Inflammation-induced reorientation of reward versus punishment sensitivity is attenuated by Minocycline

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HIGHLIGHTS

- LPS reorients sensitivity to punishments versus rewards
- Minocycline attenuates this enhanced sensitivity to punishment versus rewards
- Implicates activated microglia in inflammation-induced motivational reorientation
- Minocycline may benefit motivational features of depression like anhedonia/ fatigue

ABSTRACT

Background: Inflammation rapidly reorients motivational state, mood is impaired, pleasurable activities avoided and sensitivity to negative stimuli enhanced. When sustained, this can precipitate major depressive episodes. In humans, this has been linked to opposing actions of inflammation on striatal/insula reward/punishment learning signals while in rodents, motivational impairments can be attenuated with minocycline, implicating a mechanistic role for microglia. Here we investigated whether minocycline also inhibits the reorienting effects of lipopolysaccharide (LPS) on reward/punishment sensitivity in humans. **Methods:** Using a crossover design, fifteen healthy volunteers underwent two experimental sessions in which they each received LPS (1ng/kg) and placebo. Half (N=8) received minocycline (100 mg bd) and half (N=7) an identical looking placebo for 3½ days before each session. Six hours post-injection participants completed a probabilistic instrumental learning task in which they had to learn to select high probability reward (win £1) and avoid high probability punishment (lose £1) stimuli to maximise their gains and minimize losses. Physiological and sickness responses were sampled hourly and blood sampled at baseline, 3 and 6 hours post-injection. **Results:** LPS induced robust peripheral physiological: temperature, heart rate and immune: differential white cell, IL-6, TNF-α, IL-8, IL-10 responses (all condition x time interactions: $p<0.005$), none were significantly modulated by minocycline ($p>0.1$). LPS also biased behavior, enhancing punishment compared with reward sensitivity ($F_{(1,13)}=6.10$, $p=0.028$). Minocycline significantly attenuated this inflammation-induced shift in reward versus punishment sensitivity ($F_{(1,13)}=4.28$, $p=0.033$). **Conclusions**: These data replicate the previous finding that systemic inflammation rapidly impairs sensitivity to rewards versus punishments in humans and extend this by implicating activated microglia in this acute motivational reorientation with implications for the development of microglial-targeted immune-modulatory therapies in depression.

INTRODUCTION

Inflammation is increasingly implicated in the pathophysiology of major depressive disorder (MDD) (Khandaker et al., 2021; Miller et al., 2009). Longitudinal and mendelian randomization epidemiological studies support a role for systemic inflammation and functional polymorphisms associated with a pro-inflammatory phenotype as risk factors for depression (Khandaker et al., 2018, 2014). Complementing this, human experimental studies show that diverse immune challenges e.g. vaccines, pro-inflammatory cytokines and lipopolysaccharide (LPS) readily induce mood, motivation and cognitive changes that closely resemble clinical features of depression (Dantzer et al., 2008) and modulate many of the same brain networks that are implicated in the pathogenesis of depression (Capuron et al., 2012; Harrison et al., 2009; Kitzbichler et al., 2021). Furthermore, during sustained therapy with Interferon-alpha (IFN-α) for Hepatitis-C or cancer, acute actions of IFN-α on amygdala and hypothalamus-pituitary axis (HPA) stress-responses predict the later emergence of true depressive episodes which occur in ~one third of patients (Capuron et al., 2003; Davies et al., 2020; Udina et al., 2012). Together, these data support an etiological role for inflammatory processes in at least some patients with depression and have stimulated the drive to develop and repurpose immunomodulatory therapies for depression (Köhler et al., 2014).

However, systemic inflammation is not present in all patients with depression, and instead appears to be more prevalent in patients who present with features of anhedonia, neurovegetative features such as fatigue or who show resistance to conventional treatments (Bekhbat et al., 2020; Cattaneo et al., 2020; Chamberlain et al., 2019; Milaneschi et al., 2021). Interestingly, disturbances in reward and punishment (typically using a pain stimulus) related behavior are a central feature of both human and rodent studies on responses to inflammation (Dantzer, 2001; Harrison et al., 2016). In humans, acute challenge with lipopolysaccharide (LPS: 0.8 ng/Kg) rapidly impairs responses to cues predicting monetary reward in the

dopamine-rich ventral striatum (VS) (Eisenberger et al., 2010). A similar reduction in ventral striatal responses to reward outcomes as well as a reduction in dopamine uptake has also been reported after chronic (4 week) treatment with interferon-alpha (Capuron et al., 2012). Further evidence that dopamine rich regions such as the ventral striatum are particularly sensitive to systemic inflammation has also come from a study of mild inflammation induced using typhoid vaccination. Here, inflammation was associated with a reorientation in learning to rewards versus punishments which was associated with a reciprocal reduction in ventral striatal encoding of reward learning signals (reward prediction error: rPE) and a converse increase in encoding of punishment learning signals (punishment prediction error; pPE) in the insula (Harrison et al., 2016). Further, by using Bayesian model selection we were able to show that these effects of inflammation on encoding of rPE and pPE likely serve to modulate how subjective values associated with available choices are updated and providing an efficient mechanism for rapidly reorienting behavior during infection. These data were also consistent with previous evidence implicating ventral striatal and insula neurons in reward- and punishment reinforcement learning respectively (Pessiglione et al., 2006).

