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Differential effects of lipopolysaccharide on cognition, corticosterone and cytokines in socially-housed vs isolated male rats

Brittney Russell [a,c,1], Kelly M. Hrelja [a,*,1], Wendy K. Adams [a,c], Fiona D. Zeeb [a,c], Matthew D. Taves [a,b,c], Sukhbir Kaur [a,c], Kiran K. Soma [a,b,c], Catharine A. Winstanley [a,c,*]

[a] Department of Psychology, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada
[b] Department of Zoology, University of British Columbia, Vancouver, BC, Canada
[c] Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada

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ABSTRACT

Social isolation is an established risk factor for mental illness and impaired immune function. Evidence suggests that neuroinflammatory processes contribute to mental illness, possibly via cytokine-induced modulation of neural activity. We examined the effects of lipopolysaccharide (LPS) administration and social home cage environment on cognitive performance in the 5-Choice Serial Reaction Time Task (5CSRTT), and their effects on corticosterone and cytokines in serum and brain tissue. Male Long-Evans rats were reared in pairs or in isolation before training on the 5CSRTT. The effects of saline and LPS (150 μg/kg i.p.) administration on sickness behaviour and task performance were then assessed. LPS-induced sickness behaviour was augmented in socially-isolated rats, translating to increased omissions and slower response times in the 5CSRTT. Both social isolation and LPS administration reduced impulsive responding, while discriminative accuracy remained unaffected. With the exception of reduced impulsivity in isolated rats, these effects were not observed following a second administration of LPS, revealing behavioural tolerance to repeated LPS injections. In a separate cohort of animals, social isolation potentiated the ability of LPS to increase serum corticosterone and IL-6, which corresponded to increased IL-6 in the orbitofrontal and medial prefrontal cortices and the nucleus accumbens. Basal IL-4 levels in the nucleus accumbens were reduced in socially-isolated rats. These findings are consistent with the adaptive response of reduced motivational drive following immune challenge, and identify social isolation as an exacerbating factor. Enhanced IL-6 signalling may play a role in mediating the potentiated behavioural response to LPS administration in isolated animals.

1. Introduction

The impact of social isolation on health and well-being is widely recognized [15], and there is a growing emphasis on its contribution to negative mental health outcomes (see [77] for review). For example, Chou, Liang, and Sareen [19] found that social isolation was associated with an increased risk for the development of Major Depressive Disorder (MDD), dysthymic disorder, social phobia, and generalized anxiety disorder in over 30,000 adults. Indeed, depressive disorders like MDD are common, globally affecting more than 264 million people [69], and are strongly associated with the absence of social relationships [4,13,126]. Furthermore, the World Health Organization (WHO) recognizes mental illness, especially depression, as one of the leading causes of disability worldwide, underscoring the need to understand the factors which facilitate or contribute to a decline in mental wellness.

Recent literature has begun to recognize an exacerbated inflammatory response, resulting in increased circulating pro-inflammatory cytokines, as a risk factor for various psychiatric disorders, most notably mood disorders, such as depression, anxiety, and bipolar disorder, and schizophrenia [49,57,63,75,78,82]. This inflammatory response is driven by both peripheral and central macrophages, which function to phagocytose invading pathogens. When this system is overactive it can result in the degeneration of neuronal circuitry, thus giving rise to detrimental behavioral effects [64]. The macrophage theory of depression posits that overactive cytokine secretion by macrophages drives the neuroendocrine disruptions that have been observed in MDD [106]. In

* Corresponding authors at: Department of Psychology, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada.
E-mail addresses: kellyhre@mail.ubc.ca (K.M. Hrelja), cwinstanley@psych.ubc.ca (C.A. Winstanley).
[1] B.R. and K.M.H contributed equally to this work.

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support, several clinical studies that have found a higher prevalence of mood disorders among those with chronic inflammatory diseases including diabetes, asthma, and arthritis [53,82,85,88], and correlative evidence from multiple clinical studies suggests that subsets of psychiatric patients have higher levels of circulating pro-inflammatory cytokines, such as interlukin-1-β (IL-1β), IL-6, and tumor necrosis factor-α (TNFα) [27,42,76]. The evidence from both clinical and animal studies implicates inflammation in the development of the depression [96], with experimental research finding that depressive and anxiety symptoms can be produced in healthy humans by artificially increasing cytokine levels with either lipopolysaccharide (LPS) or typhoid vaccination [65,97,109,133]. In agreement, Capuron et al. [17] found that 45 % of patients met the diagnostic criteria for MDD following prolonged administration of the cytokine interferon (IFN)-α. Additionally, most depressive symptoms occur almost immediately after cytokine administration and disappear with termination of cytokine treatment, suggesting that cytokines play a causal role in the development of psychiatric symptoms [140]. Likewise, stress has been linked to disadvantageous changes the physical and functional properties of neurons and glia. Specifically, psychosocial and environmental stress increase both neuronal dystrophy and microglial activation, which have both been linked to depressive-like behavioural changes (see [131] for review).

LPS, an endotoxin derived from the outer membrane of gram-negative bacteria, causes transient increases in peripheral cytokines and induces flu-like behaviour in rodents that typically lasts 24 hrs post-injection. In contrast, central cytokine levels can remain elevated for prolonged amounts of time following LPS administration due to the activation of microglia, the brain's resident macrophages [90]. Moreover, the activation of microglia by LPS can trigger alterations in neuronal circuitry, thus permanently affecting behaviour [10]. Research has utilized LPS injections to model some aspects of psychiatric disorders in rodents, notably depressive symptoms. In such models, acute administration of cytokines or cytokine inducers, like LPS, have been shown to reliably produce sickness behaviour that may include loss of appetite and decreased body weight [93], cognitive deficits [113], decreased motor and exploratory activity, anhedonia as determined by saccharin preference [31], and altered sleeping patterns [30,71]. These behavioral changes share a striking resemblance to symptoms present in depressive disorders [35], and the majority of these behaviours can be abolished by treatment with antidepressants [18,31]. Additionally, chronic treatment with LPS has been shown to induce depression and anxiety-like symptoms, while inhibiting neuronal activity in the entorhinal cortex via astrocyte and microglial-mediated increase in IL-1R1/NF-kB/CCL5 signaling [115], indicating a causal role of glial-mediated cytokine signalling in LPS-induced behavioural effects.

Although there is cogent evidence for the role of cytokines in the development of numerous psychiatric disorders, not all patients exhibit high cytokine loads, nor does mental health deteriorate in all subjects with high levels of inflammation. It is likely that mental health problems are precipitated by several environmental factors, acting in concert with neurobiological mechanisms [35,44]. Both longitudinal human studies and manipulations of isolation in nonhuman social species have found that social isolation negatively impacts normal stress and immune responses, with the resulting immunosuppression and pro-inflammatory effects being especially harmful to overall health and well-being [16]. Indeed, Beck and Bredemeier's [6] latest model of depression unifies the relationship between precipitating stressors, like social isolation, and "sickness behaviour" that is produced by immune challenge. This model suggests that social isolation can act as a stressor, exacerbating the release of pro-inflammatory cytokines, which negatively impact cognition. While it is evident that immune challenges in adulthood are capable of triggering symptom onset in vulnerable individuals, further research is required to elucidate the effects of social isolation on this complex relationship.

