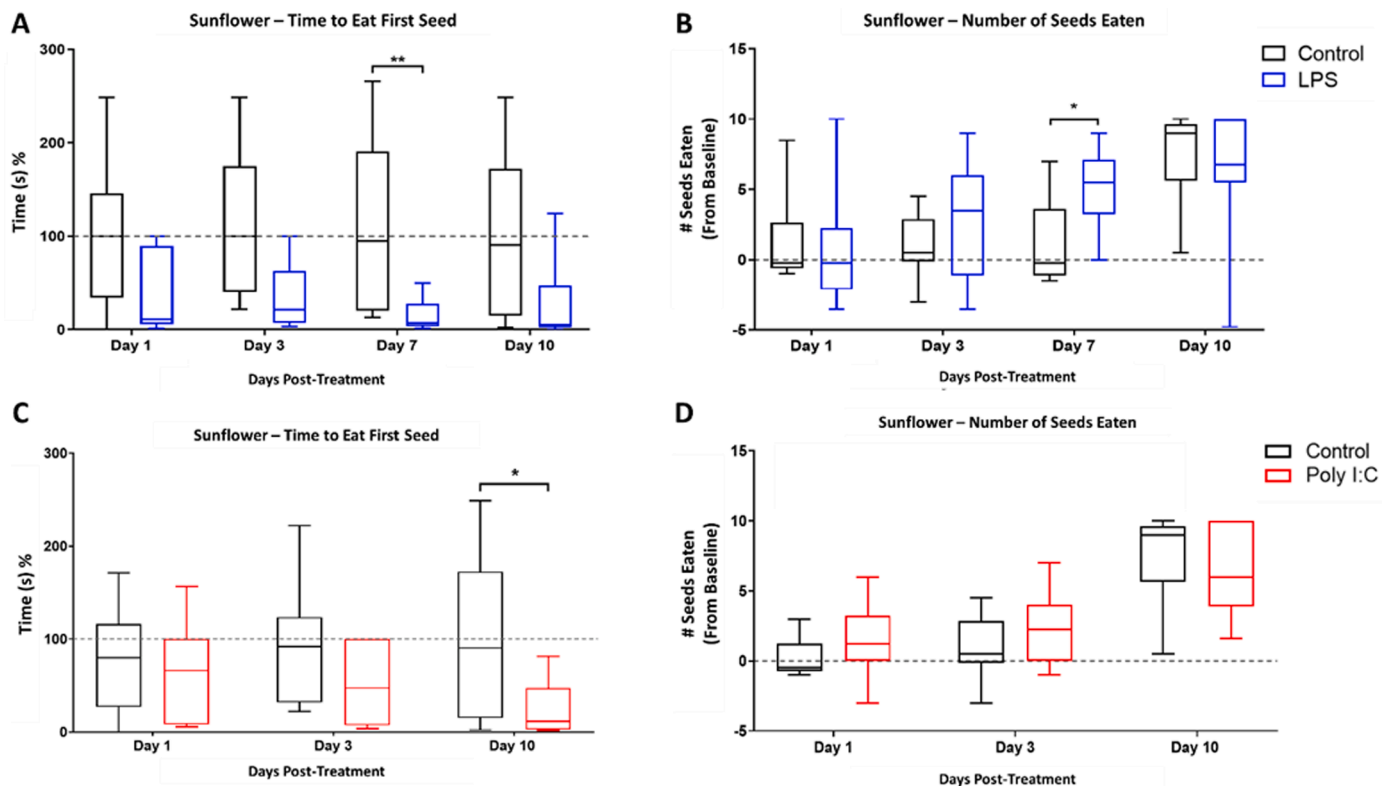


Fig. 4. Sunflower Seed Behavioral Tests Post-Treatment with LPS and Poly I:C. Box and whisker plots of sunflower seed behavioral tests, normalized to individual, with baseline (dotted line), for (A, C) time to eat first seed and (B, D) number of seeds eaten for: (A, B) 3 mg/kg of LPS and (C, D) 4.5 mg/kg of Poly I:C treatment groups compared to controls. The mean is represented as the line within the box, with the box representing the upper and lower quartiles, and the minimum and maximum percentages represented by the whiskers. Comparisons showing significance are denoted by * $p < 0.05$, ** $p < 0.01$. $N = 10$ animals per group. In panels A and C, two outliers were excluded on day 1 and day 3 (not the same animal) in the control group. In panel A, outliers were excluded from the LPS group on day 1 (1 outlier), day 3 (2 outliers), and day 10 (1 outlier). In panel C, one outlier was excluded from the poly I:C group on day 1 and day 3. In panels B and D, one outlier was excluded on day 1 in the control group.

persistent fatigue-like phenotype.

