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Experimental sepsis-associated encephalopathy is accompanied by altered cerebral blood perfusion and water diffusion and related to changes in cyclooxygenase-2 expression and glial cell morphology but not to blood-brain barrier breakdown

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► **To cite this version:**

Marion Griton, Ibtihel Dhaya, Renaud Nicolas, Gérard Raffard, Olivier Periot, et al.. Experimental sepsis-associated encephalopathy is accompanied by altered cerebral blood perfusion and water diffusion and related to changes in cyclooxygenase-2 expression and glial cell morphology but not to blood-brain barrier breakdown. *Brain, Behavior, and Immunity*, 2020, 83, pp.200-213. 10.1016/j.bbi.2019.10.012 . hal-03029674

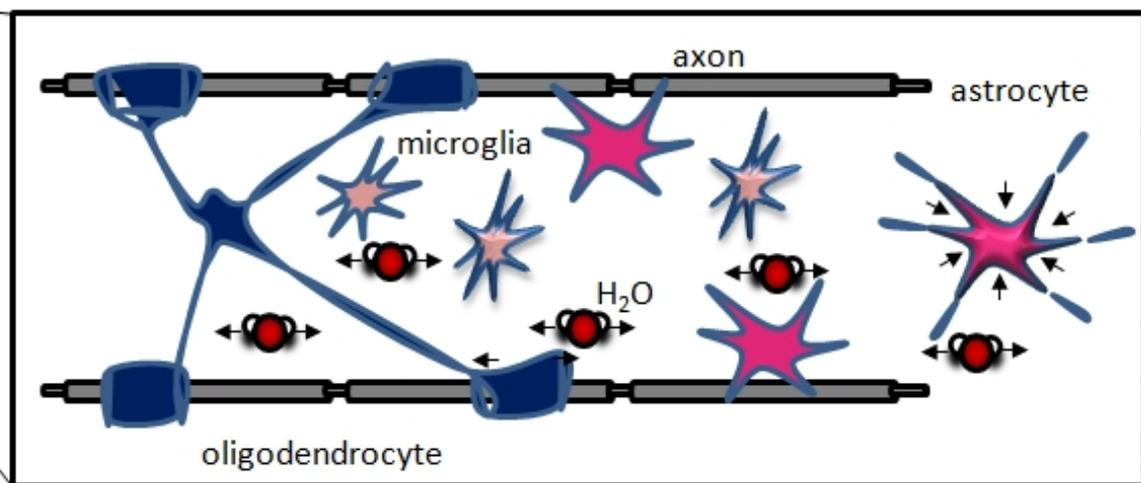
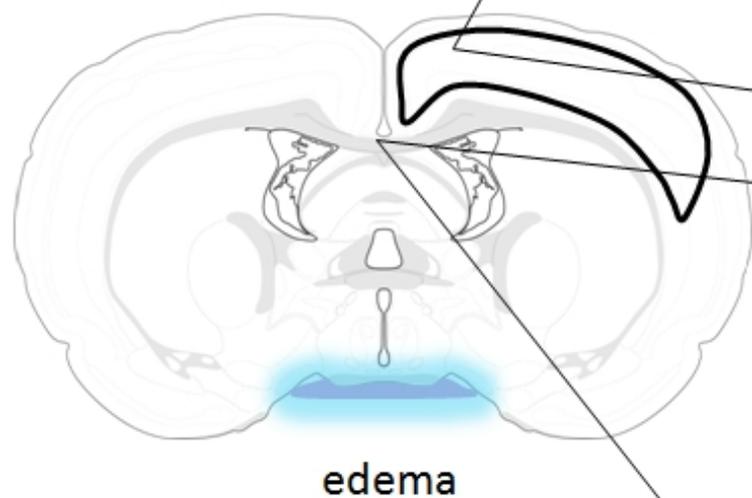
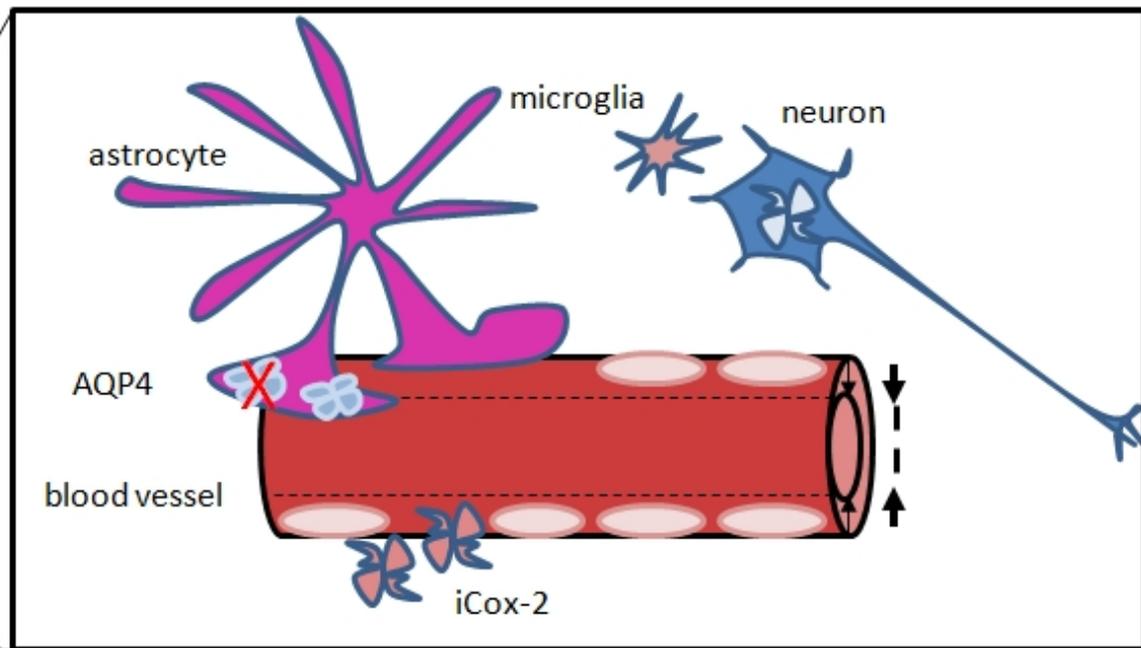
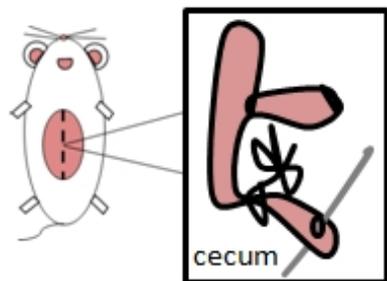
HAL Id: hal-03029674

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Submitted on 28 Nov 2020

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1 **Experimental sepsis-associated encephalopathy is accompanied by altered cerebral**
2 **blood perfusion and water diffusion and related to changes in cyclooxygenase-2**
3 **expression and glial cell morphology but not to blood-brain barrier breakdown**