In the present study, we examined the effects of social isolation on cognition, corticosterone and cytokines in rats receiving an acute immune challenge. We used the 5-Choice Serial Reaction Time Task (5CSRTT) to evaluate cognitive function. The 5CSRTT is a well-established, translationally validated test of visuospatial attention, motivation, and impulse control [89,99,102,123,124,132]. Task performance is highly sensitive to damage or alterations in the frontal cortex and nucleus accumbens, key nodes in the affective corticostriatal loop [20,21,66,99,129]. Many of the cognitive and emotional symptoms, including anhedonia, anergia and executive dysfunction, associated with MDD and other psychiatric disorders are thought to arise when functioning of this circuit is compromised [43,56,70,116]. Furthermore, numerous psychiatric medications and disease models have already been evaluated on this task, making it a useful comparative cognitive screen [1,5,61,67,95,103]. We hypothesized that social isolation would augment the effects of LPS on the 5CSRTT, corticosterone and cytokines.

2. Materials and methods

2.1. Animals

Testing and housing procedures were in accordance with the standards of the Canadian Council of Animal Care, and all experimental protocols were approved by the Animal Care Committee of the University of British Columbia. 49 male Long-Evans rats, received at postnatal day 21 (PND 21; Charles River Laboratories, Saint-Constant, QC, Canada), were housed in a colony room at a temperature of approximately 22ºC under a reverse 12 hr light-dark cycle (lights off at 8:00 am) with tap water freely available. 24 animals were randomly assigned to be pair-housed ("PAIR") and the remaining 25 animals were housed alone ("ISOLATE") for the duration of the study. Animals were fed ad libitum until they weighed approximately 350 g (PND 56). To prepare them for behavioural testing, animals in Experiment 1 (Pair: $n = 12$; Isolate: $n = 12$) were then restricted to 14 g of rat chow per day to maintain approximately 85–90 % of free-feeding body weight. Body weights did not significantly differ between housing groups at the onset of food restriction (Pair: 361.6 ± 12.1 g; Isolate: 335.2 ± 9.2 g; no group differences), corresponding to studies in the literature [100,119]. Pair-housed animals were transported from the colony room to the behavioural testing suite with their cage partners and two other rats; the isolates were transported alone and were never exposed to other rats. Animals in Experiment 2 (Pair: $n = 12$; Isolate: $n = 13$) were handled 5 days a week but were not food restricted as they were not used for behavioural assessment.

2.2. 5-Choice serial reaction time task (5CSRTT)

As per previous publications [111,130], behavioural testing took place in standard five-hole operant chambers, each enclosed within a ventilated sound-attenuating cabinet (Med Associates Inc., Fairfax, VT, USA). Food-restricted rats were trained to make a nose-poke response in one of five holes in which a stimulus light was briefly illuminated (0.5 s) to earn a food reward (45 mg pellet; Bioserv, Flemington, NJ, USA). The spatial location of the stimulus light varied randomly from trial to trial, with each session consisting of 100 trials and lasting up to 30 min. Animals initiated a trial by making a nose-poke response at the food tray, after which a 5 s inter-trial interval (ITI) ensued before presentation of the stimulus light. Responses made during the ITI were classified as 'premature' and were punished by a 5 s time-out period during which the chamber light was turned on and no further trials could be initiated. A 'correct' response was rewarded with delivery of a food pellet, whereas an incorrect or lack of response ('omission') was not rewarded and was punished in the same manner as premature responses. Other variables included repeated, 'perseverative' responding and response latencies. Animals were trained for 5 sessions/week until a stable baseline performance of ≥ 80 % correct responses and < 20 % omissions was reached. One rat was excluded from analysis as it was unable to learn the task but was retained for the duration of the experiment as he

was pair-housed (final numbers for Experiment 1: Pair n = 11, Isolate n = 12). All other animals reached training criterion by session 55; analysis of variance (ANOVA) confirmed stable baseline performance for all variables across sessions 51–55 (no significant effects of the within-subjects variable 'session'). Both pair- and isolation-housed rats required a similar number of training sessions (Pair: 22.9 ± 1.3; Isolate: 21.7 ± 1.7; no group differences) to reach the final stage (0.5 s stimulus light duration), indicating that housing conditions did not impair the ability to learn the 5CSRTT as per previous studies [28]. Animals were tested for a further 2 weeks, during which they received saline or LPS injections prior to 5CSRTT testing.

2.3. Lipopolysaccharide (LPS) administration and sickness behaviour

E. coli LPS (serotype 0127:B8; Sigma-Aldrich, Oakville, ON, Canada) was dissolved in 0.9 % sterile saline at 150 μg/mL and injected i.p. (1 mL/kg). This strain and dosage of LPS has been previously shown to elicit sickness behaviour in rats [137].

To assess the effects of LPS administration and housing condition on 5CSRTT performance, Experiment 1 animals were administered saline or LPS in a counterbalanced sequence with 3–4 days in between. After injection, animals were returned to their home cages for 90 min prior to the onset of 5CSRTT testing; this wait time was chosen on the basis of pilot tests that indicated the behavioural effects of LPS at this dose were strongest 90 min post-injection. LPS-induced sickness behaviour in the home cage was scored for 10 s every 15 min using an approach adapted from the literature [58]; the template designed for scoring is provided in Appendix A. Symptoms of ptosis, lethargy and piloerection were scored as either absent (0), mild (1) or severe (2), and sleep was scored as either absent (0) or present (1); scores were then summed to give an overall index of sickness behaviour for the 90 min period. After the first dosing round, 5CSRTT testing continued for 2 weeks before the saline/LPS dosing round was repeated. After an additional 2 weeks, a third injection of saline (Pair: n = 5; Isolate: n = 6) or LPS (Pair: n = 6; Isolate: n = 6) was given 90 min prior to blood collection for corticosterone assays.

Given the tolerance-like effects of repeated LPS injections on behaviour, behaviourally-naïve rats in Experiment 2 were used to formally assess the effects of LPS administration and housing condition on corticosterone and cytokine levels. Rats received either an LPS injection (Pair: n = 6; Isolate: n = 7) or a saline injection (Pair: n = 6; Isolate: n = 6) 90 min prior to blood and brain tissue collection. Within the pair-housed group, 1 animal per cage was administered LPS while the other received saline. Sickness behaviour was not scored in this experiment.

