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1 **Diffusion-weighted MR Spectroscopy (DW-MRS) is sensitive to LPS-**
2 **induced changes in human glial morphometry: A preliminary study**

3 Riccardo De Marco¹, Itamar Ronen², Francesca Branzoli^{3,4}, Marisa Amato¹, Iris Asllani^{1,5},
4 Alessandro Colasanti¹, Neil A Harrison^{*1,6}, Mara Cercignani^{*1,6}

5 * Joint senior authors

6 **AFFILIATIONS**

7 1. Department of Neuroscience, Brighton and Sussex Medical School, Brighton, UK

8 2. C. J. Gorter Center for High Field MRI, Department of Radiology, Leiden University
9 Medical Centre, Leiden, The Netherlands

10 3. Centre for NeuroImaging Research - CENIR, Brain and Spine Institute - ICM, Paris, France

11 4. Sorbonne University, Univ Paris 06 UMR S 1127, Inserm U 1127, CNRS UMR 7225, Paris,
12 France

13 5. Department of Biomedical Engineering, Rochester Institute of Technology, Rochester, New
14 York, USA

15 6. Cardiff University Brain Research Imaging Centre (CUBRIC), Cardiff University, Cardiff,
16 UK

17 **CORRESPONDING AUTHOR:** cercignanim@cardiff.ac.uk

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21 **ABSTRACT**

22 **Background:** Low-dose lipopolysaccharide (LPS) is a well-established experimental method
23 for inducing systemic inflammation and shown by microscopy to activate microglia in rodents.
24 Currently, techniques for in-vivo imaging of glia in humans are limited to TSPO (Translocator
25 protein) PET, which is expensive, methodologically challenging, and has poor cellular
26 specificity. DW-MRS sensitizes MR spectra to diffusion of intracellular metabolites,
27 potentially providing cell-specific information about cellular morphology. In this preliminary
28 study, we applied DW-MRS to measure changes in the apparent diffusion coefficients (ADC)
29 of glial and neuronal metabolites to healthy participants who underwent an LPS administration
30 protocol. We hypothesized that the ADC of glial metabolites will be selectively modulated by
31 LPS-induced glial activation. **Methods:** Seven healthy male volunteers, (mean 25.3±5.9 years)
32 were each tested in two separate sessions once after LPS (1ng/Kg iv) and once after placebo
33 (saline). Physiological responses were monitored during each session and serial blood samples
34 and Profile of Mood States (POMS) completed to quantify white blood cell (WBC), cytokine
35 and mood responses. DW-MRS data were acquired 5-5½ hours after injection from two brain
36 regions: grey matter in the left thalamus, and frontal white matter. **Results:** Body temperature,
37 heart rate, WBC and inflammatory cytokines were significantly higher in the LPS compared to
38 the placebo condition ($p < 0.001$). The ADC of the glial metabolite choline (tCho) was also
39 significantly increased after LPS administration compared to placebo ($p=0.008$) in the
40 thalamus which scaled with LPS-induced changes in POMS total and negative mood (Adj
41 $R^2=0.83$; $p=0.004$). **Conclusions:** DW-MRS may be a powerful new tool sensitive to glial
42 cytomorphological changes in grey matter induced by systemic inflammation.

43 INTRODUCTION

44 Effects of systemic inflammation on the brain are implicated in the etiology of a range of
45 common mental illnesses and neurodegenerative disorders (Khandaker et al., 2021). Multiple
46 parallel neural and humoral immune-brain communicatory pathways have been identified,
47 though the ultimate mediator of central effects is increasingly recognized to be microglia
48 (Critchley and Harrison, 2013), brain specialized macrophages which constitute ~10-15% of
49 all brain parenchymal cells. In rodents, peripheral immune challenges, typically using a gram-
50 negative bacterial endotoxin such as lipopolysaccharide (LPS), increase blood brain-barrier
51 permeability (Clawson et al., 1966; Varatharaj and Galea, 2017). It also rapidly triggers a shift
52 in microglial morphometry from a 'resting' to an 'activated' phenotype which is accompanied
53 by an increase in proinflammatory cytokine release (Savage et al., 2019). Though, inter-species
54 sensitivity differs markedly (up to many orders of magnitude), LPS-induced changes in
55 microglial morphometry are also observed in rodents at doses that are broadly species
56 equivalent to those used in human studies. In rodents, these inflammation-induced changes in
57 microglial morphology and secretory profile can be quantified ex-vivo using microscopy,
58 immunohistochemistry, and single cell transcriptomics, and in-vivo using multi-photon
59 imaging through a cranial window (Savage et al., 2019).

60

61 In humans, in-vivo methods for imaging microglia are currently limited to Positron Emission
62 Tomography (PET) using tracers that bind to Translocator protein (TSPO), which is
63 overexpressed on the outer mitochondria membrane of activated microglia. TSPO-PET has
64 been used to demonstrate widespread increases in grey matter TSPO binding 3-5 hours after
65 inflammatory challenge (using LPS) in humans (Sandiego et al., 2015) and 4-6 hours in non-
66 human primates (Hannestad et al., 2012). However, TSPO PET is an invasive procedure,
67 involves exposure to radioactivity and TSPO is also expressed in other glial cells as well as on

68 endothelium. Furthermore, precise quantification of the TSPO PET signal in the brain is
69 substantially complicated by LPS-induced redistribution of TSPO radiotracers across
70 compartments (Yoder et al., 2015) limiting its more widespread use in research and its potential
71 as a viable clinical tool (Schubert et al., 2021). MRI based approaches have been used to index
72 effects of systemic inflammation on brain functional reactivity (functional MRI: fMRI),
73 functional connectivity (resting state fMRI: rs-fMRI), neurochemistry (magnetic resonance
74 spectroscopy: MRS) and microstructure (quantitative magnetization transfer: qMT and
75 diffusion weighted: DW-MRI). While these approaches have highlighted a matrix of brain
76 regions sensitive to peripheral inflammation (Critchley and Harrison, 2013; Garcia-Hernandez
77 et al., 2020; Kraynak et al., 2018), they cannot directly inform on the likely cellular substrates
78 underpinning these inflammation-related changes.