4.2. Activity & stress

Reductions in activity or locomotion are common symptoms of fatigue, in both humans and animal models, and are a typical behavioral response arising from tiredness or muscle weakness from ‘sickness behavior’ [43]. Based on our results, we observed reductions in activity of LPS-treated rats in both the open field test and in-cage monitoring. These reductions in activity occurred during ‘sickness behavior’ of elevated temperature and weight loss and dissipated once temperature and weight returned to baseline values. However, no changes in activity were observed in poly I:C animals for both open field and in-cage monitoring. This reduction in activity in LPS animals was associated with abolition of normal diurnal fluctuations, which were clearly evident in poly I:C-treated and control animals. Reductions in activity have been shown previously in both the LPS and poly I:C animal models during sickness behavior [39,43] but only in relation to the open field or the wheel-running task [20,31], both of which can have a level of external interference. Interestingly, open field activity was most reduced following LPS administration at day 3, when body temperature was elevated but not to the extent as seen on day 1. However, in-cage monitoring indicates that LPS-treated animals are displaying clear signs of reduced activity on both day 1 and day 3 when elevated temperature and weight loss are present, which the open field testing could not detect on day 1. Changes to the levels of activity in LPS-treated animals may be due to the excessive cytokine production and increased vascular permeability that are produced following administration of LPS [11,42].

It is also worth noting that there was no indication of stress in these animals. Evidence has suggested that stress and cognitive impairment contribute to depressive-like symptoms in animals [33]. Determining if depressive-like symptoms influenced our results was possible with our behavioral tests and in-cage monitoring. Animals displayed no stress related behaviors in open field or in-cage monitoring, suggesting that reductions in activity are not due to depressive-like behaviors but through systemic inflammation induced ‘sickness behavior’.

4.3. Reward seeking & motivation

To test for reward seeking and motivation, we employed both the sunflower seed test and the sucrose preference test which involves the animal engaging in a non-essential activity in order to be rewarded. These tests have been used to assess reward seeking and motivation behavior in animal models of diseases or illnesses associated with fatigue, such as stroke [44,45] and depression [46]. However, to our knowledge, both the sunflower seed test and sucrose preference test have never been used to assess reward-seeking and motivation in an animal model of fatigue. This study showed that for the LPS group there was increased reward seeking and motivation behaviors to find and eat the sunflower seeds compared to controls, whereas the poly I:C group displayed only a reduction in time to eat the seeds. Interestingly for the LPS group, these increased reward-seeking and motivation appeared when ‘sickness behavior’ was absent. These results are in contrast with previous studies that have assessed the sunflower seed test on diseased animals with fatigue-associated symptoms and reported reductions in the number of seeds diseased animals ate and the latency in these animals seeking the seeds [44,45]. There is the possibility that the apparent