2.4. Tissue collection

Blood and/or brain tissue collection took place over two days with injection times staggered such that all samples were harvested 90 min post-injection. Following rapid live decapitation, trunk blood was immediately collected on ice and placed in a refrigerator (4 °C) overnight; samples were centrifuged at 10,000 g for 10 min before serum was removed in multiple aliquots. For Experiment 2, tissue samples from the medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC) and nucleus accumbens (NAc) were immediately dissected on a cold surface and frozen in dry ice following live decapitation. Anesthesia was not utilized in order to reduce the time between disturbing the cage and euthanasia, and thus to spare induction of stress or other confounds caused by anesthesia. All samples were stored at − 80 °C.

2.5. Steroid extraction and corticosterone RIA

Steroids were extracted from serum samples from both experiments using solid phase extraction with C18 columns as described previously [114]. Thawed samples were first diluted in 10 mL MilliQ deionised water, and columns were primed with 3 mL HPLC-grade methanol and equilibrated with 10 mL water. Samples were loaded onto the columns then washed with 10 mL 40 % HPLC-grade methanol to remove interfering substances. Steroids were then eluted with 5 mL 90 % HPLC-grade methanol and dried in a vacuum centrifuge at 60 °C. Corticosterone concentrations were measured in duplicate using a commercial RIA kit (07120102; MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions.

2.6. Multiplex cytokine assays

Serum samples from Experiment 2 were outsourced for multiplex cytokine analysis (#RD23, Rat 23-Plex Cytokine Assay; Eve Technologies, Calgary, AB, Canada) to assist in identifying molecular targets for assessment of brain tissue. All samples were run in duplicate. Of the 23 cytokines assayed, 7 were not reliably quantified (IL-4, IL-5, IL-17, Eotaxin, GM-CSF, G-CSF, RANTES). Concentrations were obtained for the following 16 analytes: IL-1α, IL-1β, IL-2, IL-6, IL-10, IL-12(p70), IL-13, IL-18, MCP-1, Leptin, MIP-1α, IFN-γ, IP-10, GRO/KC, TNF-α and VEGF. Serum cytokine levels are reported as pg/mL.

Samples from the mPFC, OFC and NAc were defrosted in ice-cold lysis buffer (150 mM NaCl, 20 mM Tris pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 % Triton X-100), which was prepared fresh with the addition of 1 complete mini protease inhibitor cocktail tablet (#11836153001, Roche Diagnostics, Indianapolis, IN, USA), 200 μL phosphatase inhibitors 2 and 3 (200 μL each; Sigma-Aldrich, St. Louis, MO, USA), 100 μL 1 M NaF, and 40 μL PMSF (from 500 mM stock in DMSO) per 10 mL volume. Samples were homogenised by ultrasonication and centrifuged for 15 min (16,110 g, 4 °C). Aliquots of supernatant were removed for cytokine analyses and stored at − 20 °C. The remaining supernatant was subject to protein quantification using the Pierce BCA Protein Assay Kit (#23227, Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. Samples were diluted 1:20 in working reagent to account for limited volumes. Samples were run in duplicate and averaged to give the total protein content (mg/mL).

Brain homogenates were simultaneously assayed for IL-1β, IL-4, IL-6, IL-10 and TNF-α in-house using a custom 5-plex kit (#K153A0H-2, Meso Scale Discovery, Rockville, MD, USA), as per the manufacturer's protocol. Samples were diluted 1:2 in diluent 42 and assayed in duplicate; samples from each brain region were run on separate plates. Plates were read using a Sector Imager 2400 (Meso Scale Discovery) and data were analysed using the Discovery Workbench software v. 4.0 (Meso Scale Discovery). The lower limit of detection (LLOD) for the assays varied by plate and analyte. The following LLOD ranges were observed (pg/mL): IL-1β, 3.65–7.34; IL-4, 0.0525–0.167; IL-6, 3.04–6.51; IL-10, 0.281–0.834; TNF-α, 0.0933–0.43. Tissue levels were adjusted and reported as pg (cytokine)/mg of protein.

2.7. Statistical analyses

Data were assessed by ANOVA using Systat 13 (Systat Software, Inc., Chicago, IL, USA) to determine the effects of LPS as a function of housing condition, and the effects of isolation housing per se. All behavioural and molecular data analyses used the between-subjects factor 'Housing' (2 levels: Pair, Isolate). Assessment of 5CSRTT behaviour compared responses following LPS or saline injection, with 'LPS' as a within-subjects, repeated measures factor, whereas such 'Treatment' effects on corticosterone and cytokine levels were assessed between-subjects (2 levels: Saline, LPS). Significant main effects of housing, or housing and LPS/treatment interactions, were further examined by comparing saline-treated pair- and isolation-housed animals, or LPS and saline treatments within each housing condition, respectively.

Sickness behaviour scores and 5CSRTT variables in Experiment 1 were analysed separately. 5CSRTT variables included the percentage of trials in which correct, omitted or premature responses were made; the total number of perseverative responses or trials completed; and latencies for correct responses and reward collection, as described

Table 1
5CSRTT behavioural variables at baseline in pair- or isolation-housed rats.

	Pair	*Isolate*
% Premature responses	12.64 ± 2.33	8.39 ± 1.72
% Correct responses	85.38 ± 1.93	88.01 ± 1.58
% Omitted responses	2.13 ± 0.68	3.13 ± 1.21
Correct response latency (s)	0.42 ± 0.01	0.46 ± 0.01#
Reward collection latency (s)	1.59 ± 0.09	1.67 ± 0.08
Perseverative responses	3.98 ± 1.09	5.15 ± 0.78
Trials completed	99.98 ± 0.02	99.38 ± 0.58

Data are expressed as mean ± SEM across 5CSRTT sessions 51–55 for rats housed in pairs (n = 11) or isolation (n = 12). #p = 0.053.

previously [111,130]. Corticosterone data from Experiments 1 and 2, and cytokine data from Experiment 2, were analysed separately; outlying data points identified in the initial ANOVAs were removed. To meet normality assumptions, data were transformed for analyses as appropriate: 5CSRTT variables expressed as percentages were arcsine transformed, whereas all cytokine data were log transformed. Untransformed data are presented for clarity.

All data are expressed as mean ± Standard Error of the Mean (SEM). Differences were considered significant where $p < 0.05$; trend level differences where $p \leq 0.08$ are reported.

3. Results

3.1. Effects of housing condition on baseline 5CSRTT behaviour

Table 1 shows the average behavioural responses for all seven 5CSRTT variables at baseline in pair- and isolation-housed rats in Experiment 1. Compared to pair-housed rats, socially-isolated rats tended towards slower speeds of responding ($F_{(1,21)}= 4.208$, $p = 0.053$). Otherwise, housing condition did not overtly influence baseline 5CSRTT performance (all F-values≤3.1, all p-values≥0.095).