The mechanisms underlying this inflammation-mediated shift in sensitivity to punishments versus rewards remain unclear. However, preclinical studies have demonstrated that in rodents, endotoxin induced sickness and anhedonia can be mitigated by minocycline (Henry et al., 2008) a centrally penetrant tetracycline which can inhibit the activation of microglia (Soczynska et al., 2012). Furthermore, pro-inflammatory cytokines released following microglial activation can impair the synthesis of dopamine via inhibition of the essential cofactor tetrahydrobiopterin (BH4) providing a potential mechanistic role for activation of microglia in inflammation-mediated motivational re-orientation (Neurauter et al., 2008).

In order to address this, we evaluated the effect of experimental endotoxemia and placebo (saline injection) on monetary reward versus punishment sensitivity using a repeated-measures within subject study design in healthy subjects. In addition, half of the participants received minocycline and half an identical looking placebo before each testing session. We hypothesized that similar to typhoid vaccination (Harrison et al., 2016) endotoxin would impair reward versus punishment-based learning and further that minocycline would attenuate this inflammation-induced behavioural reorientation.

METHODS

Participants: Sixteen healthy non-smoking male participants were recruited through posted advertisement (mean age: 24.7±5.0 (std) years; mean BMI: 24.4 ± 1.6 (std) kg/m2, three had BMIs in the overweight range (>25-30), none were in the obesity range (>30)). One participant failed to complete the experimental sessions and was excluded from the study. All underwent screening including medical history, Mini-International Neuropsychiatric Interview, physical examination, electrocardiogram (ECG) and blood sampling for full blood count, C-reactive protein, renal, thyroid, and liver function testing to exclude any medical or psychiatric condition. All were medication free. Participants were asked to avoid use of non-steroidal anti-inflammatory drugs (NSAIDs) for a week prior to each session. The study was approved by the London Queen Square Research Ethics Committee (REF 17/LO/0936), and all participants provided written informed consent.

Study Design: We used a mixed within/between subject study design in which all participants underwent two separate experiment sessions. During one session they received an intravenous injection of LPS (1 ng/kg) prepared from Escherichia coli O:113 (U.S. Standard Reference Endotoxin, manufactured for the Clinical Center, NIH) and in the other session an intravenous injection of 0.9% saline (placebo) (within-subjects factor). Sessions (LPS versus placebo) were

separated by a minimum of 2 weeks (3.7±3.3 (mean ± std) weeks), session order was randomised and participants (but not researchers) were blind to session order (Single blind). The LPS dose was informed by a previous study reporting a marked increase in expression of the TSPO PET imaging marker of microglial activation after the same dose (Sandiego et al., 2015). Participants were randomly divided into two groups, n=8 received minocycline (Sigma Pharmaceuticals) administered via oral tablets (100 mg bd) for 3½ days prior to each testing session and n=7 participants an identically appearing placebo (between-subjects factor). Both researcher and participant were blind to administration (double blind). Minocycline dose and dosing schedule were informed by data from human pharmacokinetic studies and clinical use (e.g. in prophylaxis of meningococcal meningitis), and rodent data on the effects of minocycline in blocking LPS-induced neuroinflammation. In humans, minocycline has a half-life of ~13 hours, readily crosses the blood-brain barrier and is consequently dosed twice daily for clinical indications (Agwuh and MacGowan, 2006). For most indications a dose of 200 mg per day is well-tolerated. In rodents, three days treatment has been shown to be sufficient to block LPS-induced neuroinflammation, more prolonged treatments (4 weeks) affect the microbiome (Yang et al., 2020). Of note, a between subject design was used to investigate effects of minocycline to avoid multiple administrations of LPS to each participant and consequent tolerance effects. The study design and the experiment sessions protocol are illustrated in **Figure 1**.

Experimental sessions were conducted in an observation room with participants lying on a clinical bed for the duration of the experiment. Participants had continuous heart rate monitoring (Mindray iMEC10) throughout each 8-hour testing session, with temperature, heart- and respiration rate, systolic and diastolic blood pressure additionally recorded every 15-60 minutes. Two venous catheters were inserted at the beginning of each testing session and remained inserted for the duration of the session. Blood samples were collected from one of

the cannulas at baseline, 3 and 6 hours post injection to measure differential white cell and cytokine responses. Subjective sickness responses were recorded hourly (at baseline, 1, 2, 3, 4 and 6 h post injection) using the profile of mood states (POMS), Karolinska Sickness Scale (Andreasson et al., 2018) and a fatigue visual analogue scale (fVAS). Timing of the experiment protocol was identical across the two sessions. Participants were asked to abstain from alcohol for 24 h prior each session and have a light breakfast prior to arrival to minimize risk of bradycardia.