4
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21
22 Running title: CLP increases white matter axial diffusion

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32 **Abstract**

33 Sepsis-associated encephalopathy (SAE) refers to brain dysfunction, including *delirium*,
34 occurs during severe infection and is associated with development of post-traumatic stress
35 disorder. SAE has been proposed to be related to reduced cerebral blood flow (CBF), blood-
36 brain barrier breakdown (BBB), white matter edema and disruption and glia cell activation,
37 but their exact relationships remain to be determined. In the present work, we set out to study
38 CBF using Arterial Spin Labeling (ASL) and grey and white matter structure with T2- and
39 diffusion magnetic resonance imaging (dMRI) in rats with cecal ligation and puncture (CLP)-
40 induced encephalopathy. Using immunohistochemistry, the distribution of the vasoactive
41 prostaglandin-synthesizing enzyme cyclooxygenase-2 (COX-2), perivascular
42 immunoglobulins G (IgG), aquaporin-4 (AQP4) and the morphology of glial cell were
43 subsequently assessed in brains of the same animals. CLP induced deficits in the righting
44 reflex and resulted in higher T2-weighted contrast intensities in the cortex, striatum and at the
45 base of the brain, decreased blood perfusion distribution to the cortex and increased water
46 diffusion parallel to the fibers of the corpus callosum compared to sham surgery. In addition,
47 CLP reduced staining for microglia- and astrocytic-specific proteins in the corpus callosum,
48 decreased neuronal COX-2 and AQP4 expression in the cortex while inducing perivascular
49 COX-2 expression, but did not induce widespread perivascular IgG diffusion. In conclusion,
50 our findings indicate that experimental SAE can occur in the absence of BBB breakdown and
51 is accompanied by increased water diffusion anisotropy and altered glia cell morphology in
52 brain white matter.

54 **Introduction**

55 Sepsis-associated encephalopathy (SAE) refers to brain dysfunction ranging from mild
56 *delirium* to deep coma that occurs in up to 70% of patients with severe systemic infection
57 (Gofton and Young, 2012; Jones and Griffiths, 2013; Lamar et al., 2011; Young, 2013). In
58 addition to acute mental dysregulation in the form of *delirium*, sepsis also has long-term
59 detrimental effects on mental health. Indeed, clinical sepsis is associated with the
60 development of post-traumatic stress disorders (PTSD) months later (Boer et al., 2008;
61 Rosendahl et al., 2013; Schelling et al., 2001; Wintermann et al., 2015).

62 In spite of its impact on mental and cognitive health, the mechanisms underlying SAE are
63 poorly understood (Chaudhry and Duggal, 2014). SAE, including *delirium*, has been
64 proposed to be related to reduced cerebral blood flow (CBF) and oxygen extraction, blood-
65 brain barrier breakdown (BBB), brain inflammation and edema (Chaudhry and Duggal, 2014;
66 Papadopoulos et al., 2000; Tsuruta and Oda, 2016). Indeed, baseline CBF has been shown to
67 be reduced during SAE (Bowton et al., 1989; Maekawa et al., 1991; Pfister et al., 2008;
68 Pierrakos et al., 2013; Pierrakos et al., 2014; Smith et al., 1998; Yokota et al., 2003).
69 Furthermore, systematic assessment of middle cerebral artery (MCA) blood velocity to
70 variations in arterial blood pressure indicates that impaired cerebral autoregulation is
71 associated with sepsis-associated *delirium* (Pfister et al., 2008; Schramm et al., 2012). Of
72 note, some authors have also suggested that loss of cerebral autoregulation sepsis-associated
73 contributes to edema during sepsis (Piazza et al., 2009).

74 Interestingly, and concomitantly with vasospasms in the MCA of patients with sepsis and
75 brain dysfunction, T2- and diffusion-weighted Magnetic Resonance Imaging (MRI) have
76 indicated white matter vasogenic edema (Bartynski et al., 2006; Sharshar et al., 2007).
77 Moreover, in patients who died of sepsis, dilation of the perivascular space accompanied by
78 dissociation of myelinated and white matter microglial activation have been observed

79 (Lemstra et al., 2007; Sharshar et al., 2007; Zrzavy et al., 2019). Finally, *delirium* has been
80 found to be associated with disruption of white matter organization at hospital discharge and
81 3 months later, which, in turn, was associated with long-term cognitive impairment (Morandi
82 et al., 2012). So among the proposed underlying mechanisms, clinical SAE has been shown
83 to be associated with reduced CBF, signs of edema formation and white matter changes.
84 However, the relationships between these phenomena and the occurrence and role of BBB
85 breakdown as well as glial activation remain to be determined.

86 Experimental models allowing to systematically vary factors of interest have the potential to
87 unravel the pathophysiological mechanisms underlying SAE. Intravenous administration of
88 bacterial lipopolysaccharide (LPS) fragments in human volunteers is sufficient to reduce
89 global CBF (Moller et al., 2002; Pollard et al., 1997). However, after systemic administration
90 of LPS in animals, some studies have also observed rapidly decreased global or incoming
91 CBF (Bryan and Emerson, 1977; Christenson et al., 1986; Ekstrom-Jodal et al., 1982; Tempel
92 et al., 1986; Villega et al., 2017; Wyler et al., 1969; Wyler et al., 1972), whereas others
93 studies have shown increased CBF in cortical MCA territories, but dose-dependent impaired
94 autoregulation of CBF (Rosengarten et al., 2007; Rosengarten et al., 2008). And although
95 peripheral LPS administration in rodents induces microglial activation in different brain
96 structures (Hoogland et al., 2015), no study so far seems to have assessed white matter.

97 Cecal ligation and puncture (CLP) is a more clinically-relevant sepsis model than systemic
98 LPS administration in that it involves live bacteria and mimics the different hemodynamic
99 phases observed in clinical sepsis. Interestingly, during the first 24 h after CLP, CBF has
100 been shown to be preserved (Hinkelbein et al., 2007) while increased T2-weighted contrast at
101 the base of the brain suggested vasogenic edema and decreased apparent diffusion coefficient
102 (ADC) indicated cytotoxic edema in the cortex and hippocampus (Bozza et al., 2010).

103 The aim of the present work was to establish temporal and spatial relationships between CNS

104 hemodynamic, metabolic and structural changes during experimental sepsis using imaging
105 and histological approaches on brains of the same animals. We studied CBF and brain
106 microstructure using Arterial Spin Labeling (ASL) and Diffusion-weighted Magnetic
107 Resonance Imaging (dMRI), respectively, in animals with SAE 24h after CLP. In addition,
108 we also detected the vasoactive prostaglandin-synthesizing enzyme cyclooxygenase-2 (COX-
109 2), which is upregulated under inflammatory response, the presence of perivascular
110 immunoglobulins G (IgG) as a measure of BBB integrity, and modifications of glia cell
111 morphology in the brains of the same animals.