3.2. Effects of LPS administration on sickness behaviour and 5CSRTT performance in pair- and isolation-housed rats

Compared to pair-housed animals, the severity of sickness behaviour elicited by LPS administration was more pronounced in rats housed in isolation (Fig. 1A; Housing: $F_{(1,21)}= 8.6$, $p = 0.008$). This enhanced LPS-induced sickness response in socially-isolated rats translated to increased omissions and slower response times in the 5CSRTT (Fig. 1D, E; Omissions–Housing × LPS: $F_{(1,21)}=8.4$, $p = 0.009$, Pair–LPS: $F_{(1,10)}= 0.7$, $p = 0.412$, Isolate–LPS: $F_{(1,11)}= 9.0$, $p = 0.012$; Correct response latencies–Housing × LPS: $F_{(1,21)}= 5.5$, $p = 0.028$, Pair–LPS: $F_{(1,10)}= 0.0$, $p = 0.885$, Isolates–LPS: $F_{(1,11)}= 10.0$, $p = 0.009$). While social isolation per se reduced levels of impulsive responding, LPS administration reduced this measure to a similar extent in animals in both housing groups (Fig. 1B; Housing: $F_{(1,21)}= 9.4$, $p = 0.006$, LPS: $F_{(1,21)}= 15.9$, $p = 0.001$, Housing × LPS: $F_{(1,21)}= 0.4$, $p = 0.540$, Saline–Housing: $F_{(1,21)}=7.0$, $p = 0.015$). Unlike its selective effect on response times in isolate rats, LPS administration increased latencies to collect food rewards in animals under both housing conditions (Fig. 1F; LPS: $F_{(1,21)}= 10.2$, $p = 0.004$, Housing × LPS: $F_{(1,21)}= 0.0$, $p = 0.886$). LPS administration also increased the number of perseverative responses in pair-housed rats, yet this effect was absent in socially-isolated animals (Fig. 1G; Housing × LPS: $F_{(1,21)}= 5.3$, $p = 0.032$, Pair– LPS: $F_{(1,10)}= 5.5$, $p = 0.041$, Isolate–LPS: $F_{(1,11)}= 1.1$, $p = 0.307$). In contrast, neither housing condition nor LPS administration was found to alter discriminative accuracy or the total number of trials completed (Fig. 1C,H; all F-values≤2.2, all p-values≥0.157).

In the second LPS dosing round, these selective effects on sickness behaviour and 5CSRTT performance were absent in socially-isolated animals (Fig.S1, Appendix B Supplementary Data), reflecting the tolerance-like effects of LPS on behaviour [120], and supporting the use of a second cohort for molecular analyses.

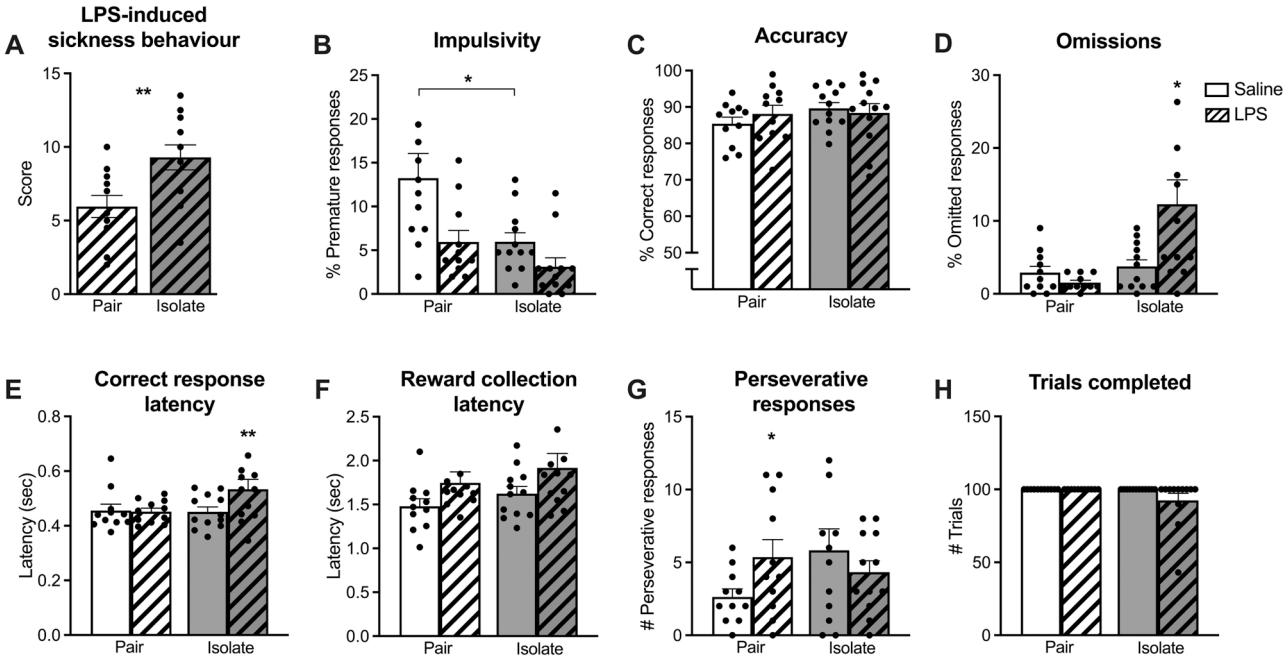

Fig. 1. Social isolation increased sickness behaviour and reduced motivational drive in the 5CSRTT after LPS administration. Graphs show A) sickness scores and B-H) 5CSRTT performance in pair- and isolation-housed rats ('Pair' n = 11, 'Isolate' n = 12) after i.p. injection with saline or 150 μg/kg LPS. Social isolation augmented A) LPS-induced sickness behaviour, which translates to increased D) omissions and E) response latency in the 5CSRTT. Both social isolation and LPS administration reduced B) motor impulsivity. LPS administration increased G) perseverative responding in pair-housed rats only, and increased F) latency to collect food rewards across both housing groups. C) Discriminative accuracy and H) total trials completed were unchanged by either isolation housing or LPS administration. Data are expressed as mean ± SEM. *p < 0.05, **p < 0.01 compared to pair-housed rats and/or saline response within housing group; main effects of LPS are not highlighted.