Cytokine Analyses: Blood was drawn into purple top (EDTA) BD Vacutainer tubes (Becton, Dickson and Company, Franklin Lakes, New Jersey, United States), centrifuged at 2000 rpm for 20 min, then plasma removed, aliquoted, and frozen at -80 °C. Plasma Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8) and interleukin-10 (IL-10) were measured using Quantikine® High Sensitivity ELISA kits (R&D Systems inc., Minneapolis, United States). Limits of detection were 0.031 pg/mL, 0.022 pg/mL, 0.13 pg/mL and 0.09 pg/mL respectively and intra- and inter-assay coefficients of variation were 4.1% and 6.5% (IL-6), 2.0% and 6.5% (TNF-α), 5.5% and 5.5% (IL-8) and 6.6 % and 9.8% (IL-10). For IL-10, ~30% of the saline session and pre-LPS samples measured below the lowest standard. All other samples were in the quantifiable range. IL-10 samples that measured below the lowest standard were assigned a value of half the lower limit of detection (Breen et al., 2011). All samples were tested in duplicate.

Effects of LPS and minocycline on physiological responses were assessed in SPSS27 using mixed ANOVAs with condition (Saline/LPS) and time (pre injection, post injection as described) as within-subject factors and Group (Mino/PLAC) as a between-subjects factor. Post-hoc comparisons between individual time points were run using Bonferroni correction.

Reinforcement learning task: Six hours after receiving either the LPS or saline (placebo) injection participants completed a probabilistic instrumental learning task previously shown to be sensitive to inflammation-induced changes in sensitivity to rewards versus punishments (Harrison et al., 2016). In this task participants were shown three pairs of abstract stimuli. Each pair was associated with a different pair of outcomes. In the gain condition (outcomes gain £1 or gain nothing), participants had a chance of winning money, in the lose condition (outcomes lose £1 or lose nothing) a risk of losing money, and in the neutral condition (outcomes look at £1 or nothing) neither win nor lose money (see **Figure 3A**). Within each pair, the two stimuli corresponded to reciprocal probabilities (0.8/0.2 and 0.2/0.8) of the associated outcome. One pair of stimuli was randomly presented on a laptop screen on each trial. The two stimuli were presented to the left and right of a central fixation cross with relative positions counterbalanced across trials. Participants used a button press to choose the right-sided stimulus (go response) and absence of a response (no-go response) to choose the left-sided stimulus. Their choice was then circled in red and the outcome displayed after a 4-second delay. Participants had to use trial and error to learn the stimulus-outcome associations and aimed to maximize their wins (by selecting the high probability win stimulus) and minimize their losses (by avoiding the high probability lose stimulus). They were told that they would be remunerated their winnings at the end of the last session, though were given the same fixed amount at the end of the study. Each condition (gain, lose, neutral) consisted of 24 trials. As previously described, performance was quantified as the proportion of the last 50% of trials in which participants correctly selected the (high probability) gain stimulus in the reward trials and correctly avoided the lose stimulus in the punishment trials. Data were analyzed in a 2 (Group (Mino/PLAC)) × 2 (Treatment (PLAC/LPS)) × 2 (Valence (Gain/Lose)) ANOVA with treatment and valence as within-subject factors.

RESULTS

Vital signs: LPS induced significant increases in body temperature and heart rate (**Figure 2A)**: Treatment (PLAC/LPS) × Time interactions: $F_{(4.65,60.45)}$=61.23, p<0.001 and $F_{(4.17,54.27)}$=17.10, p<0.001 respectively. Post-hoc paired t-tests demonstrated significant treatment-associated differences in temperature from 30 minutes to 7 hours and heart rate from 2 hr to 7 hr after drug administration (all p<0.05). We observed no significant effects of LPS on systolic or diastolic blood pressure, and no significant main effect or interactions of minocycline on any vital sign.

Differential white blood cell counts: LPS induced significant changes in total and differential white cell counts (**Figure 2B)**: Treatment (PLAC/LPS) × Time (0/3/6 Hrs) interaction for total white cell count (WCC): $F_{(2,26)}$=37.27, p<0.001); Neutrophils: $F_{(2,26)}$=63.58, p<0.001; Lymphocytes: $F_{(2,26)}$=74.08, p<0.001; Monocytes: $F_{(2,26)}$=63.52, p<0.001; Eosinophils $F_{(2,26)}$=21.35, p<0.001. There was no significant main effect or interaction with minocycline for any of these measures.