112

114 **Materials and methods**

115 *Animals*

116 Twenty-one male 3.5 month old Wistar rats (Charles River, l'Abresle, France) weighing
117 340.3 ± 3.6 g were used. Animals were treated according to European recommendations on
118 animal research (European Council Directive of 24 November 1986 (86/609/EEC) and
119 European Parliament and Council Directive of 22 September 2010 (2010/63/UE)), housed 2
120 per cage in a temperature-controlled room ($22.0 \pm 1.0^\circ\text{C}$) with lights on from 8 AM to 8 PM
121 with free access to water and food. After arrival rats were left undisturbed for at least a week
122 except for daily handling starting three days before surgery.

123

124 *Surgery*

125 On the day of surgery, animals were anesthetized with isoflurane (induction 3-5%;
126 maintenance 1.5% in air). Once anesthetized, seventeen rats underwent polymicrobial intra-
127 abdominal infection induced by CLP as previously described (Rittirsch et al., 2009;
128 Wichterman et al., 1980). Briefly, after a 2-3-cm midline abdominal incision, the cecum was
129 exposed in sterile saline on laboratory film (Parafilm, Neenah, WI, USA) and ligated with a
130 4-0 silk suture (Ethicon, Issy les Moulineaux, France) just below the ileocecal valve and
131 subsequently punctured twice with a 22 G sterile needle. Next, a small drop of cecal contents
132 was gently pushed out, before the cecum was gently placed back into the peritoneal cavity
133 and the abdomen was sutured in two layers. At the end of the operation, animals received 5
134 ml of saline and analgesia (butorphanol, Torbugesic®; 2 mg/kg subcutaneously) and were
135 placed in a clean individual cage. For the ten rats that were sham-operated rats, the same
136 manipulations were performed except cecal ligation and puncture and saline injection. Thirty
137 minutes after the end of surgery animals were awake and moving around.

138

139 *Behavioral evaluation*

140 To study non-specific sickness responses during sepsis, daily food and water intake and body
141 weight were measured at several time points prior and posterior to surgery.

142 *Delirium* involves, according to the *Diagnostic and Statistical Manual of Mental Disorders*,
143 disturbances of consciousness or awareness and in attention (Meagher et al., 2014), and is
144 therefore hard to assess in animals. However, SAE is routinely monitored through assessment
145 of reflexes, for example as part of the Glasgow coma scale (Ebersoldt et al., 2007), and can
146 thus be easily and robustly implemented in animals. Following Kadoi & Goto (2004), sham
147 and CLP -operated rats were tested at different time points before and after surgery for two
148 simple non-postural (pinna and corneal reflexes) and one complex postural somatomotor
149 reflex (righting reflex).

150 The pinna reflex was assessed by lightly touching the auditory meatus with a cotton stick tip
151 and recording the occurrence and rapidity of ear retraction or head shake. The corneal reflex
152 was tested by gently touching the cornea with a cotton stick tip and scoring the occurrence
153 and rapidity of eye blinks. The righting reflex consisted of picking up the animal behind the
154 forepaws and holding its trunk horizontally back upwards before putting the animal's back
155 down and evaluating the time required to turn over to an upright position.

156

157 *Magnetic Resonance Imaging (MRI) acquisition and image processing*

158 Twenty-four hours after surgery, animals were anesthetized again with isoflurane (induction
159 3-5%, maintenance 1.5%) and introduced into a 7T horizontal-bore scanner (Advance III
160 console, Bruker, Ettlingen, Germany) equipped with a magnetic field gradient system
161 providing a maximum magnetic field gradient amplitude of 650 mT/m. A quadrature coil was
162 used for radio-frequency emission and a 4-element phased-array surface coil for signal
163 reception. Intracolonic temperature was constantly monitored and the animal was heated

164 when colonic temperature dropped below 35.5°C by warm water circulating in the bed used
165 to position the rat inside MRI scanner. Respiration was assessed with a ventral pressure
166 sensor and heart rate recorded using an MRI compatible electrode. After careful second-order
167 shimming, T1-weighted MRI, T2-weighted MRI, Arterial Spin Labeling (ASL) and
168 diffusion-weighted MRI (dMRI) images were acquired (see supplementary Materials and
169 Methods for details of MRI acquisition sequences).

170 Analysis of T2-weighted images was performed using mipav (<https://mipav.cit.nih.gov>). In
171 this study, relative T2-weighted intensities were compared to determine the presence rather
172 than the quantity of edematic fluid (Abdel-Aty et al., 2005). Volumes Of Interest (VOIs)
173 were defined in the brain and in the skull muscles and expressed as ratios. The VOI at the
174 base of the brain was made up of 3 parts (right, left and center) to avoid blood vessels. The
175 corresponding VOIs were summed together to cover the base of the rat brain (in bregma ~ -
176 0.46mm). The other VOIs were cortex, striatum (right and left) and corpus callosum.
177 Maximum and average intensities obtained in brain VOIs were then divided by those
178 obtained in the skull muscle.

179 ASL data processing was performed using ParaVision software (Bruker, Ettlingen, Germany)
180 unless otherwise indicated. Two (selective and global) T1 maps were computed using slice-
181 selective and global FAIR data sets. Relative cerebral blood flow (rCBF) map was then
182 computed pixel by pixel according to Kober et al. (2004). Blood T1 measured on rat blood at
183 7 T was set at 2070 ms (Dobre et al., 2007; Esparza-Coss et al., 2010). To analyze perfusion
184 data, Regions Of Interest (ROIs) corresponding to the global brain, brain hemispheres, grey
185 matter of the cortex and striatum as well as to the white matter of the corpus callosum and
186 external capsule were traced manually on each individual's selective inversion 1100ms FAIR
187 image using a rat brain atlas (Swanson, 1998) for guidance. These ROIs were then copied
188 onto perfusion maps images (Fig. 1).

189 A quality control was first performed on raw dMRI data and diffusion tensor imaging (DTI)
190 maps. The *3dSkullStrip* command included in Analysis of Functional NeuroImages software
191 (AFNI) (<https://afni.nimh.nih.gov>) was used for image processing 1) to extract the brain from
192 surrounding tissues and 2) to create a mask. The twenty-one DW images and the B0 images
193 were corrected for eddy currents using *eddy* and fitted to the DTI model with *dtifit* (both part
194 of the FMRIB's Software Library (FSL) (<http://www.fmrib.ox.ac.uk/fsl>)).