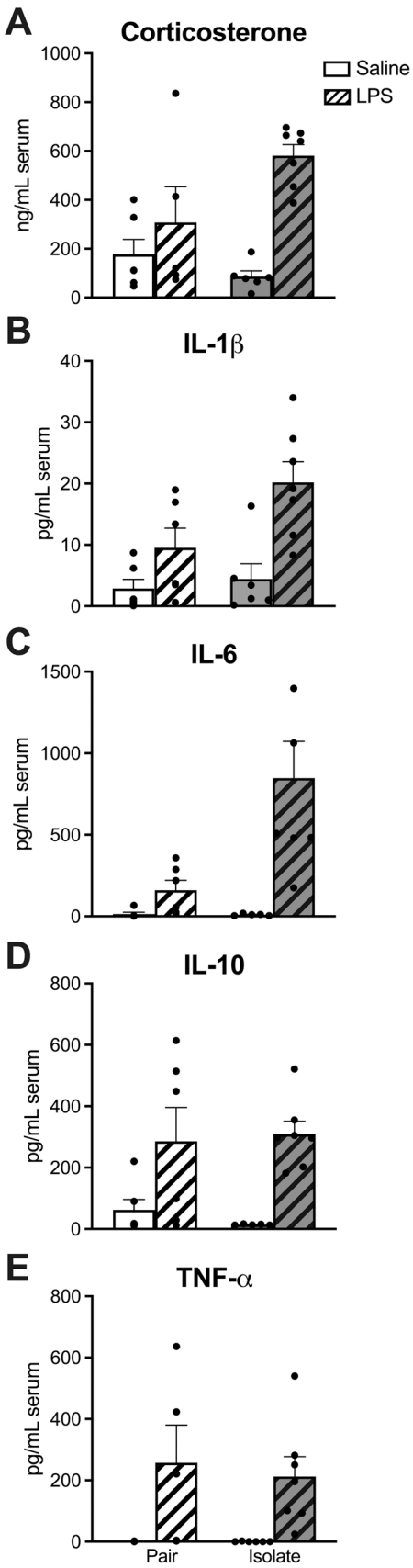

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Fig. 2. Social isolation increased circulating corticosterone and cytokines following LPS administration. Graphs show serum levels of A) corticosterone, B) IL-1β, C) IL-6, D) IL-10 and E) TNF-α in behaviourally-naïve, pair- and isolation-housed rats 90 min after injection with saline or 150 μg/kg LPS (n = 5–6 per group). LPS administration increased serum A) corticosterone levels in socially-isolated, but not pair-housed, rats. A similar pattern of results was observed with serum B) IL-1β and C) IL-6 levels (see text for detailed statistics). LPS administration also increased serum D) IL-10 and E) TNF-α levels similarly in both housing conditions. Data are expressed as mean ± SEM. ***$p < 0.001$ compared to saline response within housing group; main effects of LPS are not highlighted.

3.3. Effects of LPS administration and housing condition on circulating corticosterone and cytokines

In the behaviourally-naïve cohort in Experiment 2, serum corticosterone levels at 90 min following LPS administration were augmented in socially-isolated, but not pair-housed, rats (Fig. 2 A; Housing × Treatment: $F_{(1,20)} = 6.1$, $p = 0.022$, Pair–Treatment: $F_{(1,9)} = 0.8$, $p = 0.400$, Isolate–Treatment: $F_{(1,11)} = 84.9$, $p < 0.001$). As expected, this effect was absent in those animals tested in the 5CSRTT in Experiment 1, following a third dose of LPS that did not impair 5CSRTT performance or saline prior to serum collection (Fig.S2, Appendix B Supplementary Data).

Across both housing groups, LPS administration increased serum levels of IL-1β, IL-6, IL-10 and TNF-α (Fig. 2B-E; main effects of Treatment: IL-1β–$F_{(1,21)} = 16.0$, $p = 0.001$; IL-6–$F_{(1,20)} = 67.9$, $p < 0.001$; IL-10–$F_{(1,20)} = 26.2$, $p < 0.001$; TNF-α–$F_{(1,20)} = 67.5$, $p < 0.001$). In addition, overall serum IL-1β concentrations tended to be higher in isolates compared to pair-housed rats, although social isolation did not significantly potentiate the magnitude of LPS' effects (Fig. 2B; Housing: $F_{(1,21)} = 3.4$, $p = 0.080$, Housing × Treatment: $F_{(1,21)} = 1.0$, $p = 0.332$). In contrast, the LPS-induced elevation of serum IL-6 content was enhanced in socially-isolated rats (Fig. 2C; Housing: $F_{(1,20)} = 8.1$, $p = 0.010$, Housing × Treatment: $F_{(1,20)} = 3.6$, $p = 0.074$); although the interaction was trend level, the main effect of housing on IL-6 was driven by the enhanced LPS response in isolated rats, since basal IL-6 levels did not differ between housing groups under saline (Saline–Housing: $F_{(1,9)} = 0.5$, $p = 0.511$). A trend level interaction also suggested that the magnitude of the LPS-induced increase in circulating IL-10 was enhanced in socially-isolated, compared to pair-housed, rats (Fig. 2D; Housing: $F_{(1,20)} = 0.0$, $p = 0.988$; Housing × Treatment: $F_{(1,20)} = 3.5$, $p = 0.074$). However, LPS' effects on serum TNF-α concentrations were not moderated by housing condition (Fig. 2E; Housing: $F_{(1,20)} = 0.5$, $p = 0.490$; Housing × Treatment: $F_{(1,20)} = 0.8$, $p = 0.382$).

LPS administration also increased serum levels of IL-12(p70), MCP-1, MIP-1α and GRO/KC across all subjects (Table 2; main effects of Treatment: IL-12(p70)–$F_{(1,20)} = 4.7$, $p = 0.043$; MCP-1–$F_{(1,21)} = 53.3$, $p < 0.001$; MIP-1α–$F_{(1,19)} = 100.7$, $p < 0.001$; GRO/KC–$F_{(1,21)} = 51.5$, $p < 0.001$). Levels of IFN-γ also tended to be enhanced following LPS administration (Table 2; Treatment: $F_{(1,20)} = 3.7$, $p = 0.070$). Housing condition did not modulate these LPS effects, nor influence basal levels of these cytokines (Table 2; all F-values≤3.2, all p-values≥0.087).

Compared to pair-housed animals, serum IP-10 content was reduced in isolation-housed rats overall; however, this effect was absent in saline-treated animals only (Table 2; Housing: $F_{(1,21)} = 4.6$, $p = 0.044$, Saline–Housing: $F_{(1,10)} = 0.1$, $p = 0.774$). Otherwise, LPS administration did not significantly alter circulating IP-10 levels in animals in both housing groups (Table 2; Treatment: $F_{(1,21)} = 2.7$, $p = 0.113$, Housing × Treatment: $F_{(1,21)} = 2.8$, $p = 0.109$).

Neither isolation housing nor LPS administration was found to alter serum levels of IL-1α, IL-2, IL-13, IL-18, Leptin or VEGF (Table 2; all F-values≤2.8, all p-values≥0.110).

Effects of LPS administration and housing condition on cytokines in the OFC, mPFC and NAc.

Contrasting the serum results, IL-1β levels in the OFC, mPFC and NAc

Table 2
Serum levels of cytokines in pair- and isolation-housed rats following saline or LPS.