Cytokines: Significant treatment (PLAC/LPS) × Time (0/3/6 Hours) interactions were observed for each cytokine (**Figure 2C)**: IL-6: $F_{(2,28)}$=109.80, p<0.001; TNF-α: $F_{(2,28)}$=120.60, p<0.001; IL-8: $F_{(2,28)}$=32.05, p<0.001; IL-10: $F_{(2,28)}$=44.63, p<0.001. Post-hoc paired t-tests confirmed significantly higher concentrations of all cytokines at both 3 and 6 hours after LPS compared to placebo: plasma IL-6: increase was 186.56±2.71% mean (± SE) at 3hr (t_{14}=-10.70, p<0.001) and 86.29±13.79% at 6hr (t_{14}=-4.81, p<0.001); TNF-α: 161.59±10.89% at 3hr (t_{14}=-11.16, p<0.001) and 154.94±7.56% at 6hr (t_{14}=-4.83, p<0.001); IL-8: 184.66±3.19% at 3hr (t_{14}=-6.01, p<0.001) and 151.98±6.31% at 6hr (t_{14}=-9.12, p<0.001); IL-10: 198.49±0.87% at 3hr (t_{14}=-6.71, p<0.001) and 116.14±14.78% at 6hr (t_{14}=-8.43, p<0.001).

Similar to findings for vital signs and differential white cell counts, there were no significant main effects or interactions with minocycline on any of the cytokines measured (all $p>0.05$) confirming no effect of minocycline on baseline or systemic immune responses to LPS.

Behavioral responses: Consistent with results previously reported following a milder inflammatory challenge (Typhoid vaccination), LPS was associated with a shift in sensitivity to rewards versus punishments expressed as reduced selection of high probability reward, yet increased avoidance of high probability punishment stimuli in the last 50% of trials (**Figure 3C-D).** This was confirmed by a significant treatment (LPS/Placebo) × valence (Gain/Lose) interaction: $F_{(1,13)}=6.10$, $p=0.028$. Post-hoc t-tests for reward and punishment conditions separately were $t_{14}=1.48$, $p=0.161$ and $t_{14}=-2.03$ $p=0.062$ respectively, indicating that similar to the milder Typhoid vaccination model of inflammation, LPS induced a relative increase in sensitivity to punishments versus rewards. Importantly, there was no significant main effect of inflammation ($F_{(1,14)}=0.07$, $p=0.797$) nor inflammation by valence interaction ($F_{(1,14)}=0.45$, $p=0.513$) for go versus no-go responses, confirming equal task engagement across conditions ($p=0.972$), and no significant time (Session1/Session2) by condition (Gain/Lose) interaction ($F_{(1,14)}=3.22$, $p=0.094$) indicating that task order did not influence reward/punishment learning.

To investigate our hypothesis that LPS-induced shifts in reward versus punishment sensitivity are driven by activation of (micro)glia (which we hypothesized would be blocked by minocycline) we next performed a Group (Mino/PLAC) × Treatment (PLAC/LPS) × Valence (Gain/Lose) interaction analysis. This confirmed that minocycline significantly interacted with the effects of LPS on reward versus punishment sensitivity: $F_{(1,13)}=4.28$, $p=0.033$ serving to attenuate the inflammation-induced shift in reward versus punishment sensitivity (**Figure 3C-D**). Of note, there was no main effect of minocycline $F_{(1,13)}=0.61$, $p=0.808$. Post-hoc analysis for the reward and punishment conditions separately revealed non-significant interactions for

minocycline to simultaneously attenuate both the LPS-induced impairment in reward sensitivity ($F_{(1,13)}$=3.33, p=0.091) and the LPS-induced enhancement of punishment sensitivity ($F_{(1,13)}$=5.29, p=0.095).

LPS was also associated with a significant increase in fatigue reported on the fVAS: Treatment (PLAC/LPS) × time ($F_{(5,70)}$=4.05, p=0.003). Minocycline did not significantly interact with reported fatigue.

DISCUSSION

Consistent with previously reported findings with the Typhoid model of mild systemic inflammation (Harrison et al., 2016), endotoxin (1ng/Kg) was associated with an acute shift in human monetary reward- versus punishment-learning, serving to enhance participants' sensitivity to punishments versus rewards. Furthermore, this acute behavioral reorientation was attenuated by minocycline, a centrally penetrant tetracycline that has been shown to block inflammation-induced activation of microglia in rodents (Henry et al., 2008).

These data are in line with preclinical results showing that minocycline improves inflammation induced anhedonia and sickness in rodents (Reis et al., 2019). The mechanism through which minocycline exerts these beneficial effects on sickness behavior is not yet fully understood, though is believed to relate to inhibition of inflammation-induced microglial activation (Nettis, 2021). Causally, minocycline inhibits the release of pro-inflammatory cytokines such as IL-1β IL-6, IL-2, TNF-α and IFN-γ in the brain and promotes the production of anti-inflammatory cytokines IL-10 (Soczynska et al., 2012). One mechanism through which minocycline is thought to exert its anti-inflammatory function in the CNS is through inhibition of p38 mitogen-activated protein (MAP) kinase, a key enzyme for the production of inflammatory mediators. Of note, minocycline inhibits LPS-induced p38 MAP kinase activation (phosphorylation) in

microglia cell culture (Nikodemova et al., 2006) though the specific molecular targets have not been characterized. Other putative mechanisms proposed to mediate the behavioral effects of minocycline in preclinical studies are via inhibition of microglial proliferation or effects on the microbiome-gut-axis (Schmidtner et al., 2019). The acute nature of the LPS challenge model makes it unlikely that our effects relate to changes in microglial cell number and though actions on the microbiome are reported after sustained i.e. two-week use in rodents reported actions after short e.g. 3½ day administration are minimal (Schmidtner et al., 2019). Furthermore, in our current study we failed to show any effect of minocycline on peripheral immune responses (including differential cell counts and cytokines), peripheral sickness symptoms such as aching or tender muscles or joints, or use of button press (rather than no-press) responses either at baseline or in response to LPS argues strongly for a central rather than peripheral action for the behavioral differences we observed.