79

80 Diffusion-weighted MRS (DW-MRS) offers the potential to address these shortcomings.
81 Briefly, DW-MRI has high sensitivity to tissue microstructure and water compartmentalization
82 (Pierpaoli and Basser, 1996). However, its cellular specificity is limited, as water diffuses
83 similarly through all cell types as well as the extra-cellular environment. In contrast, MRS
84 probes the signal from metabolites, which are predominantly intra-cellular, and in some cases
85 cell-specific. Of the commonly measured metabolites, N-Acetyl-Aspartate (NAA) is
86 exclusively confined within neurons, total creatine (creatinine + phosphocreatine = tCr) is found
87 in each brain cell type, while choline compounds (Choline, phosphocholine and
88 glycerophosphocholine = tCho) predominantly reside in glial cells (with a ~3-fold higher
89 concentration than in neurons), O-2A progenitor cells and meningeal cells (Urenjak et al.,
90 1993). The specificity of DW-MRI to different cell types can thus be dramatically improved
91 by combining it with MRS, enabling the morphological properties of specific cell types to be

92 probed by measuring the apparent diffusion coefficient (ADC) of different metabolites (Ingo
93 et al., 2018; Palombo et al., 2018).

94

95 In line with this, DW-MRS has been shown to be sensitive to cell-specific metabolite diffusion
96 changes in the brain occurring in chronic inflammatory conditions. For example, in the
97 cuprizone model of multiple sclerosis, apparent diffusion of both tCho and myo-inositol was
98 observed after 6 weeks of cuprizone with these changes showing moderate to strong
99 correlations with histological measures of microglial and astrocytic area fractions respectively
100 (Genovese et al., 2021b). In humans, patients with systemic lupus erythematosus (SLE) exhibit
101 increased intracellular diffusion of total creatine and total choline in the white matter compared
102 with healthy controls (Ercan et al., 2016). Diffusion of tCr and tCho has also been found to be
103 increased in the primary motor cortex of patients with amyotrophic lateral sclerosis (ALS),
104 suggesting the presence of reactive glia (Reischauer et al., 2018). Data from the thalamic grey
105 matter of multiple sclerosis (MS) patients has shown a decrease in ADC(NAA), which is
106 believed to reflect accompanying neuronal-axonal loss or mitochondrial dysfunction, and a
107 decrease in ADC(tCr), thought to reflect impaired cell energy metabolism (Bodini et al., 2018).

108

109 Here we employed DW-MRS to assess the effect of peripherally administered LPS on the ADC
110 of NAA, tCr and tCho within a grey and a white matter region of interest in healthy humans.
111 The thalamus was used as it is an homogeneous grey matter (GM) region known to have among
112 the highest microglial density in human brain (based on TSPO PET) (Schubert et al., 2021)
113 and to be susceptible to peripheral LPS (Buttini et al., 1996). The corona radiata, which is a
114 homogenous white matter (WM) region with relatively low microglial density and reactivity
115 to LPS in humans (Sandiego et al., 2015) (based on TSPO PET) was selected as a control
116 region with low expected signal. Of note, though white matter inflammatory responses are a

117 feature of some inflammatory models (e.g. Cuprizone) this is not the case with LPS (Hannestad
118 et al., 2012). LPS is a cell-wall component of Gram-negative bacteria. It triggers innate immune
119 activation through the Toll-like receptor 4 (TLR-4) signaling cascade (Chow et al., 1999) and
120 is a well-established method for eliciting systemic inflammation in both animals and humans
121 (Lasselin et al., 2020). In healthy humans, intravenous injection leads to a rapid, dose-
122 dependent increase in circulating white blood cells (particularly neutrophils) and release of
123 proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6),
124 which are accompanied by raised body temperature and heart rate (Fullerton et al., 2016; van
125 Deventer et al., 1990). It also reliably elicits transient sickness behaviors (that resemble
126 depressive symptoms), facilitating investigation of how regional changes in brain
127 microstructure may perturb behavior and potentially contribute to depression (Dowlati et al.,
128 2010).

129 MATERIALS AND METHODS

130 **Participants:** Seven healthy male subjects (mean age: 25.2 ± 5.9 (std) years, mean body
131 weight: 80.6 ± 10.3 (std) Kg, mean BMI: 24.6 ± 1.7 (std) Kg/m²) were recruited through public
132 advertisement. All were screened to exclude any underlying neurological or psychiatric
133 condition, including substance misuse. All participants were medication free. At screening,
134 participants underwent physical examination and blood samples were taken for full blood
135 count, differential white blood cell (WBC) count (neutrophils, lymphocytes, monocytes,
136 eosinophils, basophils) as well as renal, thyroid, and liver function. Volunteers were asked to
137 abstain from alcohol for 24h before the start of each session. The study was approved by the
138 London-Queen Square Research Ethics Committee (REF 17/LO/0936), and all participants
139 provided written informed consent.

140

141 **Study Design:** We adopted a randomized, double-blind, crossover, repeated measure
142 experimental study design. All participants received an intravenous injection of either LPS
143 (1ng/Kg) prepared from *Escherichia coli* O:113 (U.S. Standard Reference Endotoxin,
144 manufactured for the Clinical Center, NIH) or saline (placebo control) in random order in two
145 separate study sessions spaced a minimum of 2 weeks apart (mean(\pm std) 3.7 ± 3.8 weeks).
146 Three participants received placebo in the first session and four LPS. Participants' systolic and
147 diastolic blood pressure, body temperature and heart-rate were monitored throughout each 8-
148 hour testing session. Blood samples were collected at baseline and at 3 and 6 hours post
149 injection to measure differential WBC count. A DW-MRS scanning session was scheduled for
150 5-5½ hours after each injection. This timing was informed by: 1) prior human and baboon
151 TSPO PET studies that report increased TSPO uptake at 3-5 and 4-6 hours post LPS
152 respectively as well as sustained subjective sickness and systemic inflammatory responses at
153 this time-point confirming an ongoing peripheral and central inflammatory response. 2)
154 Evidence from prior human studies using 1ng/kg LPS that show a significant reduction in
155 peripheral limb and joint discomfort coupled with less pronounced pyrexia at this timepoint
156 meaning that participants would be better able to comply with requirements to remain very still
157 during the DW-MRS scanning session (which is highly sensitive to motion).

158

159 **Cytokine Analyses:** Blood drawn into purple top (EDTA) BD Vacutainer tubes (Becton,
160 Dickson and Company, Franklin Lakes, New Jersey, United States) was centrifuged at 2000
161 rpm for 20 min, then plasma removed, aliquoted, and frozen at -80 °C. Plasma (IL-6), tumor
162 necrosis factor- α (TNF- α) and interleukin (IL-10) were measured using Quantikine® High
163 Sensitivity ELISA kits for IL-6 (R&D Systems inc., Minneapolis, United States). Limits of
164 detection were 0.031 pg/mL, 0.022 pg/mL and 0.09 pg/mL respectively and intra- and inter-
165 assay coefficients of variation were 6.9% and 9.6% (IL-6), 2.2% and 6.7% (TNF- α) and 9.3%

166 and 13.1% (IL-10). For the IL-10 analyses, 5 samples measured below the lowest standard and
167 a value of one-half of the lower limit of detection was assigned to these samples (Breen et al.,
168 2011). All samples were tested in duplicate. Values were natural log transformed before
169 analysis.