	Pair		*Isolate*	
	Saline	*LPS*	*Saline*	*LPS*
IL-1α	2.2 ± 0.2	4.4 ± 1.4	2.7 ± 0.7	2.2 ± 0.3
IL-2	2.5 ± 0.7	11.6 ± 5.6	3.4 ± 0.9	4.2 ± 1.1
*IL-12 (p70)**	4.2 ± 2.0	5.6 ± 1.5	2.3 ± 0.4	4.5 ± 0.8
IL-13	29.6 ± 14.5	46.0 ± 11.5	25.6 ± 12.7	23.7 ± 6.6
IL-18	79.6 ± 6.0	75.3 ± 11.1	81.3 ± 7.8	69.3 ± 8.7
*MCP-1 * **	339.1 ± 58.2	2851.7 ± 1038.8	294.0 ± 79.7	4273.7 ± 751.4
Leptin	19495.3 ± 1353.2	21923.7 ± 2600.6	19468.9 ± 1573.5	17727.0 ± 1681.4
MIP-1α **	8.6 ± 0.7	47.7 ± 17.7	7.7 ± 0.6	59.5 ± 5.1
IFN-γ#	4.2 ± 0.7	12.2 ± 5.5	4.4 ± 2.4	6.0 ± 1.2
IP-10	1.2 ± 0.9	4.1 ± 1.5	1.0 ± 0.9	0.6 ± 0.3
GRO/KC **	1431.0 ± 132.2	7275.7 ± 1177.0	1790.1 ± 270.0	5957.3 ± 1481.6
VEGF	1.3 ± 0.9	1.0 ± 0.4	2.4 ± 1.2	2.7 ± 1.0

*$p < 0.05$, * **$p < 0.001$, #$p = 0.070$, main effect of LPS administration. Data are expressed as mean pg/mL ± SEM (n = 4–6 per group).

were not altered by either isolation housing or LPS administration (Fig. 3A; all F-values≤2.6, all p-values≥0.123). Similarly, in the brain regions examined, levels of IL-10 were unchanged by housing condition or LPS administration, except for a trend level LPS-induced reduction of OFC IL-10 content across all subjects (Fig. 3D; OFC–Treatment: $F_{(1,19)}= 4.3$, $p = 0.053$; otherwise, all F-values≤ 3.1, all p-values≥ 0.095).

LPS administration did not affect levels of IL-4 in the OFC, but reduced these in the mPFC across both housing conditions (Fig. 3B; OFC–Treatment: $F_{(1,20)}= 1.3$, $p = 0.264$, Housing × Treatment: $F_{(1,20)}= 0.0$, $p = 0.851$; mPFC–Treatment: $F_{(1,19)}= 8.6$, $p = 0.009$, Housing × Treatment: $F_{(1,19)}= 0.1$, $p = 0.743$). Whereas isolation housing did not influence cortical IL-4 content, basal levels of IL-4 in the NAc were lower in socially-isolated rats compared to pair-housed animals (Fig. 3B; main effects of Housing: OFC–$F_{(1,20)}= 1.1$, $p = 0.306$; mPFC–$F_{(1,19)}= 0.3$, $p = 0.616$; NAc–$F_{(1,20)}= 10.8$, $p = 0.004$, NAc–Saline: $F_{(1,10)}= 7.2$, $p = 0.023$). However, LPS administration did not alter NAc IL-4 content in rats under either housing condition (Fig. 3B; Treatment: $F_{(1,20)}= 0.3$, $p = 0.579$, Housing × Treatment: $F_{(1,20)}= 0.0$, $p = 0.942$).

Social isolation and LPS administration had region-specific, interacting effects on brain IL-6 content. In the OFC, IL-6 levels were increased by LPS administration in isolation-housed, but not pair-housed, rats (Fig. 3C; Housing × Treatment: $F_{(1,20)}= 12.4$, $p = 0.002$, Pair–Treatment: $F_{(1,10)}= 0.4$, $p = 0.522$, Isolate–Treatment: $F_{(1,10)}= 20.6$, $p = 0.001$). Social isolation also potentiated the increase in mPFC IL-6 levels following injection with LPS (Fig. 3C; Housing: $F_{(1,20)}= 17.7$, $p < 0.001$; Treatment: $F_{(1,20)}= 11.0$, $p = 0.003$; Housing × Treatment: $F_{(1,20)}= 4.0$, $p = 0.058$); however, like the effects observed with serum IL-6, the main effect of housing reflects the enhanced LPS response in isolated rats, as mPFC IL-6 content was similar in saline-treated animals across housing groups (Fig. 3 C; Saline–Housing: $F_{(1,10)}= 2.1$, $p = 0.176$). In contrast, LPS administration had bidirectional effects on IL-6 levels in the NAc, reducing IL-6 content in pair-housed rats and increasing IL-6 content in socially-isolated rats (Fig. 3 C; Housing × Treatment: $F_{(1,19)}= 12.8$, $p = 0.002$, Pair–Treatment: $F_{(1,9)}= 5.9$, $p = 0.038$, Isolate–Treatment: $F_{(1,10)}= 8.7$, $p = 0.014$). Basal levels of IL-6 in the NAc also tended to be lower in socially-isolated rats overall (Fig. 3C; Housing: $F_{(1,19)}= 4.3$, $p = 0.053$).

Finally, LPS administration significantly increased TNF-α in all brain regions investigated to a similar extent in both pair- and isolation-housed rats (Fig. 3E; main effects of Treatment: OFC–$F_{(1,20)}= 17.8$, $p < 0.001$; mPFC–$F_{(1,19)}= 15.4$, $p = 0.001$; NAc–$F_{(1,19)}= 8.1$, $p = 0.010$; otherwise, all F-values≤ 2.1, all p-values≥ 0.159).

4. Discussion

Here we show that social isolation increased the severity of LPS-induced sickness behaviours, resulting in an increase in omissions and slower responding on the 5CSRTT compared to pair-housed animals. Follow-up analyses indicate that social isolation exacerbated LPS-induced increases in serum corticosterone and IL-6, the latter of which was also increased in all brain regions examined. These findings indicate that the combination of environmental stress, in the form of social isolation, and immune activation produces substantial changes in motivation and related cognitive behaviours. As such, it is likely that a lack of social interactions could potentiate the deleterious impact of immune activation on mental health.