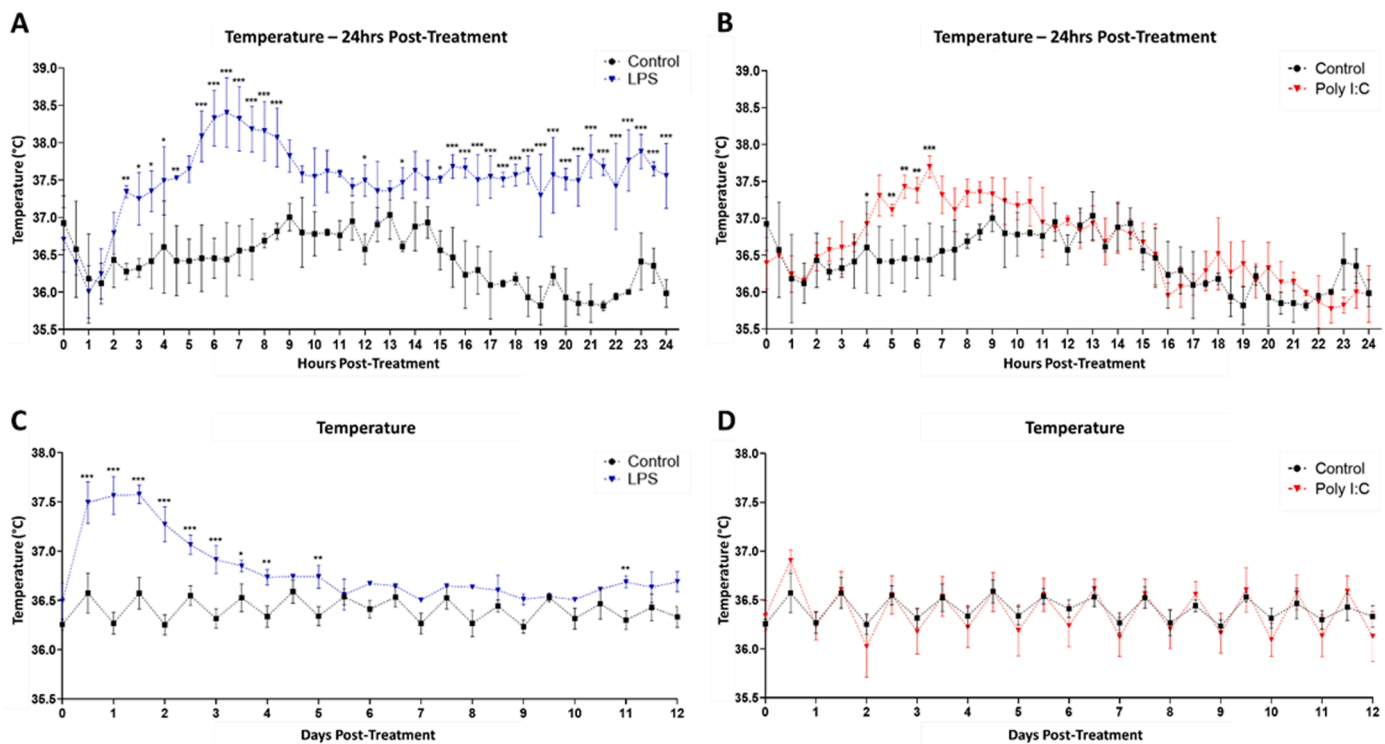


Fig. 5. Body temperature following treatment with LPS or poly I:C. Using in-cage monitoring, body temperature for rats was recorded over 24 h (A, B) and over 12 days (C, D) following treatment with 3 mg/kg LPS (A, C) or 4.5 mg/kg poly I:C (B, D) compared to vehicle-treated controls. Data for each timepoint are shown as mean \pm SD. Comparisons showing significance are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. $N = 3$ animals per group. Please note that the same control group is displayed across both panels A and B, as well as panels C and D.

increase in reward-seeking and motivation may be attributed to learning the sunflower seed task and improved ability to complete the task. Previous studies have shown that repeated behavioral testing can lead to learned behavior improving performance in specific tasks [47,48], but to mitigate this, we carried out a habituation period for both the open field and sunflower seed tasks. Another possibility is that rodents are motivated to seek carbohydrate rich foods [49,50] due to a possible energy deficit, or due to the animal recovering from treatment and seeking a substance it perceives as a reward.

In addition, we found that in the sucrose preference test there was no difference between sucrose and water consumption after treatment with LPS or poly I:C. This indicates that there are no changes in reward-seeking or motivation towards the sucrose solution following these treatments. This is in contrast with previous studies employing the sucrose preference test where diseased animals with fatigue-associated symptoms exhibited increases in sucrose solution consumption compared to water [39,46]. It is possible that we lacked sufficient power to detect any changes, while protocol differences and the time of assessment post-LPS or poly I:C administration may also have influenced our results. Further studies are required to fully delineate the extent of motivational and reward-seeking behaviors in these fatigue models, and careful consideration must be given to the test and approach taken.