195 The following diffusion-tensor maps were computed: 1) Eigenvalues (λ_1 , λ_2 and λ_3)
196 corresponding to water molecule diffusivities along the principal axes of the diffusion tensor,
197 2) Axial Diffusivity ($AD=\lambda_1$) representing water diffusivity along the length of the white
198 matter fibers, 3) Radial Diffusivity ($RD=(\lambda_2+\lambda_3)/2$) representing water diffusivity
199 perpendicular to the white matter fibers. 4) Mean Diffusivity ($MD=(\lambda_1+\lambda_2+\lambda_3)/3$) of water
200 molecules, 5) Fractional Anisotropy (FA calculated following Alexander (2000)) indicating
201 the degree of anisotropy of water diffusivity and 6) residuals maps as a quality assessment
202 tool for DTI regression accuracy showing artefacts that are not always visible on above maps.
203 Additional maps were then calculated to describe the shape of diffusion including 7)
204 Coefficient of sphericity (C_s) corresponding to the roundness of tensor ellipsoid, 8)
205 Coefficient of planar anisotropy (C_p) measuring planarity, and 9) Coefficient of linear
206 anisotropy (C_l) that assesses the tendency of the tensor ellipsoid to have a cigar shape
207 (Alexander et al., 2000).

208 ROIs were positioned manually on the white matter of the corpus callosum and external
209 capsule and the grey matter of the cortex and striatum at two different rostrocaudal levels: 1)
210 bregma -0.51mm (Swanson, 1998) and 2) bregma -1.08mm (Swanson, 1998) because the
211 cytoarchitecture of these structures is known to vary along the rostrocaudal axis (Barazany et
212 al., 2009). On Color-coded FA maps showing white matter in different colors according to
213 fiber orientations, additional ROIs were placed on the cortex and on the external capsule,

214 after which the part of the external capsule where axons run horizontally and the part where
215 axons are oriented vertically were analyzed.

216 To explore diffusion data in a non-hypothesis driven manner, a whole brain voxelwise
217 statistical method adapted to the rat brain was performed using Tract-Based Spatial Statistics
218 (TBSS) which is part of FSL (Smith et al., 2006). FA maps from each rat were co-registered
219 to the DTI Rat Atlas for Postnatal Day 72 (DTI-RAPnD72) template
220 (https://www.nitrc.org/projects/dti_rat_atlas/) using *fnirt* a nonlinear registration command
221 adapted to take into account rat brain. After image registration, FA maps were averaged to
222 produce a group mean FA image. A skeletonization algorithm was applied to the group mean
223 FA image to define a group template of the lines of maximum FA, thought to correspond to
224 centers of white matter tracts. FA values for each individual subject were then projected onto
225 the group template skeleton by searching along perpendiculars from the skeleton to find local
226 maxima. Non-FA maps (AD, RD, and MD) were skeletonized according to non-linear warps
227 of the FA registrations described above.

228

229 *Tissue processing and immunohistochemistry*

230 At the end of MRI, rats were deeply anaesthetized by intraperitoneal injection of 60mg/kg of
231 sodium pentobarbital. Bodies were rinsed from blood by intracardiac perfusion with 0.1 M
232 phosphate-buffered saline (PBS; pH 7.4) and subsequently fixed by 300 ml of 4%
233 paraformaldehyde in 0.1 M PBS. Brains were removed from the skull, post-fixed for 4 h in
234 the same fixative, and then cryoprotected in 30% sucrose in 0.1 M phosphate buffer. Thirty-
235 micrometer thick sections were cut on a cryostat (CM3050 S, Leica Microsystems, Nussloch,
236 Germany) from the rostral limit of the optic chiasm to the caudal end of the central amygdala
237 (Swanson, 1998) and collected in cold cryoprotectant solution (0.05 M PBS, 20 % glycerol,
238 30% ethylene glycol) and stored at -20°C until processing.

239 Immunohistochemistry was performed as previously described (Konsman and Blomqvist,
240 2005; Konsman et al., 2008; Konsman et al., 2004). Briefly, free-floating sections were
241 washed four times in 0.1 M PBS (pH 7.4). Non-specific binding sites were blocked by a 45-
242 minute incubation of sections in PBS containing 0.3% Triton X-100 and 2.0% normal serum
243 or 1.0% BSA. The first antibody was then diluted (indicated below for every antiserum used)
244 in the same buffer and added to the sections overnight at room temperature.

245 Commercially available antisera raised against COX-2 (goat anti-COX-2; M-19 sc-1747, lot#
246 F2512, Santa Cruz Biotechnology, Heidelberg, Germany) diluted 1:750, the microglia-
247 macrophage specific ionized calcium-binding adaptor molecule (Iba-1; rabbit anti-Iba-1; 019-
248 19741, Wako Chemicals GmbH, Neuss, Germany) diluted 1:1000 and rat immunoglobulin G
249 (IgG; biotinylated goat anti IgG, BA-9401, Vector Laboratories, Burlingame, CA, USA)
250 diluted 1:2000, the astrocytic aquaporin-4 water channel (rabbit anti-AQP4, cat # AB2218,
251 lot # 2506432, Millipore, Fontenay sous Bois, France) diluted 1:2000, the intermediate
252 filament Glial fibrillary acidic protein GFAP (mouse anti-GFAP mAb, clone GA5, MAB360,
253 Merck Millipore, Fontenay sous Bois, France) diluted 1:500, the myelin-associated enzyme
254 2',3'-Cyclic-nucleotide 3'-phosphodiesterase also known as CNPase (mouse anti-CNPase,
255 Abcam, Paris, France) diluted 1:8000 were used.

256 After four rinses in PBS, sections were treated for 30 minutes with 0.3% (v/v) hydrogen
257 peroxide to block endogenous peroxidases followed by four rinses in PBS. With the
258 exception of those sections exposed to biotinylated rat IgG, sections were then incubated for
259 2h with biotinylated antisera raised against IgGs of the species of the first antibody (Vector
260 Laboratories, Burlingame, CA, USA) diluted 1:500 in PBS, 0.3% Triton X-100, 1% BSA or
261 normal serum. After four washes in PBS, sections were incubated for 2 h with avidin and
262 biotinylated peroxidase complex (Vector Laboratories, Burlingame, CA, USA) diluted in
263 PBS to 1:750 in IgG immunostaining and 1:500 in others. Finally, sections were transferred

264 to a sodium acetate buffer (0.1 M; pH 6.0) and stained using diaminobenzidine as a
265 chromogen in the presence of Ni-ions, thus yielding a dark grey to black precipitate.

266

267 *Microscopy and image analysis*

268 Staining reactions were stopped by rinses in sodium acetate and PBS, after which sections
269 were mounted on slides in 0.5% gelatin in distilled water containing chromo-potassium
270 sulphate, dehydrated in alcohol, defatted in xylene and coverslipped with Eukitt (O.Kindler
271 GmbH & CO, Freiburg, Brisgau, Germany). Stained sections were examined with a light-
272 microscope (Leica DM5500B, Leica Microsystems, Nanterre, France) and images were
273 captured by a high-resolution digital camera system (Leica DFC425C, Leica Microsystems,
274 Nanterre, France) and stored onto a personal computer. In every brain section between
275 bregma +4,85 and -2,85 mm the occurrence and extent (scored as restricted (1), intermediate
276 (2) or extensive (3)) of perivascular IgG diffusion in a given structure was noted. As each
277 region or structure considered covered at least two, but a varying number (depending on the
278 length of the structure), of brain sections (with two consecutive sections being separated by
279 240 μm), the number of occurrences of perivascular IgG diffusion for a structure were then
280 divided by the number of brain sections in which the structure was present. Similarly, the
281 cumulative score of the extent of IgG diffusion for a given brain region or structure was also
282 divided by the number of sections that contained the region or structure in question.