In contrast to previous studies [84,138], LPS administration did not impair accuracy of target detection. The discrepancy between our findings may be caused by methodological differences, including differences in the task being performed, as well as the acute use of a substantially (over 2x) lower dose of LPS in our experiment. The current data suggest that neither housing condition nor LPS administration influence attentional functioning on the standard 5CSRTT. The increase in both omissions and reward collection latency observed in response to LPS are therefore more likely caused by motivational, rather than attentional, impairments. However, inflammation also causes a range of behavioural changes which are not specific to any particular cognitive process, including psychomotor slowing, anergia, and fatigue [30,52,96]. These behavioural changes may result in elevated omissions and longer response latencies on the 5CSRTT. It is therefore difficult to determine to what extent our results map onto psychological constructs like anhedonia, which may be modelled better through assessing rodents' unwillingness to exert more effort in order to obtain higher value rewards. For example, both LPS and systemic administration of IL-6 or IL-1β shift rats' preference toward low-effort options that yield smaller rewards, consistent with behaviour that would be expected in an anhedonic state [36,37,92,121,134,139,141].

Environmental stressors, like chronic mild stress (CMS), decrease responding for reward [101,128], which may be caused by a generalized CMS-induced decrease in reactivity to rewards (for review, refer to [128]). Our finding that social isolation exacerbates cognitive deficits produced by LPS are consistent with the few studies that examine the contribution of concurrent inflammation and environmental stress to the pathogenesis of psychological disorders [44,137]. Thus, it is reasonable to infer that environmental stress, like social isolation, can potentiate changes in motivation produced by inflammation. Interestingly, saline injections, which can be interpreted as a mild stressor, reduced premature responding in isolated rats during both dosing rounds, which may suggest that an interaction between two environmental stressors is sufficient to produce changes in motivation but not as globally as the changes observed in the socially isolated rats administered LPS.

According to nearly every molecular and behavioural metric recorded here, socially isolated rats showed a more pronounced response to LPS, yet only pair-housed rats increased perseverative responding in response to LPS administration. This form of behavioural inflexibility is mediated by the mPFC and OFC [23,25,26,99]; [3,11]. Increases in mPFC TNF-α results in decreased cognitive flexibility on a set-shifting task [142], which may contribute to these findings. However, we found TNF-α to be elevated in both housing conditions, making this explanation less likely.

In line with previous studies, we found behavioural tolerance to repeated LPS exposure [44,45,55], and therefore had to analyse the cytokine changes caused by LPS in a separate cohort of animals. Consistent with previous research [138], we found that LPS administration increased serum levels of IL-1β, IL-6, IL-10, TNF-α, IL-12(p70),

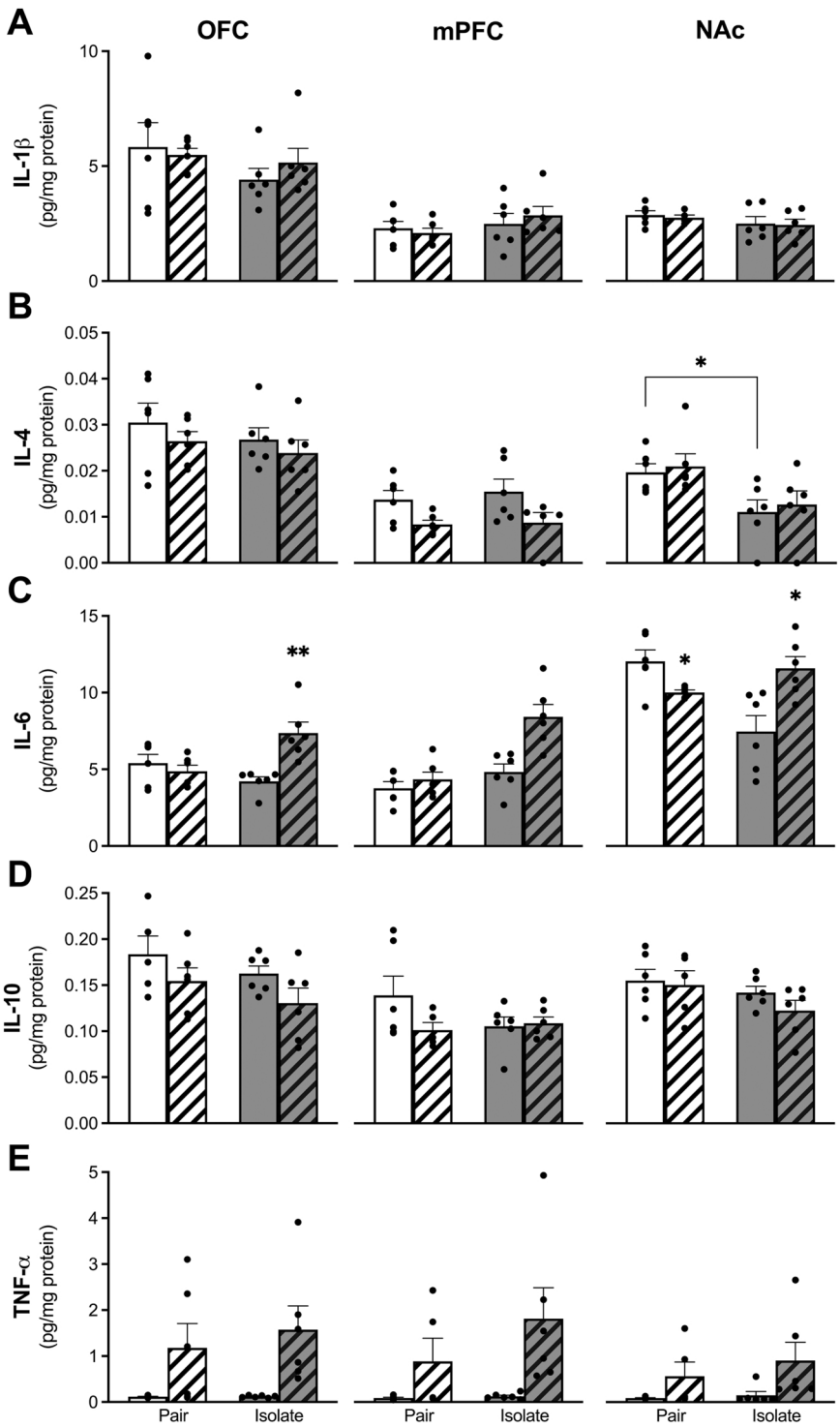

Fig. 3. Social isolation leads to region-specific changes in central cytokines at baseline and after LPS administration. Graphs show tissue content of A) IL-1β, B) IL-4, C) IL-6, D) IL-10 and E) TNF-α in the orbitofrontal and medial prefrontal cortices, and nucleus accumbens (OFC, mPFC, NAc), of pair- and isolation-housed rats 90 min after saline or 150 μg/kg LPS injection (n = 5–6 per group). Social isolation reduced basal B) IL-4 levels in the NAc, and LPS administration increased levels of C) IL-6 across regions in isolated rats only. LPS administration also reduced mPFC IL-4 content and increased E) TNF-α levels in all regions in animals of both housing groups. Brain levels of A) IL-1β and D) IL-10 were unchanged by isolation housing and/or LPS administration. Data are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$ compared to pair-housed rats or saline response within housing group; main effects of LPS are not highlighted.