One of the downstream consequences of minocycline's anti-inflammatory activity is that it appears to inhibit inflammation-induced upregulation of indolamine 2,3 dioxygenase (IDO) (Henry et al., 2008; O'Connor et al., 2009). However, though IDO is central to the regulation of serotoninergic and glutamatergic neurotransmission, which are believed to play a role in mediating LPS-induced mood symptoms, and may relate to alterations in insula-based punishment learning signals, it is less clear how this would modulate dopaminergic pathways that are believed to be central to anhedonia and reward learning (Gorwood, 2008; Schultz et al., 1997). Though, in line with our results, minocycline treatment in a rat model of depression with decreased level of dopamine in the amygdala induced an antidepressant effect and increased dopamine in the amygdala, even though serotonin concentrations were unchanged (Arakawa et al., 2012). This implicates at least part of the antidepressant properties of minocycline to an action on dopaminergic pathways.

Though we did not directly measure effects on dopamine turnover in our current study, our results coupled with evidence from previous imaging studies (Capuron et al., 2012; Eisenberger et al., 2010; Harrison et al., 2016; Pessiglione et al., 2006) suggest an effect of minocycline on dopaminergic activity in humans. In this regard, it is also known that inflammation can lead to a reduction in dopamine through blockade of tetrahydrobiopterin (BH4), a key enzyme co-factor in dopamine synthesis and the conversion of L-phenylalanine to L-tyrosine and L-tyrosine to L-DOPA, the precursor of dopamine. BH4 is oxidation-labile, therefore inflammation-induced reactive oxygen species (ROS) can readily reduce BH4 level and ultimately inhibit dopamine synthesis (Felger and Miller, 2012; Neurauter et al., 2008). Supporting the relevance of inflammation with dopamine level in humans, chronic IFN-α treatment in hepatitis-C patients has been shown to reduce plasma L-phenylalanine turnover (reflecting lower BH4 concentration) which negatively correlated with CSF dopamine concentration. Moreover, decreased CSF BH4 concentration correlated with increased CSF IL-6 (Felger et al., 2013). However, to our knowledge it is yet to be shown whether minocycline can affect dopamine biosynthesis.

Though this study and previous data using the same reinforcement learning task (Harrison et al., 2016) provide further evidence that systemic inflammation can alter reward learning signals and sensitivity to rewards, other studies have reported an effect of inflammation that is mediated via actions on effort sensitivity rather than reward sensitivity per se (Draper et al., 2017; Lasselin et al., 2016). It is worth reflecting on what may underlie these apparent differences. For example, task designs in studies reporting effects on effort sensitivity do not have a learning component during the trial sequence and use two different levels of reward (high vs low) (Lasselin et al., 2016) or 25 different conditions (combination of 5 effort and 5 stake levels) (Draper et al., 2017) whereas our reinforcement learning task includes only a single type of reward (win or nothing). Furthermore, in some studies LPS-induced somatic

symptoms (e.g. aching joints, muscular pain and muscle fatigue) rather than a motivation reorientation could, at least in part, account for the decreased willingness to engage in high effort trials. This would be particularly relevant for tasks performed acutely e.g. within 2 hours of LPS injection, when local physical symptoms are at their peak, or when using higher doses of LPS (e.g. 2ng/Kg).

To mitigate this, in our current study participants completed the reinforcement learning task approximately six hours after LPS injection when local physical symptoms such as muscle aches and pain had completely resolved, yet central symptoms such as raised body temperature and fatigue persisted. Interestingly, Draper and colleagues reported an LPS effect on effort sensitivity at 2 hours post injection but not at 5 hours suggesting that inflammation-associated effort sensitivity may predominate in the more pronounced phase of the immune response when peripheral sickness symptoms are at their peak rather than when they are improving. Furthermore, the physical effort required to complete our reinforcement learning task was minimal (button press/ no press response) and was not significantly affected by LPS (similar go/ no-go responses across conditions $p=0.97$). Of note, TSPO PET data from other groups confirm substantial glial activation in humans at 3-5 hr post LPS (1 ng/kg) (Sandiego et al., 2015) and at 4-6 hours in baboons (Hannestad et al., 2012) confirming sustained LPS-induced glial activation at this testing time window.