283 The counts and relative surfaces of AQP4, CNPase, COX-2, GFAP and Iba-1-
284 immunoreactive elements were quantified after application of fixed particle size and intensity
285 criteria on 8-bit images with Image J (<http://imagej.nih.gov/ij/>). Iba-1- and GFAP-
286 immunostaining was also quantified using the Image J plugin Fraclac
287 (<http://rsb.info.nih.gov/ij/plugins/fraclac/fraclac.html>). Briefly, on binary images of brain
288 sections between bregma -0.11 and -1.33 mm, Iba-1-positive cells (size between 300-6000

289 pixels) and GFAP-immunoreactive cells (size between 300-8000 pixels) were enclosed by a
290 Hull convex (a boundary enclosing the foreground pixels of an image using straight line
291 segments to each outermost point) and bounding circle after which the surface, perimeter,
292 diameter and circularity (defined as $4*\pi*Area / Perimeter^2$) were determined in addition to
293 the width and height of the cells. In addition, Iba-1 cell size and activation was also evaluated
294 with Image J based on staining intensity as described by Hovens et al (2014). Briefly, a
295 minimum size filter was applied on cell body size (>150 pixels) before cell and cell body size
296 were distinguished based on an intensity criterion. Finally, three different categories of sizes
297 of GFAP-positive elements (GFAP: <300 pixels considered as astrocytic fragments;
298 300<pixels<8000 considered as astrocytes; pixels>8000 considered as astrocytes in contact)
299 were quantified in terms of number and relative surface after application of a fixed intensity
300 threshold.

301 Image editing software (Adobe Photoshop, Adobe Systems, San Jose, CA, USA) was used
302 only to adjust contrast and brightness for photomicrographs composing illustrating figures.

303

304 *Data representation and analysis*

305 Food intake and water intake were expressed relative to 100g of body weight and 100g of
306 food intake respectively. Maximum intensity obtained from T2-weighted images analysis was
307 expressed relative to skull muscle. To assess blood redistribution between brain structures,
308 ASL values in cerebral structures were also expressed relative to perfusion of brain
309 hemispheres or to that of the global brain parenchyma in the case of the corpus callosum that
310 is situated between the two hemispheres. All data, except TBSS data, were expressed as
311 means \pm standard error of the mean (SEM) and analyzed by a one-way ANOVA with surgery
312 as a between factor. When data were not normally distributed, a log₁₀ or square root
313 transformation was performed, and if still not normally distributed then the non-parametric

314 Mann-Whitney U test was used. Pearson linear correlation coefficients were calculated
315 between MRI (T2, ASL and DTI) parameters and immunohistochemical (AQP4-, COX-2-,
316 GFAP- and Iba-1-immunoreactivity) data. In all cases, a level of $p < 0.05$ was considered as
317 statistically significant.

318 For statistical analysis of TBSS data, a nonparametric voxel-wise statistical procedure was
319 carried out using *randomise*, a permutation testing algorithm, with 500 and 5000
320 permutations. Threshold-free cluster enhancement (TFCE) method (Smith and Nichols, 2009)
321 was used to define clusters with significant differences at $p < 0.05$ fully corrected for multiple
322 comparisons across space. Contrasts were performed for sham-operated vs. CLP animals.
323 TBSS results were displayed on top of the group mean_FA map, the DTI-
324 RAPnD72_template and the DTI-RAPnD72_labels.

325

327 **Results**

328 *CLP reduced food intake and induced encephalopathy*

329 Within the 23h following the end of surgery, no mortality was observed in the sham-operated
330 group (One animal did die however at the induction of anesthesia for imaging) while CLP
331 resulted in 12.5% mortality. One-way ANOVAs on food consumption relative to body weight
332 during the 24h before surgery did not reveal any differences between animals to be allocated
333 to treatment groups. However, a Mann-Whitney U-test on food consumption relative to body
334 weight during the 24h after the start of surgery showed a significant lower food intake in
335 animals that underwent CLP as compared to sham-operated rats (U: 26.0, $p < 0.05$) (Fig. 2A).
336 Mann-Whitney tests on corneal, pinna and righting reflexes showed that CLP significantly
337 reduced the righting reflex 8 (U: 10.0, $p < 0.001$) and 24h (U: 4.50, $p < 0.001$) later as compared
338 to sham surgery (Fig. 2B) without affecting the pinna and corneal reflexes (Fig. 2C, D).

339

340 *CLP increased T2-weighted intensities in cortex and striatum*

341 One way ANOVAs on T2-weighted intensity ratios (VOIs of interest relative to that of skull
342 muscle) showed significantly greater maximum and average intensities at the base of the
343 brain ($F_{1,17}$: 8.98, $p < 0.01$; Fig. 3A; $F_{1,17}$: 16.4, $p < 0.001$ (data not shown), respectively) in
344 CLP animals as compared to sham-operated rats. Similar analyses in the cortex also indicated
345 a greater maximum ($F_{1,18}$: 5.41, $p < 0.05$; Fig. 3B) in CLP rats compared to sham-surgery
346 controls, but no effect on average intensity. In the striatum, one way ANOVAs showed
347 greater maximum and average intensities ($F_{1,18}$: 5.39, $p < 0.05$; Fig. 3C; $F_{1,18}$: 6.99, $p < 0.05$
348 (data not shown), respectively) 24h after CLP compared to sham-surgery. Finally, one way
349 ANOVAs only revealed a trend for an increased maximum in the corpus callosum ($F_{1,18}$:
350 4.08, $p = 0.059$; Fig. 3D) of CLP animals as compared to sham-operated rats with no
351 differences in average intensities.

352

353 *CLP decreased perfusion towards the cerebral cortex*

354 One-way ANOVAs or Mann-Whitney tests on absolute perfusion values in the ROIs
355 covering the cortex (Sham: $105.75 \pm 7.29\text{ml}/100\text{g}/\text{min}$; CLP: $97.97 \pm 5.46\text{ml}/100\text{g}/\text{min}$),
356 striatum (Sham $102.56 \pm 10.71\text{ml}/100\text{g}/\text{min}$; CLP: $96.7 \pm 7.24\text{ml}/100\text{g}/\text{min}$), corpus callosum
357 (Sham: $112.85 \pm 9.07\text{ml}/100\text{g}/\text{min}$; CLP $101.28 \pm 7.77\text{ml}/100\text{g}/\text{min}$) and external capsule
358 (Sham: $94.21 \pm 8.48\text{ml}/100\text{g}/\text{min}$; CLP $87.07 \pm 4.88\text{ml}/100\text{g}/\text{min}$) did not reveal significant
359 differences between CLP- and sham surgery groups 24 h after surgery.