170

171 **Behavioral Analyses:** Participants completed self-rating questionnaires at baseline then at 5
172 further time-points after each injection (1, 2, 3, 4 and 6 hours post injection) to assess LPS-
173 induced mood changes. These included: profile of mood states (POMS), fatigue visual
174 analogue scale (fVAS) and the Karolinska Sickness Questionnaire (Andreasson et al., 2018).

175

176 **DWMRS acquisition and analysis:** MRI and MRS were obtained at 3T (Siemens Magnetom
177 Prisma, Siemens Healthineers, Erlangen, Germany). After a standard localizer, a T1-weighted
178 magnetization prepared rapid acquisition gradient echo (TR=1900 ms; TE=3.97 ms; TI=904
179 ms, flip angle=8 deg, FOV 220 × 220 mm², matrix size 192 × 192) was acquired in sagittal
180 orientation and reconstructed in 3 orthogonal planes. These high-resolution scans were used to
181 position two 4.5 cm³ DW-MRS volumes of interest (VOIs) on the left thalamus and left corona
182 radiata. VOIs were chosen based on prior data indicating high microglial density and reactivity
183 to LPS in thalamus and low microglial density and LPS reactivity in corona radiata (Schubert
184 et al., 2021; Sandiego et al., 2015; Buttoni et al., 1996). The left hemisphere was chosen as it
185 is more commonly implicated in inflammation-induced cognitive changes (Haroon et al., 2014;
186 Harrison et al., 2015). The DW-MRS sequence used was a bipolar sequence based on a semi-
187 Localization by Adiabatic SElective Refocusings (semi-LASER) sequence (Genovese et al.,
188 2021a) with TE=100ms, TR=5s, spectral width=2500 kHz, number of complex points=1024.
189 The following diffusion weighting conditions were used: one at b=0 s/mm² and three at b=3823
190 s/mm² with diffusion gradients applied in three orthogonal directions ([1, 1, -0.5], [1, -0.5, 1],

191 [-0.5, 1, 1] in the VOI coordinate system, diffusion gradient duration=14 ms, diffusion
192 time=50 ms). The number of signals averages (NSA) was 32 for each condition. A short
193 (NSA=4) scan without water suppression was performed for eddy current correction. B₀
194 shimming was performed using a fast automatic shimming technique with echo-planar signal
195 trains utilizing mapping along projections, FAST (EST) MAP (Gruetter and Tkáč, 2000).

196

197 The spectra were transferred off-line for analysis with customized software implemented in
198 Matlab (Mathworks, Natick MA, USA). Spectral analyses were performed with linear
199 prediction singular value decomposition (LPSVD), and the peak area estimates for the three
200 orthogonal gradient directions at high b value were averaged and the resulting mean values
201 were used to compute the ADC values for the three metabolites. The operator was blind to
202 participant condition (i.e. LPS or placebo). The spectra at b=0 s/mm² were used to estimate
203 relative tCho and tNAA (tNAA = NAA + NAAglutamate) concentrations, expressed as the
204 ratio between their peak area to the tCr peak area.

205

206 **Statistical Analysis:** Analyses was conducted using SPSS statistics 24. Physiological,
207 cytokine and behavioral effects of LPS were analyzed using repeated-measures factorial
208 ANOVAs (factors: treatment (LPS, placebo), time (pre injection, post injection as described))
209 with significant effects followed up with paired sample t-tests. The ADC of the three
210 metabolites as well as the tCho and tNAA concentration relative to tCr ([tCho]/[tCr] and
211 [tNAA]/[tCr]) were compared between sessions (LPS vs placebo) using a paired-sample t-test.
212 Associations between changes in ADC(tCho) and changes in mood (POMS), temperature and
213 peripheral immune measures (cell counts and cytokines) were assessed using Pearson's
214 correlation coefficient.

215 RESULTS

216 **Physiological effects:** Effects of LPS on physiological parameters are summarized in **Figure**
217 **1.** Significant condition (LPS/Placebo) x time interactions were observed for body temperature
218 ($F_{(13,78)}=21.6$, $p<0.001$) and heart rate ($F_{(13,78)}=8.48$, $p<0.001$). LPS significantly increased
219 body temperature, from 60 minutes, peaking at 3 hours post injection, and significantly
220 increased heart rate which peaked between 2 and 4 hours post-injection. Significant condition
221 (LPS/Placebo) \times time interactions were also observed for total WBC count ($F_{(2,12)}=30.6$,
222 $p<0.001$) as well as for each differential WBC count (Neutrophils: $F_{(2,12)}=54.9$, $p<0.001$;
223 lymphocytes: $F_{(2,12)}=26.2$, $p<0.001$; monocytes: $F_{(2,12)}=23.7$, $p<0.001$) with the greatest changes
224 observed at 6 hours. Post-LPS cell count increases at 6 hours (compared to baseline) were:
225 $113 \pm 14.5\%$ and $230.6 \pm 32.3\%$ for Total WBC and Neutrophils respectively. Lymphocytes
226 showed the opposite pattern: $-54.9 \pm 6.1\%$ at 6hr (relative to baseline) while monocytes
227 displayed a $-60.9 \pm 8.2\%$ decrease at 3 hrs after LPS followed by a $51.4 \pm 11.6\%$ increase at 6
228 Hrs after LPS (both relative to baseline) (Table 1) consistent with results reported in other LPS
229 challenge studies (Peters van Ton et al., 2021).

230

231 **Cytokine levels:** LPS induced significant increases in circulating cytokines shown by
232 significant condition (LPS/Placebo) \times time (baseline, 6 hours) interactions for IL-6:
233 $F_{(1,6)}=14.91$, $p=0.008$; TNF- α : $F_{(1,6)}=50.05$, $p<0.001$; and IL-10 $F_{(1,6)}=27.67$, $p=0.002$ (**Figure**
234 **2**).