The major limitation of the present study is the modest sample size. For example, though the relative potency of our 1ng/kg LPS dose coupled with our mixed within/betweeen-subject study optimised the efficiency of the study, we were still only powered to detect main effects of inflammation and/or interactions with minocycline of moderate to large effect size. For example, we had 80% power to detect an interaction between minocycline and the effects of LPS on reward versus punishment sensitivity of large effect (i.e. *partial eta^2=0.14 or f=0.4)*.

Though previous studies have reported LPS-induced behavioral changes in the same sample size using lower doses of LPS e.g. 0.8 and 0.4 ng/Kg (Grigoleit et al., 2011), it is likely that we were underpowered to detect any potential correlations between effects on reward versus punishment learning and peripheral immune markers. Moreover the absence of a significant interaction of minocycline with learning to rewards ($p=0.091$) or punishments ($p=0.095$) when tested separately was also likely due to the limited sample size.

Endotoxemia is increasingly used as a method for investigating effects of inflammation on the brain, and has been proposed as a method for investigating the use of immunomodulating agents for depression (Lasselin et al., 2020). In this regard, our present methods could serve as an experimental model for screening new drugs purported to act as inhibitors of neuroimmune pathways in depression. However, that said, LPS has a number of limitations as a model of inflammation-associated depression, as unlike depression where mild systemic inflammation is chronic and by definition symptoms sustained for at least two weeks, LPS induces a marked short lived immune and sickness responses that resolve within a few hours.

LPS-induced increases in cytokine levels have been reported to be more marked in female versus male participants (Wegner et al., 2017). Further, low-dose LPS has been associated with decreased ventral stiatal activation in anticipation of rewards in female but not male subjects (Moieni et al., 2019). A limitation of the present study is that participants were only composed of male subjects and future studies will be needed to determine generalization of these results. Furthermore, the reinforcement learning task used in this study specifically investigated monetary rewards. Previous data have indicated that inflammation is also associated with social anhedonia and increased response to negative social feedback. Yet, in the case of a familiar caregiver it has been reported that inflammation heightened reward sensitivity (Moieni and

Eisenberger, 2018). A comparable task would be necessary to investigate whether the present findings can be extended to using positive and negative social rather than monetary rewards.

Minocycline did not alter levels of circulating immune cells or plasma concentration of IL-6, TNF-α, IL-8 and IL-10, either at baseline or in response to LPS challenge consistent with previous data showing no alterations in plasma cytokines following prolonged minocycline treatment in depressed patients (Nettis et al., 2021). This suggests that its anti-inflammatory effect is only exerted centrally. Of note minocycline has excellent brain penetrance (Elewa et al., 2006), a feature that has been utilized clinically in its prophylactic use in individuals exposed to cases of bacterial meningitis (Kumar et al., 2016).

To conclude, to our knowledge this is the first study to assess the effect of minocycline after experimentally induced inflammation. We provide two key findings: Firstly, a replication of the finding that systemic inflammation can rapidly impair sensitivity to rewards versus punishments. Secondly, that minocycline, a centrally penetrant tetracycline that blocks microglial activation can abrogate these effects. Together, these findings suggest that using an immune challenge coupled with a cognitive task assessing reward versus punishment sensitivity could represent a useful strategy for evaluating target engagement of novel centrally penetrant immunomodulatory drugs in human early phase clinical trials. These data also provide evidence that anti-inflammatory agents such as minocycline may be efficacious in the treatment of specific depressive symptoms such as anhedonia in the context of inflammation.

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FIGURE LEGENDS

Figure 1: Study design and protocol

Figure 2: Systemic inflammatory response

(A) Vital parameters. (B) Total and differential white blood cell counts. (C) Plasma cytokines concentration. Means ± SEM are shown. Post-hoc comparisons between individual time points were run using Bonferroni correction for multiple comparisons. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. matching time between conditions.

Figure 3: Experimental task and behavioral results

(A) Experimental task. In each trial participants selected one visual stimulus of the pair from one of the three conditions (gain, loss and neutral), and observed the outcome. In the examples the choices are associated with a probability of 0.8 of receiving a gain outcome ("gain 1£") (upper images) and a lose outcome ("lose 1£") (lower images) and a probability of 0.2 of obtaining nothing. The neutral pair of stimuli (not shown) was associated with neutral outcomes ("look 1£" or "nothing"). The left/right position of the stimuli was randomized at each trial. **(B)** The learning curves show the percentage (moving average) of participants that selected the high probability stimulus associated with the gain outcome in the reward trials and avoided the loss outcome in the punishment trials. Given the lack of previous knowledge, the curves start at a value close to 0.5. As participants learn the high probability stimuli, they keep selecting the high probability win in the win/nothing trials and keep avoiding the high probability loss in the lose/nothing trials condition. Thus, the learning curve quickly increases in the reward condition and decreases in the punishment condition. The shaded area represents the last 50% of trials which were averaged within subjects and conditions and used for the analysis. **(C-D)** Proportion of the last 50% of trials in which

participants chose the high probability gain stimulus and avoided the high probability lose. Means ± SEM are shown. There was a significant Treatment (LPS/Placebo) × Valence (Gain/Lose) interaction: $F_{(1,13)}=6.10$, $p=0.028$. Analyses were repeated with Minocycline and Placebo as between subject factor in the model. We observed a significant significantly Group (Mino/PLAC) × Treatment (PLAC/LPS) × Valence (Gain/Lose) interaction: $F_{(1, 13)}=4.28$, $p=0.033$.