360 To assess the distribution of blood between brain structures, perfusion values measured in
361 brain areas were also analyzed relative to that of the hemisphere (or to that of the global brain
362 parenchyma in the case of the ROI centered on the corpus callosum that is situated between
363 the two hemispheres). One-way ANOVAs on these data indicated significant lower
364 distribution of blood to the cortex ($F_{1,18}$: 5.04, $p < 0.05$; Fig. 4A), but no changes in perfusion
365 distribution to the striatum (Fig. 4B), corpus callosum (Fig. 4C) or external capsule region
366 (Fig. 4D) in animals that had undergone CLP surgery as compared to sham surgery.

367

368 *CLP increased axial water diffusion in the corpus callosum*

369 DWI data were generally of good comparable quality in 20 out of 21 animals. One animal
370 was excluded because its head was tilted in a way that hindered processing for DTI.

371 Mann-Whitney tests on water diffusion parameters in the ROI covering the cortex at the
372 anatomical level where the white matter of the anterior commissure crosses the third ventricle
373 (corresponding to bregma -0.51mm (Swanson, 1998)) or at the level where the third ventricle
374 is divided in a ventral and dorsal part by the grey matter of the paraventricular thalamus,
375 (corresponding to bregma -1.08mm (Swanson, 1998)) did not indicate any significant
376 differences between rats that underwent CLP and those that were subject to sham surgery.

377 Similar analyses on water diffusion parameters in the striatum ROI indicated a significant
378 decrease in RD at bregma -1.08mm ($F_{1,18}$: 4.82, $p < 0.05$). Using a non-hypothesis-driven and
379 non-ROI-based TBSS approach, a significant higher FA was found in the ventral caudate
380 putamen and globus pallidus of images centered at bregmas -0.51 and -0.83mm of CLP rats
381 as compared to sham-operated animals after 5000 random permutations (Supplementary fig.
382 1).

383 One-way ANOVAs or Mann-Whitney tests on water diffusion parameters measured in the
384 corpus callosum ROI at bregma -0.51mm indicated a significant increase in λ_1 (U: 19.0,
385 $p < 0.05$; Fig. 5A) and a significant decrease in C_p (U: 18.0, $p < 0.05$) in rats that underwent
386 CLP surgery as compared to sham-operated rats.

387 Finally, one-way ANOVAs or Mann-Whitney tests on water diffusion parameters in the ROI
388 placed on the external capsule at bregma -1.08mm revealed a significant increase in FA in the
389 left external capsule ($F_{1,18}$: 5.33, $p < 0.05$; Fig. 5B) after CLP as compared to sham surgery.

390

391 *CLP did not induce widespread BBB breakdown*

392 In all animals, IgG was found in brain regions lacking a functional BBB including the
393 choroid plexus, meninges and circumventricular organs from which it spread to surrounding
394 regions (Fig. 6A, B). One-way ANOVA or Mann-Whitney tests on the occurrence of
395 perivascular plume-like diffusion clouds of IgG staining in the white matter revealed a
396 significantly increased frequency in the fimbria (U: 30.0, $p < 0.05$; not shown), but not in the
397 corpus callosum (Fig. 6C, D) or elsewhere in the white matter (Fig. 6E, F), of rats that
398 underwent sham surgery as compared to CLP animals. Similar analyses on grey matter did
399 not indicate any differences with regard to the light to moderate perivascular IgG diffusion
400 that was occasionally observed in the cortex, striatum and hippocampus of both sham-
401 operated and CLP animals (Fig. 6G-J).

402

403 *CLP decreased cortical COX-2-immunoreactivity*

404 No or very weak COX-2 immunoreactivity was found associated with blood vessels in the
405 preoptic area (Fig. 7A), caudate putamen (Fig. 7C), external capsule (Fig. 7E), hippocampus
406 (Fig. 7G) or cortex (Fig. 7I) of animals subjected to sham-surgery. However, distinct
407 perivascular disc-like COX-2-immunoreactive cells were frequently observed in these
408 structures, with the exception of the hippocampus, in rats that underwent CLP (Fig. 7B, D, F,
409 H and J).

410 Constitutive COX-2 expression was detected in neurons of the hippocampus and to a lesser
411 extent, in neurons of the cortex (Fig. 7 G-J). Interestingly, and in spite of perivascular
412 induction of COX-2 in the cortex, one-way ANOVAs on cortical COX-2-immunoreactivity
413 showed a significant decrease in the number of cells ($F_{1,11}$: 22.2, $p < 0.001$), the total ($F_{1,11}$:
414 8.37, $p < 0.05$) and relative ($F_{1,11}$: 8.90, $p < 0.05$) surface labeled after CLP in comparison to
415 sham-surgery (Fig. 7K, L). Interestingly, significant positive correlations were found between
416 the total and relative surface occupied by COX-2 immunoreactivity and perfusion distribution
417 to the cortex (both $r = 0.75$, $p < 0.005$).

418

419 *CLP altered microglial density and morphology in grey and white matter*

420 One-way ANOVAs on Iba-1 staining in the cortex revealed a significant increase in the
421 number ($F_{1,15}$: 8.68, $p < 0.05$) and relative surface ($F_{1,15}$: 7.46, $p < 0.05$) of positive elements in
422 CLP rats compared to sham-operated animals. Similar analyses on Iba-1-immunoreactivity in
423 the striatum also indicated a significant increase in number ($F_{1,16}$: 6.15, $p < 0.05$) and relative
424 surface labeled ($F_{1,16}$: 6.29, $p < 0.05$) after CLP rats as compared to sham surgery. In addition,
425 one-way ANOVAs on Iba-1 staining in the ventral part of the striatum at bregma levels -0.51
426 to -0.83mm, that was indicated by tract-based spatial statistics of water diffusion data,

427 indicated a significant increase in relative surface ($F_{1,16}: 5.48, p<0.05$) of positive elements in
428 CLP rats as compared to sham-operated animals. Finally, a significant negative correlation
429 was found between the number of Iba-1-immunoreactive elements and MD in the striatum at
430 bregma -1.08mm ($r=-0.50, p<0.05$).

431 In contrast, one-way ANOVAs on Iba-1 staining in the corpus callosum (Fig. 8A, B) did not
432 reveal a significant difference in relative surface of positive elements in CLP rats as
433 compared to sham-operated animals. However, one-way ANOVAs or Mann-Whitney tests on
434 other measures obtained by fractal analysis showed a significant increase in area (U: 21.0,
435 $p<0.05$), perimeter (U=21.0, $p<0.05$), height ($F_{1,18}: 14.9, p<0.01$; Fig. 8C) and width ($F_{1,18} :$
436 $6.93, p<0.05$; Fig. 8D) of Iba-1-positive elements in CLP rats compared to sham-operated
437 animals. One-way ANOVAs or Mann-Whitney tests on parameters generated by the method
438 of Hovens et al. (2014) indicated a significant decrease in Iba-1-positive cell size (U:18.0,
439 $p<0.05$) and cell processes (U: 18.0, $p<0.05$) and a significantly increased cell body size (U:
440 0.0, $p<0.001$) as well as a significantly increased microglial activation measure (cell body
441 size/cell size; $F_{1,17}: 6.94, p<0.05$) in CLP animals compared to sham-operated rats. Linear
442 regression analyses did not indicate any significant correlations between measures of Iba-1-
443 immunoreactivity and increased axial diffusion in the corpus callosum.