235

236 **Behavioral response:** LPS injection led to significant temporary changes in mood, fatigue,
237 and sickness symptoms as shown by POMS questionnaire, fVAS, and sickness questionnaire.
238 Specifically, LPS was associated with a reduction in total mood score: main effect of condition
239 ($F_{(1,6)}=77.73$, $p<0.001$), condition \times time interaction ($F_{(5,30)}=2.31$, $p=0.068$) and increase in

240 negative mood score: main effect of condition ($F_{(1,6)}=57.17$, $p<0.001$), condition \times time
241 interaction ($F_{(5,30)}=2.38$, $p=0.062$) (**Figure 3** Insets). LPS induced changes in both total mood
242 and negative mood scores (compared to placebo) peaked 1 hour post injection (paired-sample
243 t-tests: $t_{(6)}=3.6$, $p=0.011$; $t_{(6)}=-3.0$, $p=0.024$) respectively (**Figure 3** Insets). Moreover, LPS
244 induced a significant increase in both fatigue: significant condition \times time interaction for fVAS
245 ($F_{(5,20)}=4.06$, $p=0.01$) and sickness score ($F_{(5,25)}=6.34$, $p=0.001$). fVAS showed a rapid
246 increase in fatigue that peaked at 1 hour post LPS injection, compared to baseline (paired-
247 sample t-test $t_{(6)}=-3.6$, $p=0.011$) while sickness score peaked at 2 hours ($t_{(6)}=-5.5$, $p=0.002$)
248 followed by a gradual improvement.

249

250 **DW-MRS: Main effects of LPS:** The spectra were of good quality for all participants. As an
251 example, **Figure 4** shows spectra acquired in the thalamus of the same participant in the two
252 conditions (placebo and LPS). The average ADCs of tNAA, tCr and tCho are shown in **Table**
253 **2**; the between-condition difference in metabolite ADC is shown in **Figure 5**. Paired t-test
254 revealed a significant increase in ADC(tCho) in the thalamus for the LPS compared to the
255 placebo condition ($t_{(6)}=-3.9$, $p=0.008$), (ADC(tCho) (Thalamus): placebo ($M=1.26*10^{-4}$,
256 $SD=3*10^{-5}$), LPS ($M=1.76*10^{-4}$, $SD=1.6*10^{-5}$)). No significant difference was detected in
257 ADC(tCho) in the WM control region between the two conditions. ADC(tNAA) and ADC(tCr)
258 did not differ significantly between conditions in either VOI. No significant differences in the
259 metabolites' relative concentration (expressed as $[tCho]/[tCr]$ and $[tNAA]/[tCr]$) were
260 observed in either region between conditions. There was no significant correlation between
261 LPS-induced changes in body temperature and ADCs for any of the metabolites (all $p>0.1$).
262 Exploratory analyses investigating associations between ADCs and peripheral immune
263 measures revealed a trend association between LPS-induced changes in thalamic ADC(tCho)

264 and associated change in monocyte count at 6 hours (Adj $R^2=0.46$; $p=0.09$). All other
265 associations were non-significant ($p>0.1$).

266

267 **Correlation between mood deterioration and ADC(tCho) change:** To investigate potential
268 associations between mood change and putative changes in neuroinflammation we regressed
269 peak change in mood (1 hour post injection minus baseline) for LPS compared to placebo
270 against changes in the ADC(tCho) between conditions. LPS-associated changes in both POMS
271 total and negative mood were significantly associated with changes in ADC(tCho) of the
272 thalamus (Adj $R^2=0.79$; $p=0.007$ and Adj $R^2=0.83$; $p=0.004$, respectively) (**Figure 3**).

273 **DISCUSSION**

274 Glial activation is a hallmark of the neuroinflammatory cascade and a pathological feature of
275 a wide range of severe and disabling central nervous system (CNS) diseases. Increased density,
276 altered morphology, and/or a pro-inflammatory immune phenotype of microglia and astrocytes
277 are consistent post-mortem findings in autoimmune neuroinflammatory disorders, such as MS
278 or Neuro SLE, and neurodegenerative diseases such as ALS and Alzheimer's disease
279 (González-Reyes et al., 2017; McGeer and McGeer, 2002; Perry and Holmes, 2014).
280 Furthermore, post-mortem and neuroimaging evidence of alterations in microglia and
281 astrocytes in depression and schizophrenia have implicated glia in the neuropathology of
282 psychiatric disorders that are linked to heightened systemic inflammation (Almeida et al., 2020;
283 Dantzer et al., 2008; Lanquillon et al., 2000; Najjar et al., 2013). At a systemic level, increasing
284 evidence has linked the onset of depression with raised circulating inflammatory markers
285 (Dowlati et al., 2010; Osimo et al., 2020). Yet, the exact mechanisms underlying the brain
286 response to systemic inflammation remain elusive as sensitive and viable neuroimaging

287 methods for the *in vivo* assessment of glial response to systemic inflammation are yet to be
288 fully developed.

289

290 Here using DW-MRS, we report results from 7 healthy subjects providing preliminary
291 evidence for altered intracellular metabolite diffusion in the context of experimentally induced
292 systemic inflammation in humans. Changes of metabolite diffusion properties have been
293 considered to mirror cytomorphological cell rearrangement or, in a pathological framework,
294 tissue damage. The primary finding of this study is the increased ADC(tCho) in the gray matter
295 after LPS injection compared to placebo. This result suggests that cytomorphological changes
296 in glial cells observed using microscopy in rodents and baboons after systemic inflammation
297 can also be detected in-vivo in humans using DW-MRS. This finding is also in keeping with
298 recent DW-MRS results in rodents showing an increase in tCho and myo-inositol ADCs using
299 the Cuprizone model of neuroinflammation, which correspondingly correlate with induced
300 changes in microglial and astrocytic area fraction recorded histologically (Genovese et al.,
301 2021b), as well as in human neurological diseases, such as neuropsychiatric systemic lupus
302 erythematosus (Ercan et al., 2016) and ALS (Reischauer et al., 2018), that are each
303 characterized by glial activation. However, confirmation of our preliminary findings will
304 require future replication in larger studies.

305

306 The absence of a significant change in the neuronal marker tNAA ADC in our current study is
307 also consistent with histological studies which indicate no effect of LPS on neuronal
308 morphology. However, it is worth stressing that our small sample size may have meant we
309 were under powered to detect diffusion changes in other metabolites. Of note, reductions in
310 ADC(tNAA) have been previously reported in MS, however this finding was interpreted as
311 reflecting accompanying neuronal damage or cell loss which is a feature of this model but not

312 systemic LPS (Bodini et al., 2018). A potential bias in our experiment was the effect of LPS
313 on body temperature and consequently metabolite diffusion. However, the absence of an effect
314 of LPS on diffusion of the neuronal marker tNAA in either VOI or on tCho in our WM control
315 region, coupled with an absence of even a trend level association between changes in body
316 temperature and any of the metabolite ADCs suggests this an unlikely cause of our findings.