4.4. Behavioral testing vs in-cage monitoring

This study is the first to utilize a combination of both traditional behavioral testing and in-cage monitoring technology to observe fatigue-like behavior in the LPS and poly I:C animal models. Traditional behavioral testing, although reproducible, requires large numbers of animals to have enough statistical power for valid comparisons between groups due to high variability. These tests are also prone to human or external interference, stress effects and training effects where the animals adapt their behavior after repeated test exposure. In-cage

monitoring can circumvent these problems through enabling monitoring of animal behavior in their own home environment without human or external factors affecting behavior. This reduces subjective bias imposed by the researcher as well as limiting external variables that can influence rat behavior in an experimental setting. Our study showed that in-cage monitoring was sensitive in the detection of multiple parameters influenced by LPS and poly I:C, and that there is clear diurnal variation related to the animals' activity. Furthermore, the combination of behavioral testing and in-cage monitoring used in this study provide a complementary approach, strengthening our understanding of fatigue-behavioral outcomes. We postulate that some of the differences in outcomes observed in our study compared to previous studies may be attributed to the low-stress methods of behavior testing used (such as the use of non-invasive in-cage monitoring, paired with 7-day habituation cycles), in addition to randomization and blinding.

4.5. Limitations

This study has several limitations. The dosages of LPS (3 mg/kg) and poly I:C (4.5 mg/kg) used in this study were at the lower end of the range previously used in rodent studies. Earlier studies using LPS and poly I:C to model fatigue and systemic inflammation in animals have used dosages from 2 mg/kg to 12 mg/kg to induce a behavioral phenotype [4,19,20,38]. However, studies using higher doses of LPS and poly I:C have reported greater systemic inflammation and the induction of other behavioral changes, not associated with a fatigue phenotype [19]. Therefore, we used the lowest dosages of LPS and poly I:C consistent with producing enough inflammation to replicate a primarily fatigue phenotype without the other unwanted side effects of high-level inflammatory change. We found that the low doses of both LPS and poly I:C that we used induced systemic inflammation but only LPS produced the desired fatigue phenotype, even though the dose of LPS was lower than poly I:C. Future studies exploring a full dose response relationship