632 **FIGURES**

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634 **Figure 1: Study design and protocol**

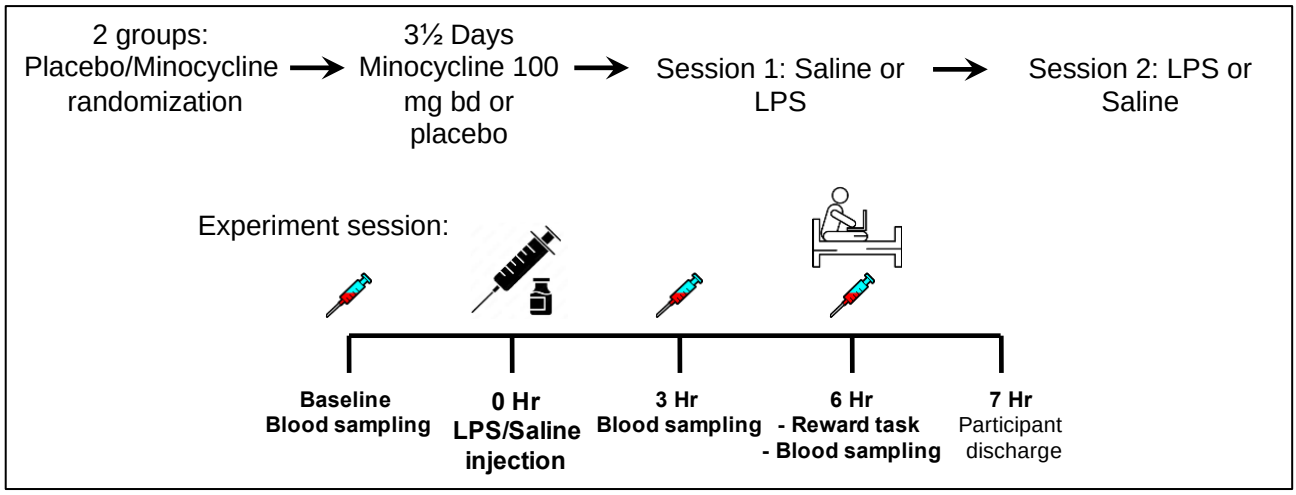

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Figure 2: Systemic inflammatory response