317

318 Though tCho has only limited specificity to glial cells, its significant ADC change following
319 LPS compared to placebo is consistent with previous studies reporting microglial and astrocytic
320 neuroinflammatory responses following peripheral LPS exposure in mice (Ryu et al., 2019).
321 The activation of central inflammation has also been reported in humans using TSPO PET
322 where it has been proposed to be mediated mainly by microglial cells (Sandiego et al., 2015).
323 Microglia are known to be the principal mediator of innate immune responses within the CNS.
324 In the healthy brain, microglial cells are highly dynamic and extend and retract multiple long
325 finger-like processes to monitor their local environment (Nimmerjahn et al., 2005). Changes
326 in brain homeostasis due to infection, injury or neurodegeneration can alter microglia gene
327 expression, morphology and motility (Helmut et al., 2011). However, during
328 neuroinflammation both microglia and astrocytes undergo metabolic, functional and
329 morphological changes (Heneka et al., 2014). In this reactive state glial cells can release
330 neurotoxic factors such as proinflammatory cytokines and reactive nitrogen and oxygen
331 species, ultimately affecting neuronal transmission and neurogenesis (Orihuela et al., 2016).
332 Once activated, microglia display a thickening and retraction of their processes and assume an
333 amoeboid spherical shape with increased cell body size (Davis et al., 1994). In a similar way,
334 reactive astrocytes are characterized by cellular hypertrophy and overlapping processes
335 (Sofroniew and Vinters, 2010). It is therefore uncertain whether the changes in tCho diffusion
336 we observed stemmed from microglial or astrocytic activation. However, in a mouse model of

337 astrocytic hypertrophy myo-inositol diffusion changes were sensitive to cytomorphological
338 rearrangement, whereas tCho was unaffected (Ligneul et al., 2019). Similarly, in the cuprizone
339 model mentioned earlier, induced changes in tCho scaled with histological changes in
340 microglia and myo-inositol with changes in astrocytes (Genovese et al., 2021b).

341

342 It is worth noting that we were unable to quantify myo-inositol diffusion using our current
343 method. The higher diffusivity of tCho we observed is also consistent with the increased
344 intracellular space that characterizes microglia in the activated state. Furthermore, although
345 microglial and astrocytic activation are both key components of neuroimmune processes,
346 microglia are thought to initiate the process triggered by LPS and then causally induce
347 astrocytic activation (Liddelow et al., 2017). In line with this finding, a DW-MRI study using
348 a multi-compartment tissue model based on microglia and astrocyte cell shape has recently
349 shown typical microglial changes at 8 hrs post LPS injection in rodents with a subsequent
350 astrocytic signal emerging later at 24 hrs (Garcia-Hernandez et al., 2020). This time-dependent
351 neuroimmune cascade allows us to speculate that the observed increase in tCho value may
352 selectively reflect microglial activation. In support of this, since the tCho concentration was
353 unchanged between sessions it is unlikely that the observed change in ADC was influenced by
354 infiltrating or peri-vascular immune cells.

355

356 A second finding from our study was the tight correlation between LPS-induced changes in
357 thalamic ADC(tCho) and the severity of induced mood changes. Despite being characterized
358 by a high density of microglial cells, the thalamus has not previously been reported as a key
359 structure for the psychological and behavioral effects associated with systemic inflammation
360 (Harrison, 2017). Instead, previous fMRI studies have reported functional changes of the
361 subgenual cingulate and amygdala (Davies et al., 2020; Harrison et al., 2009) following

362 immune challenges which correlate with the severity of mood/ depressive changes. Decreased
363 global functional connectivity has also emerged as a putative neurobiological underpinning of
364 mood deterioration driven by peripheral inflammation (Dipasquale et al., 2016). This later
365 finding suggests a potentially widespread effect of systemic inflammation on grey matter which
366 is consistent with the widespread increase in TSPO expression previously reported following
367 LPS injection in both baboons and humans (Hannestad et al., 2012) (Sandiego et al., 2015).
368 Hence, the effect that we report within the thalamus (which was selected based on its high
369 microglial density and sensitivity to LPS rather than a specific role in mood processing) is
370 likely a proxy of more general glial morphological changes in the brain grey matter. Given the
371 time implications of acquiring data in multiple VOIs we restricted this preliminary study to
372 acquisition of data in a single grey and white matter volume of interest. Nevertheless, it will be
373 important for future studies to consider data acquisition from a greater range of VOIs to further
374 address the specificity of regional changes to discrete behavioral features.

375 As discussed above, the main limitation of our study is the relatively modest sample size and
376 consequently the need to replicate our findings in future larger studies. Another limitation is
377 that our data were only acquired at a single time-point (5-5½ hours) after LPS/placebo
378 injection. Though human LPS studies typically only scan at a single time point it will be
379 important for future studies to begin to address the temporal evolution of brain changes and
380 their association with the evolution/ resolution of specific behavioral and cellular features.

381 Following LPS changes in physiological e.g. heart-rate, temperature, cytokine and sickness
382 symptoms typically peak 2-3 hours post administration. However, cellular responses e.g.
383 neutrophil and monocyte counts typically continue to increase up until at least 6 hours post
384 injection. TSPO PET studies indicate central (brain) glial activation 3-5 hours after LPS in
385 humans (Sandiego et al., 2015) and from 1-3 hours in baboons rising further at 4-6 hours before
386 returning to (or below) baseline at 22-24 hours (Hannestad et al., 2012). Thus, though many

387 indices of peripheral immune responses peak relatively early, central glial responses appear to
388 peak later and remain evident until at least 6 hours post-LPS. How and when these initial pro-
389 inflammatory responses ultimately resolve and how they relate to acute and potentially more
390 persistent symptoms in some participants will need to be addressed in future studies. Rodent
391 studies combining longitudinal DW-MRS with histological analyses will also be valuable in
392 clarifying the cell-specific basis of the signal we report.

393

394 DW-MRS is a challenging measurement, and several sources of errors need to be addressed at
395 both the acquisition and processing stages. Motion occurring during the diffusion time has a
396 significant effect on the DW-MRS signal. More specifically, simple linear translational motion
397 results in a constant signal phase shift that can be corrected in the post-acquisition stage, when
398 individual shots are acquired separately, resulting in no significant effect on the calculated
399 metabolite ADC. Rotational and compressive motion, resulting for example from cardiac
400 pulsation, causes a non-constant phase shift across the VOI that results in drop in signal
401 intensity (Anderson and Gore, 1994). This type of error cannot be corrected, and if individual
402 acquisitions during which such signal drop occurred are included in the signal average, the
403 resulting ADC is significantly overestimated. These acquisitions must be excluded, and in our
404 post-processing pipeline we do so using a simple criterion imposed on the amplitude of the
405 NAA peak (the strongest peak in the spectrum), taking into account the expected variance of
406 the peak amplitude across acquisitions based on the variance of the noise (Genovese et al.,
407 2021a).