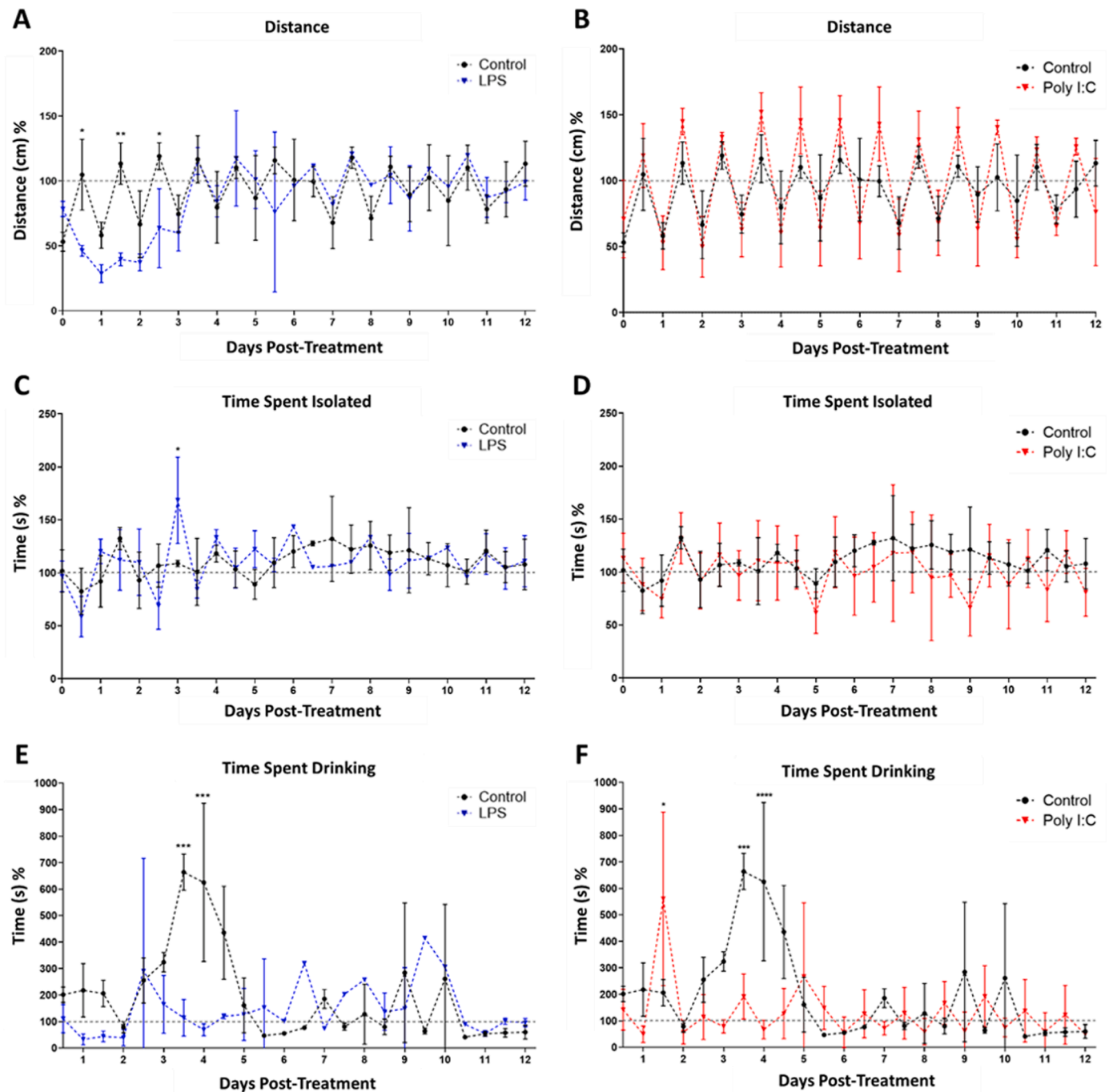


Fig. 6. In-cage Monitoring of Behavior Post-Treatment with LPS and Poly I:C. Line plots of in-cage monitoring of (A, B) distance, (C, D) time spent isolated and (E, F) time spent drinking normalized to individual with baseline (dotted line) for 12 days following treatment with (A, C, E) 3 mg/kg LPS or (B, D, F) 4.5 mg/kg poly I:C compared to controls. Data for each timepoint shown as mean \pm SD. Comparisons showing significance are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. $N = 3$ animals per group. Please note that the same control group is displayed across both panels A and B, as well as panels C and D, and panels E and F.

would help determine whether a threshold for inflammatory induction of fatigue exists and whether there is a ceiling beyond which fatigue does not change.

Another limitation in this study was the low sample size of animals used in the in-cage monitoring. Despite these low numbers, there were highly significant differences in many of the in-cage monitoring parameters observed for the LPS cohort compared to controls, indicating the in-cage monitoring system is highly sensitive to changes in behavior. However, increasing the sample size in a future experiment will enable us to further determine the sensitivity of in-cage monitoring to capture fatigue-like behaviors that may not have been seen in the present study.

Lastly, the time period chosen for behavioral tests of 12 days post-treatment may have been too short to observe all behavioral changes related to a fatigue phenotype. A significant increase in temperature was observed on day 11 for the LPS group, which may be a delayed fatigue response. This is consistent with chronic fatigue syndrome patients who have reported having short periods of fevers before, during and after bouts of fatigue [10]. There was also a delayed response with changes to sunflower seed consumption in the LPS (day 7) and poly I:C (day 10) groups, when fatigue phenotype is expected to have subsided. As this study is one of the first to extensively assess fatigue behavior in these models including beyond the peak inflammatory period [4,18–20,27], it

remains unknown as to why behavioral changes may lag the initial temperature and inflammatory responses post-LPS or poly I:C administration. It has been suggested that these longer-term behavioral changes following an inflammatory response could be due to altered neuroendocrine and neurotransmitter production in regions such as the basal ganglia, the location where reward-seeking and motivational behaviors are regulated [30]. Increasing the time of behavioral testing may help determine if a fatigue phenotype extends beyond the period where inflammatory changes are evident, and future studies could determine the mechanism of this lag between inflammation and behavior.

5. Conclusion

Currently both the LPS and poly I:C animal models are widely used in fatigue research due to both producing a systemic inflammation response hours after treatment. Although sickness behavior and reductions in activity are well documented in both these models, this study is the first to utilize behavioral tests assessing reward seeking behaviors and in-cage monitoring in both the LPS and poly I:C models. The results from this study highlight that the LPS model produces a more extensive fatigue-like phenotype, with prolonged elevation of temperature, weight loss and changes to activity, and possible reward-seeking and motivation effects, compared to the poly I:C model. Our results suggest that the LPS animal model is a more suitable candidate for further studies on fatigue-like behavior.

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Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.physbeh.2021.113347](https://doi.org/10.1016/j.physbeh.2021.113347).

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