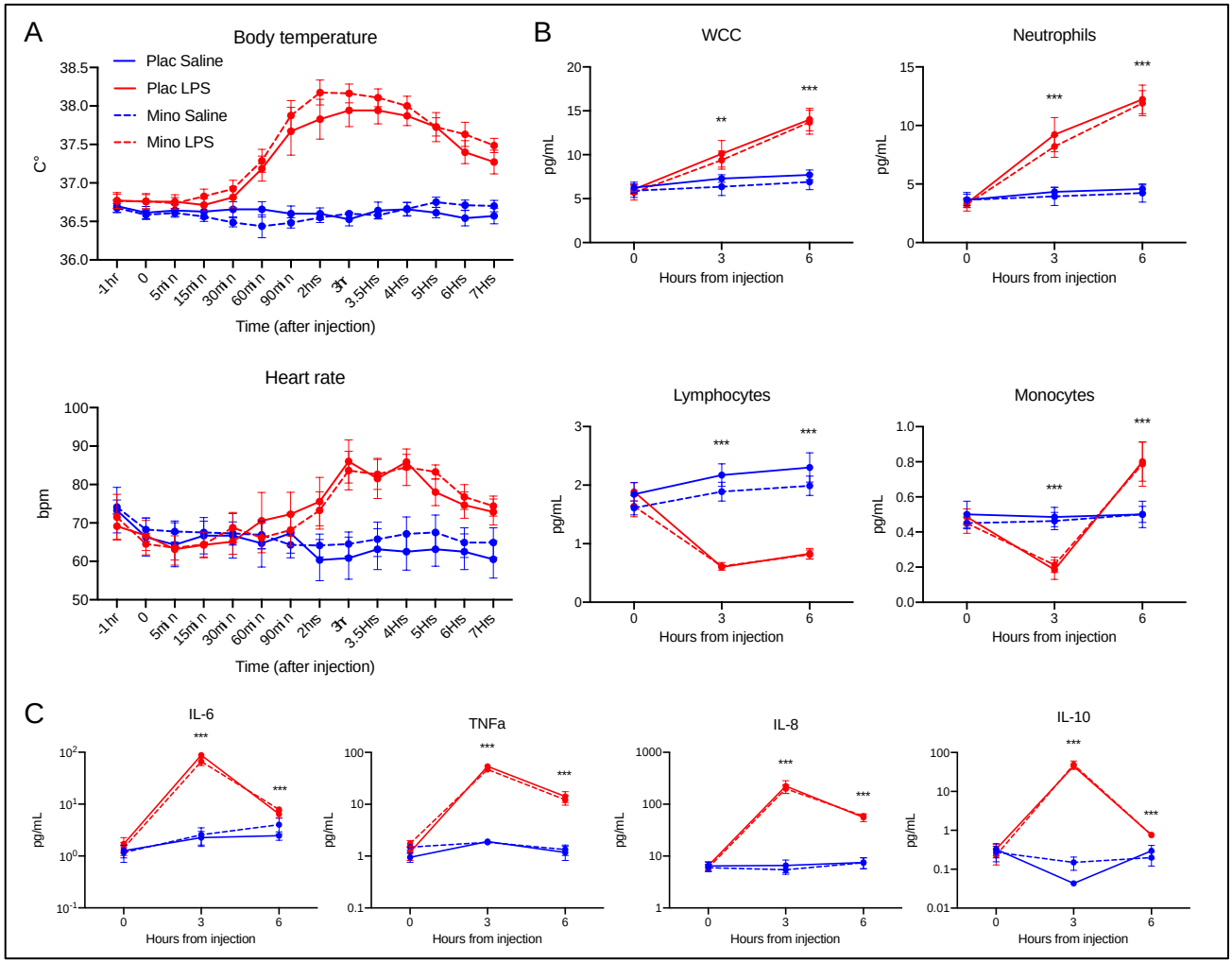
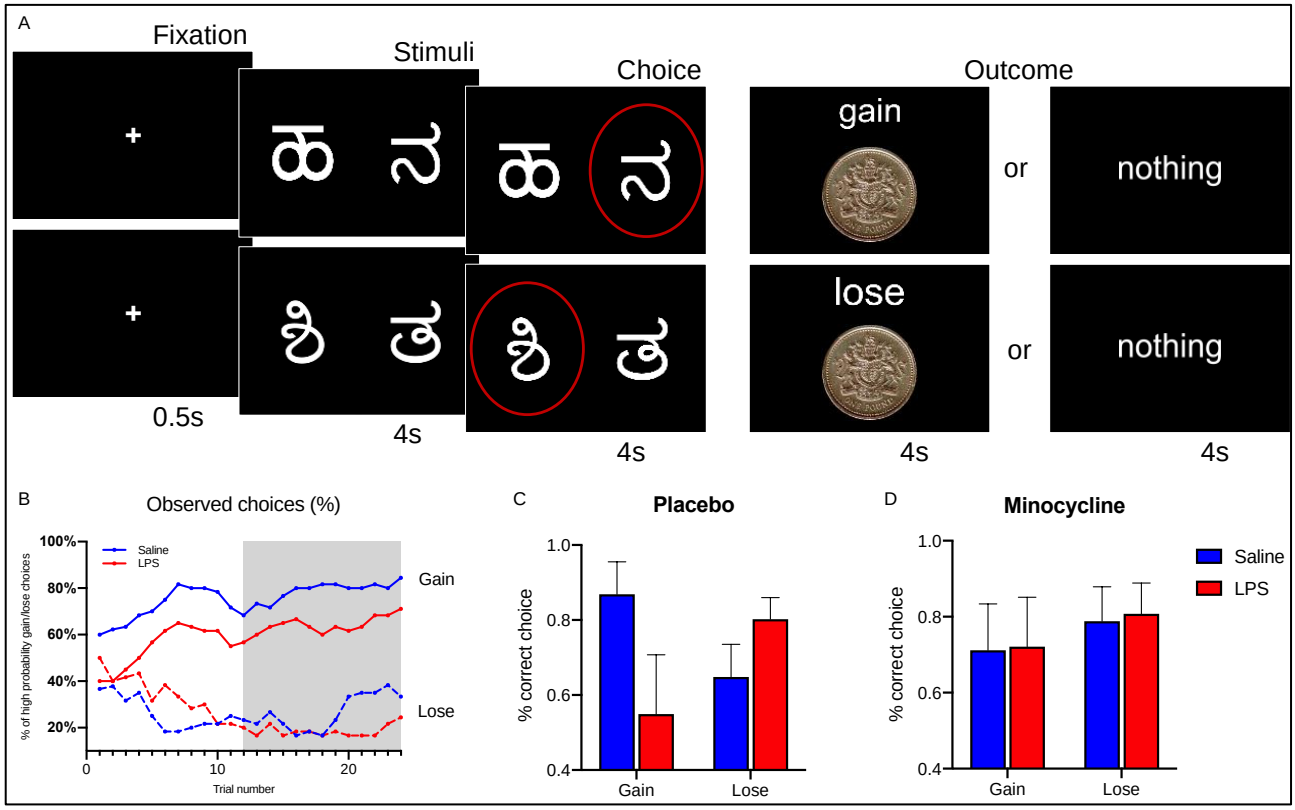


Figure 3: Experimental task and behavioral results



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