408

409 DW-MRS also suffers from low signal-to-noise ratio (SNR), compared to a simple MRS
410 acquisition: the need to accommodate the diffusion gradients within the sequence dictates a
411 longer echo time than standard “Short-TE” MRS sequences, and the need to acquire several

412 diffusion weighted conditions limits the number of averages that is possible to acquire during
413 a given scan time. SNR affects the variance in metabolite ADC values in a non-linear fashion
414 that can be estimated via calculation of error propagation in the estimation of ADC with the
415 assumption of mono-exponential decay (Ronen and Valette, 2015). Even when the b factor is
416 optimally set to be roughly $1/ADC$, the error propagation is significant and amplifies the
417 variance in metabolite ADC values several times above that of the non-diffusion weighted
418 MRS signal. A robustness/reproducibility study for single volume DW-MRS (Wood et al.,
419 2015) indicates that with carefully optimized acquisition and processing DW-MRS protocols
420 as the one we applied in this study, it is possible to reach the required statistical power for
421 detecting ADC differences in case-control studies similar to the one presented here.

422

423 Finally, the clear physical responses to LPS mean that both the participant and the researcher
424 will become aware of allocation to the LPS condition ~ 1 hour after its administration (though
425 notably not all participants receiving placebo in the first session realized this until completing
426 the second LPS session). Nevertheless, it is important to note that critically the DW-MRS data
427 were analyzed completely blind to condition.

428

429 To conclude, we present a novel MR imaging paradigm that could enable the quantification of
430 glial morphological changes in-vivo in humans. Although resident immunocompetent cells
431 have emerged as a central component in a wide array of brain disorders, a gold standard method
432 to selectively assess different biological mechanisms in vivo is still lacking. TSPO PET has
433 been widely used to quantify neuroinflammation. However, it is invasive, expensive, requires
434 the use of radioactivity and remains challenging to quantify without an arterial input function,
435 particularly in conditions where inflammation is not restricted to the CNS (Nettis et al., 2020).
436 Unlike PET, DW-MRS has the advantage of being non-invasive and it can be acquired with

437 other routine MRI images. A limitation is that it can currently only be acquired in pre-specified
438 VOIs rather than whole brain. Nevertheless, pending further validation in larger studies DW-
439 MRS could form a valuable tool for investigating neuroinflammatory processes in clinical
440 populations and for providing evidence of target engagement for novel pharmacotherapies
441 being developed to target glial cells.

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633 **TABLE LEGENDS**

634 **Table 1: Effects of LPS on white blood cell count**

635 Data represent mean ± standard error

636

637 **Table 2: effects of LPS on glial metabolites ADC**

638 Data represent mean ± standard error

639 **FIGURE LEGENDS**

640 **Figure 1: Effect of LPS on vital signs and WCC**

641 A) Body temperature. B) Blood pressure. C) WCC. D) Heart rate. Red lines represent LPS
642 condition, blue lines placebo. X Axis shows hours post LPS or Placebo injection. Significance
643 values show Treatment \times sample contrasts with baseline (* $p < 0.05$ ** $p < 0.001$).

644

645 **Figure 2: Effect of LPS on cytokines levels**

646 Changes (mean \pm SE) in plasma levels (natural log transformed) of circulating cytokines
647 (* $p < 0.05$ ** $p < 0.001$). Significance values were tested with paired sample t-tests.

648

649 **Figure 3: Effect of LPS on mood and relationship with ADC(tCho) change**

650 Correlation between ADC(tCho) change between the two sessions and the difference between
651 mood changes from baseline to 1 Hr post injection in the two session. (A) POMS total mood
652 score, (B) POMS negative mood score. Insets show the mood score throughout the two
653 sessions; grey shaded areas indicate the two timepoints used to obtain the POMS difference
654 correlated with the ADC(tCho) change.

655

656 **Figure 4: MR Spectra**

657 Example of MR spectra acquired at $b=0$ s/mm² and $b=3823$ s/mm² in the left thalamus of one
658 subject after placebo (top) and LPS (bottom) injections.

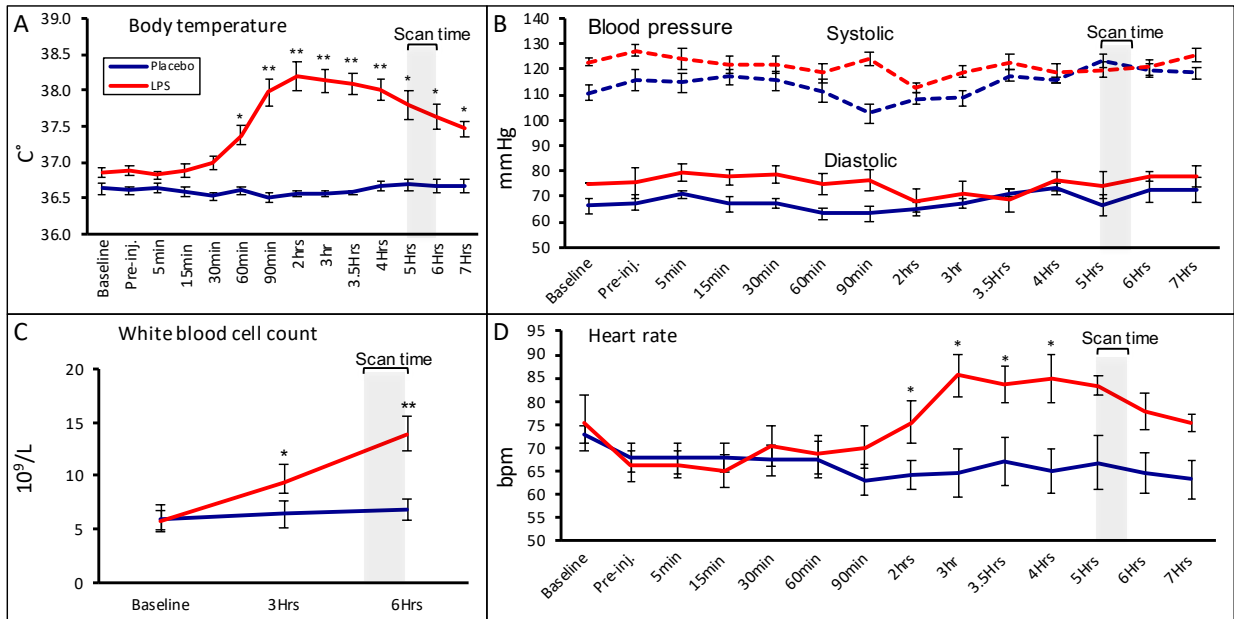
659

660 **Figure 5: Metabolites ADC responses to LPS**

661 tCho, tCr and tNAA ADC differences between LPS and placebo sessions in thalamus (A) and
662 white matter (C). Mean differences and standard deviations are reported. Volumes of interest
663 (VOIs) were located in the thalamus (B) and in the parietal white matter (corona radiata) (D).