444

445 *CLP altered corpus callosum astroglia morphology, but not density*

446 One-way ANOVAs on GFAP staining in the cortex revealed a significant increase in the
447 number ($F_{1,19}: 4.91, p<0.05$) and relative surface ($F_{1,19}: 4.55, p<0.05$) of positive elements in
448 CLP rats compared to sham-operated animals. Similar analyses on GFAP-immunoreactivity
449 in the striatum also indicated a significant increase in number ($F_{1,19}: 6.14, p<0.05$) and
450 relative surface labeled ($F_{1,19}: 5.82, p<0.05$) in after CLP as compared to sham surgery.
451 Finally, a one-way ANOVA on GFAP staining in the ventral region of the striatum at bregma

452 levels -0.51 and -0.83mm, indicated by tract-based spatial statistics to show changes in water
453 diffusion, showed a trend for an increase in number ($F_{1,13}$: 4.57, $p=0.052$) of GFAP-positive
454 elements.

455 One-way ANOVAs or Mann-Whitney tests on GFAP staining in the corpus callosum (Fig.
456 9A, B) did not reveal any differences in number or relative surface of positive elements
457 between CLP rats and sham-operated animals. One-way ANOVAs on fractal parameters of
458 GFAP-1-positive cells in the corpus callosum also did not indicate significant differences in
459 height (Fig. 9C), width, perimeter, surface and circularity.

460 One-way ANOVAs on GFAP-positive fragments (<300 pixels) in the corpus callosum using
461 a fixed threshold did however indicate a significantly lower number of counts ($F_{1,17}$: 8.45,
462 $p<0.05$) and relative surface ($F_{1,17}$: 18.49, $p<0.001$; Fig. 9D) in the CLP group as compared to
463 sham surgery. Interestingly, significant negative correlations were found between the number
464 of and the relative surface occupied by GFAP-positive fragments and axial water diffusion
465 ($r=-0.63$, $p<0.01$ and $r=-0.70$, $p<0.005$, respectively) in the corpus callosum.

466

467 *CLP decreased cortical AQP4 expression*

468 One way ANOVA showed that cortical AQP4-immunoreactivity (Fig. 10A, B) between
469 bregma -0.46 and -1.08 mm, which corresponded to the position of the T2-weighted image
470 analyzed above, was significantly lower 24h after CLP-induced sepsis than after sham
471 surgery in terms of both counts ($F_{1,14}$:9.74, $p<0.01$; Fig. 10C) and relative surface ($F_{1,14}$:8.66,
472 $p<0.05$; Fig. 10D). Similar analyses on AQP4-positive staining in the corpus callosum and
473 striatum did not indicate differences between groups. In addition, no significant correlations
474 were found between AQP4-immunoreactivity and T2-weighted images in any of these brain
475 structures.

477 **Discussion**

478 The main findings of the present work were that CLP induced sepsis-associated CNS
479 dysfunction resulted in higher T2-weighted contrast intensities in the cortex, striatum and at
480 the base of the brain, decreased blood perfusion distribution to the cortex and increased axial
481 water diffusion in the corpus callosum and fractional anisotropy in the ventral striatum
482 compared to sham surgery. In these animals, CLP induced perivascular COX-2 expression,
483 decreased cortical COX-2 and AQP4 expression and altered white matter glial cell
484 morphology. Interestingly, positive correlations were established between COX-2
485 immunoreactivity and perfusion distribution to the cortex, while negative correlations were
486 found between GFAP-positive fragments and axial water diffusion in the corpus callosum.

487 Decreased food intake is a classic non-specific symptom of systemic inflammation in
488 response to bacterial infection (Hart, 1988; Konsman and Dantzer, 2001) and was observed
489 following CLP. In addition, the loss of the righting reflex in animals that underwent CLP is in
490 agreement with previous studies (Kadoi and Goto, 2004; Kafa et al., 2010a; Kafa et al.,
491 2010b) and indicated deteriorated neurological status and SAE.

492 Using the perivascular diffusion of IgG as a marker of BBB breakdown, no indication of
493 increased BBB permeability was found in the parenchyma of the grey and white matter,
494 except for the fimbria, after CLP in the present work. This was neither due to technical failure
495 nor lack of sensitivity as perivascular IgG was readily observed in brain circumventricular
496 organs and meninges, where the endothelial BBB is absent. Moreover, we recently showed
497 increased perivascular IgG diffusion in the white matter after ip LPS administration using the
498 same staining protocol (Dhaya et al., 2018). It is important to keep in mind however that the
499 severity of CLP-induced sepsis depends on the size of the needle used to puncture as well as
500 the number of punctures (Rittirsch et al., 2009) and that the needle size used here was smaller
501 than previous work reporting BBB breakdown 24h after CLP in rodents (Avtan et al., 2011;

502 Comim et al., 2011; Imamura et al., 2011; Jeppson et al., 1981; Vachharajani et al., 2012;
503 Yokoo et al., 2012). Given that SAE has been proposed to be related to BBB breakdown
504 (Chaudhry and Duggal, 2014; Papadopoulos et al., 2000; Tsuruta and Oda, 2016), our
505 experimental model is interesting in that it induces signs of neurological impairment in the
506 absence of widespread BBB breakdown for molecules of high molecular weight.

507 Behavioral impairment was associated with signs of fluid accumulation at the base of the
508 brain as indicated by increased T2-weighted contrast, which is in accordance with results of
509 T2-weighted imaging in a murine CLP model (Bozza et al., 2010). In addition, increased T2-
510 weighted contrast intensities were also found in the cortex and striatum in the present work.
511 As diffusion imaging did not indicate altered ADC in the cortex and striatum the increase in
512 T2 intensities were considered as indicating vasogenic rather than cytotoxic edema
513 (Loubinoux et al., 1997). Interestingly, lower perivascular expression of the water channel
514 AQP4 was observed in the cortex of CLP animals as compared to those subject to sham
515 surgery. Reduced water clearance may therefore, in part, explain the increased T2-weighted
516 contrast intensity found in the cortex after CLP.