MCP-1, MIP-1α and GRO/KC in both housing groups, and there was a trend towards increased IFN-γ. In socially isolated animals, we found a potentiated increase in serum IL-6 after LPS administration, which is consistent with previous findings [44], a potentiated trend-level increase in IL-1β, and a subtle reduction in chemokine CXCL10/IP-10. Also consistent with the literature, neither LPS nor housing condition had an effect on serum levels of IL-1α, IL-2, IL-13, IL-18, Leptin or VEGF [38,90,91]. Although the current findings support the hypothesis that greater cognitive impairment resulting from LPS administration in socially isolated animals arises through a greater pro-inflammatory response, these data do not allow us to definitively conclude which, if any, cytokine(s) are primarily responsible for driving these behavioural changes.

While our serum cytokine findings were relatively consistent with the literature, our serum corticosterone findings differed. In contrast to Yee and Prendergast [137], we only observed an increase in serum CORT following LPS in socially-isolated rats, rather than across both housing conditions. CORT levels fluctuate throughout the day and are highly sensitive to environmental factors, which could contribute to unintended variation across studies. The more widespread CORT response in the previous study could also result from differences in strain of rat used, as Wistar rats are known to have higher levels of CORT than

Long-Evans rats and to differ in immune and neuroendocrine responses to stress [7,39,108,122]. Furthermore, the rats used here were handled daily for weeks during behavioural training, and were also accustomed to mild food deprivation, both of which could have altered the sensitivity of their CORT response.

The patterns of serum cytokine expression observed in our study were not always reproduced in brain regional analyses. While the LPS-induced increase in serum TNF-α was paralleled across housing conditions and in all brain regions investigated, LPS did not alter IL-1β and IL-10 levels in the OFC, mPFC and NAc, despite significant elevations in circulating levels of these cytokines in all rats. We also found that LPS, independent of housing condition, decreased levels of IL-4 in the mPFC but not OFC, while the combination of LPS and isolation stress elevated IL-6 in the mPFC and OFC. In contrast, basal levels of IL-4 were reduced in the NAc of socially isolated rats, while no group differences were observed in serum analyses. A lack of concordance between central and peripheral cytokine content is not uncommon [1,12,68,79,110]. It is known that microglial phenotype and reactivity is dependent on cues in the local environment ([33,34,41,60]; see [131] for review). This gives rise to the possibility of region-specific cytokine profiles, as well as neuronal and synaptic differences, at baseline and in response to challenges. Likewise, astrocytes and neurons are also capable of differentially mediating microglial phenotype and cytokine tone in a region-dependent manner [54,127,131].

Reduced levels of anti-inflammatory cytokines, including IL-4, are common in models of psychiatric disorders [86,87], and infusion of IL-4 has been shown to reverse IL-1β-induced anhedonic behaviour and restore social activity in rats [94]. Similarly, IL-4 is lower in some patients with MDD, and increases following treatment with anti-depressants [62,107,112]. Here we observed significantly lower levels of IL-4 selectively within the NAc of socially isolated rats. This brain region has been implicated in reward processing and motivational deficits typically observed in MDD ([8]; Diego A. [40,46,117]). It is therefore possible that decreased accumbal IL-4 contributed to the increase in omissions and slower responding on the 5CSRTT caused by LPS in socially isolated rats. mPFC levels of IL-4 also dropped following LPS administration in isolated rats. The behavioural effects of reducing IL-4 levels in specific brain regions remains largely unknown, and may warrant further investigation.

In parallel with our serum findings, IL-6 was increased in all brain regions analysed after LPS administration in socially isolated rats only. This cytokine has both pro- and anti-inflammatory effects. Increased levels of IL-6 have been observed in a very wide range of psychiatric disorders, including but not limited to depression [42,48,76,83], schizophrenia [81,104], autism spectrum disorder [125,135], and obsessive compulsive disorder [47,72]. It has been found that lithium treatment selectively reduced levels of IL-6 in the OFC, corresponding to a decrease in impulsivity - a behaviour thought to be largely mediated by the OFC [1], further supporting the existence of region-specific differences in cytokine levels following manipulation. Perhaps of particular relevance to the current study, elevated IL-6 may be predictive of cognitive symptoms of depression [32,48,59]. In further support of region-specific findings in the current study, it has been shown that symptoms of depression can be linked to neuronal and synapse degeneration in the PFC, but neuronal hypertrophy and increased synaptic density in subcortical regions, including the NAc [22]. Furthermore, Felger et al. [51] found that elevated serum IL-6 and IL-1β were associated with decreased neural connectivity within the corticostriatal reward circuit and increased anhedonia in depressed patients, providing a potential mechanism linking the peripheral and central elevations in IL-6 to the motivational deficits observed in socially isolated rats that were administered LPS.

Inflammation, particularly increases in TNF-α, IL-1, and IL-6, significantly alters HPA axis activity, resulting in increased CORT levels [98,105,118,143]. The changes in CORT noted here could therefore be driven by changes in cytokine levels. Alternatively, HPA axis dysregulation, resulting in hypercortisolism, is independently capable of mediating immune function [14,80,136]. LPS-induced changes in CORT and immune molecules may therefore be happening in series, in parallel, or through a synergistic interaction. Previous work has shown that CORT administration impairs reward-based decision--making through alterations in frontal circuitry [73,74]. Specifically, c-fos expression, a marker of neuronal activation, was increased in the lateral OFC, insular cortex, and infralimbic cortex of rats treated with systemic CORT [73], and directly infusing CORT into the infralimbic cortex was sufficient to disrupt such decision-making [74]. These frontal regions have all been implicated in motivation and impulse control on the 5CSRTT [24,29,50,130]. It is therefore possible that CORT is interacting with changing cytokine levels to produce the observed behavioural effects in our socially isolated rats. Similarly, symptoms of mental illness may be mediated by the interaction between both the immune and stress response to environmental challenges. Future work with LPS-treated rats could determine the degree to which changes in stress versus immune molecules dominate in driving the cognitive behavioural response to observed here, or if these two biological pathways work in tandem.

In summary, social isolation resulted in greater LPS-induced sickness behaviour and motivational deficits resulting in a decrease in responding and an increase in omissions on the 5CSRTT. LPS administration increased latencies to collect rewards and decreased premature responding regardless of housing condition. As evidenced in experiment 2, LPS caused an expected increase in brain TNF-α, along with a number of pro-inflammatory serum cytokines, and LPS selectively increased serum CORT and brain levels of IL-6 in socially isolated animals. Taken together, the data provide support for inflammatory theories of psychiatric illness, whereby immunological challenges can trigger symptom onset. Therefore, we provide evidence implicating both an exacerbated immune response and environmental stress in the form of social isolation in the development of motivational symptoms that are observed in a range of psychiatric disorders.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2022.114000.

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