664 P-value relates to comparison between LPS and placebo session.

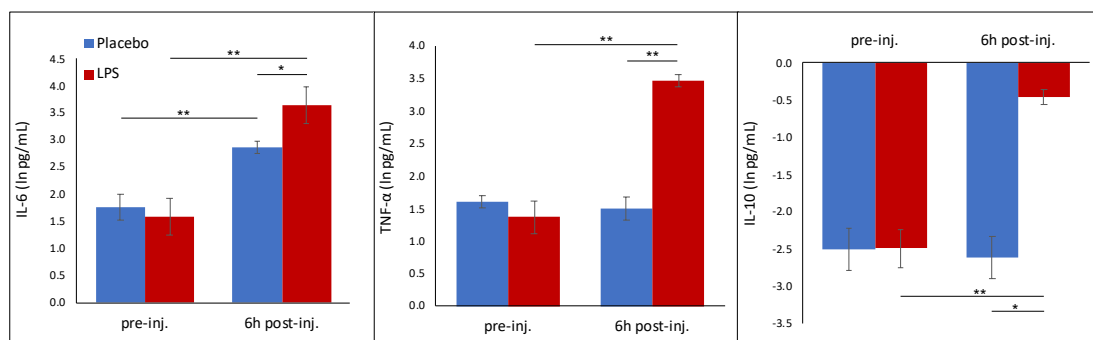
665 **Figure 1: Effect of LPS on vital signs and WCC**



666

667

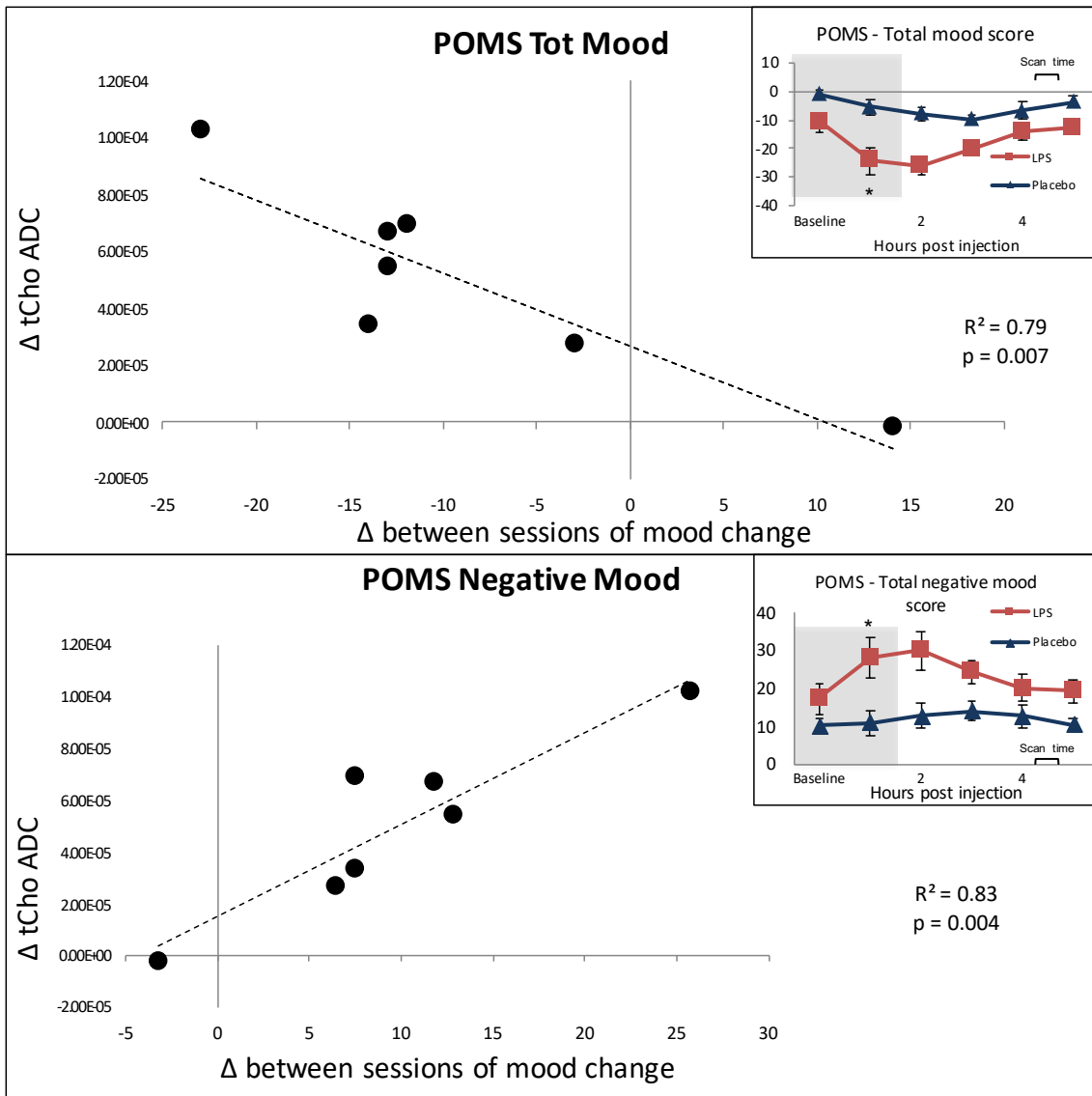
668 **Figure 2: Effect of LPS on cytokine levels**



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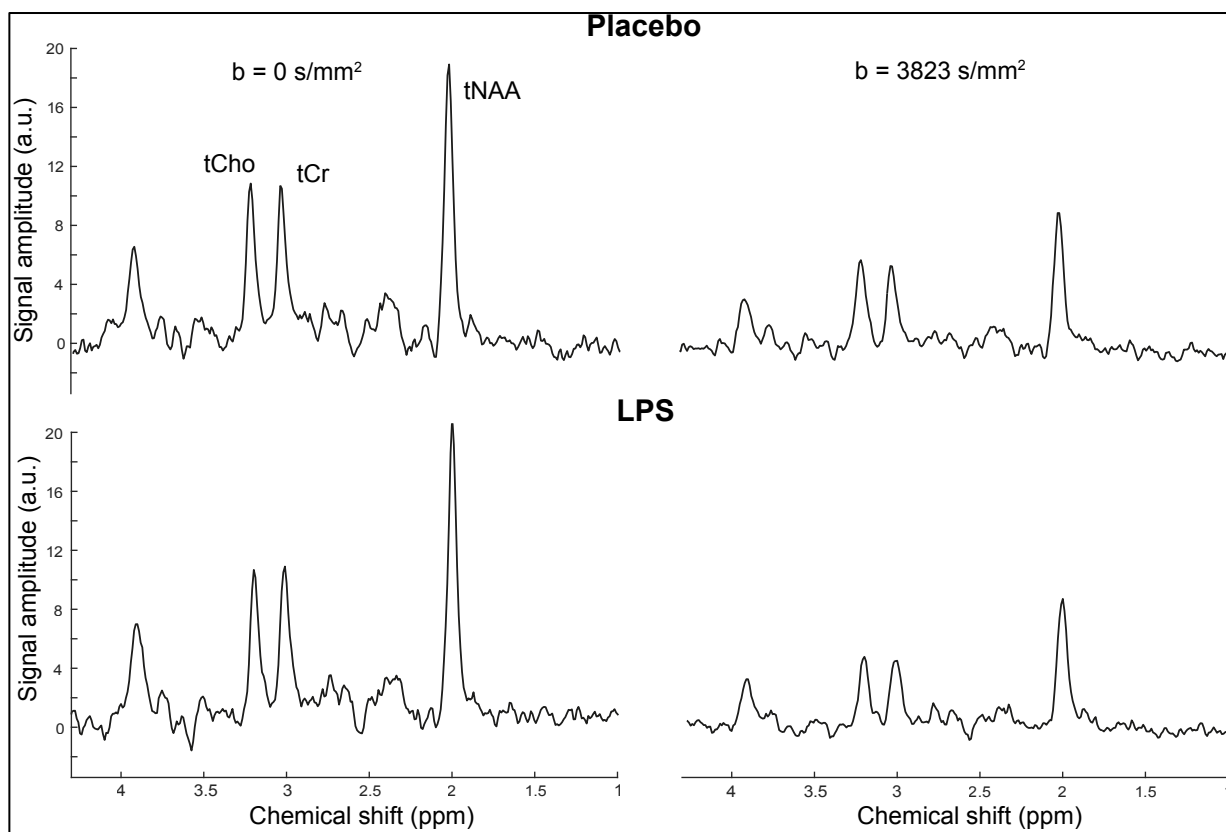
671 **Figure 3: Effect of LPS on mood and relationship with tCho ADC change**



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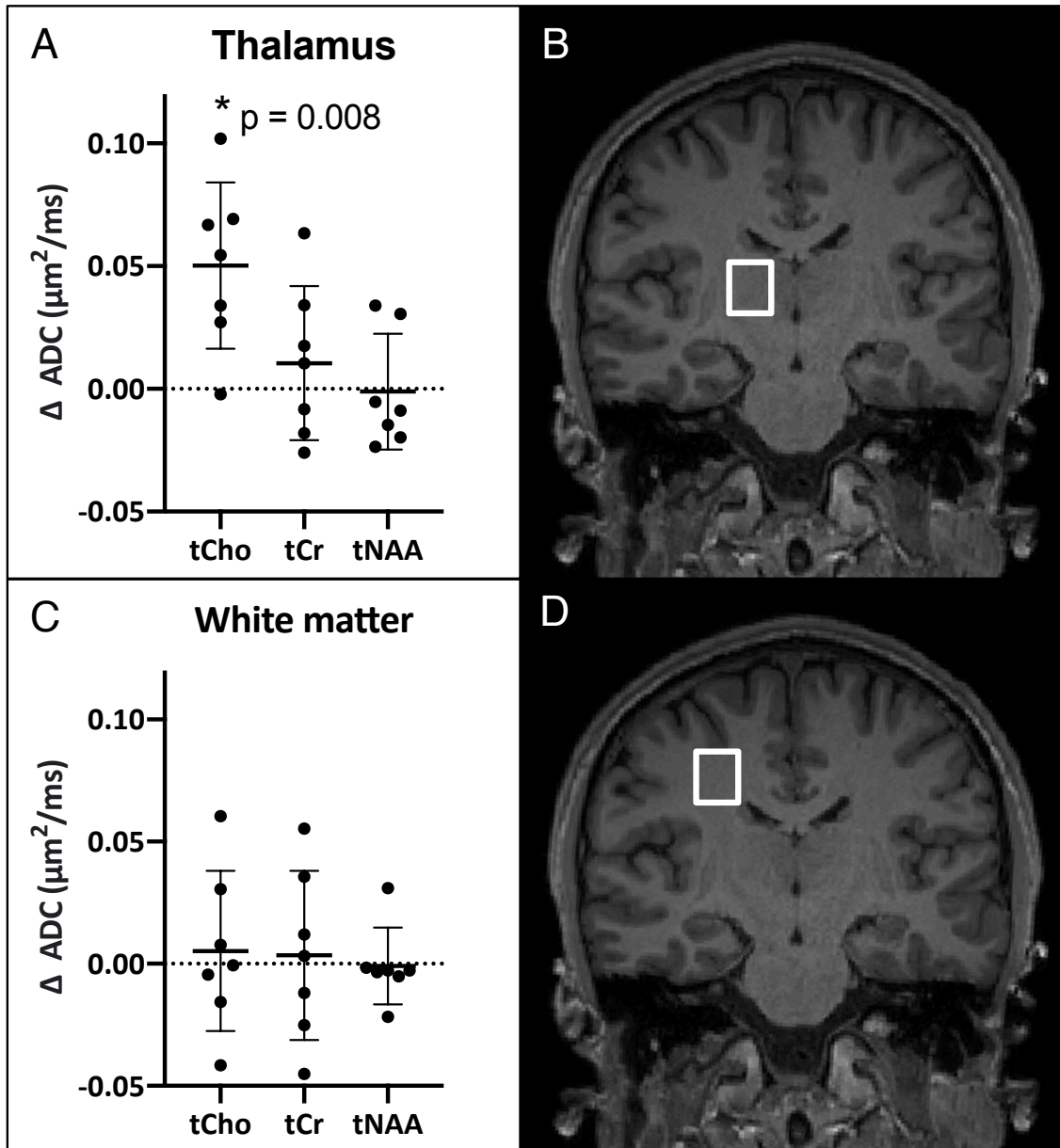
674 **Figure 4: MR Spectra**



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677 **Figure 5: Metabolites ADC responses to LPS**



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