517 Since in addition to edema, SAE has been proposed to be related to reduced CBF and oxygen
518 extraction (Papadopoulos et al., 2000), the perfusion of major brain was assessed with ASL.
519 The accuracy of ASL-FAIR to quantify brain perfusion in the rat at low, normal, and
520 moderately increased flows has been validated by a direct comparison with the cerebral
521 distribution of intravenously-injected radioactive microspheres (Bos et al., 2012). Brain
522 perfusion values in sham-operated animals were in accordance with those observed in some
523 previous studies (Detre et al., 1992; Williams et al., 1992), but higher than those described by
524 others (Danker and Duong, 2007; Shen et al., 2016), yet lower than measured in still other
525 published work (Carr et al., 2007; Moffat et al., 2005; Williams et al., 1992). These
526 discrepancies may be due to differences in anesthesia, ASL sequences used and the way the

527 brain was defined (in our case both cerebral hemispheres without the ventricular system and
528 the large vessels at the base of the brain). This being said, ASL-based perfusion data are
529 known to vary even between studies of the same group (Danker and Duong, 2007; Shen et
530 al., 2016; Sicard et al., 2003) and the aim of the present work was not to obtain absolute
531 quantitative, but to compare between CLP and sham-surgery.

532 Like in our previous work with LPS (Dhaya et al., 2018), perfusion of the global brain,
533 striatum and cortex was not found to be altered by CLP-induced sepsis. In addition, to
534 absolute perfusion values, the distribution of blood between brain structures is an important
535 value as this distribution can vary as a function of local cerebral metabolism. In this respect it
536 is interesting to note that the distribution of blood to the cortex relative to that of the
537 ipsilateral hemisphere was lower 24h after CLP as compared to sham-surgery. This relative
538 hypoperfusion of the cortex may be related to a loss of cerebral autoregulation or uncoupling
539 between CBF and cerebral metabolism (Feng et al., 2010; Rosengarten et al., 2007). The
540 reported local decrease of glucose utilization in cortical areas (Soejima et al., 1990) and
541 altered cortical electric activity (Kafa et al., 2010a) during sepsis support the possibility of
542 altered cortical metabolism. Cortical COX-2 plays an important role in neurovascular
543 coupling between neuronal activity and CBF (Niwa et al., 2000; Stefanovic et al., 2006).
544 Interestingly although increased perivascular *de novo* induction of perivascular COX-2 was
545 observed after CLP, overall cortical COX-2-immunoreactivity was decreased as compared to
546 sham-surgery. Moreover, significant positive correlations were found between the total and
547 relative surface occupied by COX-2 immunoreactivity and perfusion distribution to the
548 cortex indicating that these imaging and histological measures varied to the same extent.
549 Thus, decreased neuronal COX-2 may explain the observed decrease in blood distribution
550 towards the cerebral cortex during sepsis.

551 In accordance with and expanding our previous work on LPS, increased axial water diffusion

552 was observed in the body of corpus callosum after CLP. The body of the corpus callosum is
553 characterized by large axonal diameter distribution and a higher diffusivity suggesting that
554 the space between axons may be larger than that in the genu and splenium where axonal
555 diameter is lower (Barazany et al., 2009). The observed decrease in the coefficient of planar
556 anisotropy in the corpus callosum may also be explained by increased axial water diffusion as
557 λ_1 is part of the divider term that allows to calculate planar anisotropy ($C_p = 2(\lambda_2 - \lambda_3)/(\lambda_1 +$
558 $\lambda_2 + \lambda_3)$). Of note, septic patients that had suffered from SAE show an inverse correlation
559 between delirium duration and FA in the body of the corpus callosum three months later, but
560 not at hospital discharge (Morandi et al., 2012). The shape-oriented anisotropy measure C_p
561 has been shown to be more useful than FA to differentiate between white matter fibers and
562 abscesses, between white matter and tumors and between invasive and well-demarcated
563 tumors in the brain (Kim et al., 2008; Kumar et al., 2008; Lope-Piedrafita et al., 2008).
564 Collectively, previously-published findings together with the present observations suggest
565 that during inflammation axial diffusion increases while planar anisotropy decreases in the
566 white matter of the corpus callosum first to later give rise to decreased fractional anisotropy.
567 However, lower FA in the splenium and genu, but not in the body, of the corpus callosum at
568 hospital discharge is associated with worse verbal fluency and attention scores months later
569 (Morandi et al., 2012). Interestingly, patients with PTSD, a condition that also often occurs
570 months after sepsis (Boer et al., 2008; Rosendahl et al., 2013; Schelling et al., 2001;
571 Wintermann et al., 2015), do show decreased FA in the body of the corpus callosum when
572 compared with healthy non-trauma-exposed controls as well as a negative correlation
573 between PTSD clinical scores and the corpus callosum body FA (O'Doherty et al., 2018).
574 Thus, our present findings of reduced planar anisotropy in the corpus callosum body 24h after
575 sepsis induction may be relevant for the subsequent of development stress disorder but not for
576 that of cognitive impairment.

577 Recently, disruption of the tubulin network inside white matter axons has been shown in rats
578 made septic by intraperitoneal injection of fecal slurry (Ehler et al., 2017). If the same
579 phenomenon occurred after CLP, this could alter axial diffusion in the corpus callosum.
580 Interestingly, peripheral administration of the microtubule assembly inhibitor methylmercury
581 increases axial diffusion in rat optic nerves (Kinoshita et al., 1999) and suggests that the
582 increase in axial diffusion as observed in the corpus callosum of septic animals could be
583 related to changes in the intraneuronal tubulin network. Alternatively, retraction of the
584 processes of microglia or astrocytes that occupy the space between white matter fibers may
585 explain increased horizontal water diffusion in the corpus callosum after CLP. Indeed, in the
586 corpus callosum, microglia and astrocytes have several long cytoplasmic processes that run
587 mostly parallel, but also perpendicularly to the longitudinal axis of myelinated axons
588 (Lawson et al., 1990; Mori and Leblond, 1969a, b). In the present work, an increase in Iba-1
589 positive cell body size, a decrease in cell processes stained for Iba-1 and an increase in cell
590 body to cell size ratio were observed in the corpus callosum of CLP animals as compared to
591 that of rats that underwent sham-surgery. This increased cell body to cell size ratio has been
592 proposed as a measure of microglial activation (Hovens et al., 2014). In addition, CLP
593 decreased in the number of GFAP-positive fragments in the corpus callosum. The decrease in
594 Iba-1 positive cell processes and GFAP-immunoreactive fragments may represent retraction
595 of glial processes. This retraction would result in less hindrance for axial water diffusion in
596 the corpus callosum and thus increase it.

597 When white matter astrocytes are activated, they retract their higher order processes, increase
598 their soma and lose their transverse orientation (Sun et al., 2010). Less GFAP-
599 immunoreactive fragments were found in the corpus callosum after CLP than after sham-
600 surgery. Interestingly, significant negative correlations were found between the number of as
601 well as the relative surface occupied by GFAP-positive fragments and axial water diffusion in

602 the corpus callosum suggesting that changes in this astrocytic marker underlie, at least in part,
603 the observed modifications of dMRI parameters. Although GFAP does not mark astrocytic
604 endfeet (Sun and Jakobs, 2012), this finding can be interpreted to suggest astroglial activation
605 and retraction of their processes, which, like for microglia, allows for increased diffusion of
606 water molecules parallel to corpus callosum fibers.

607 One of the main differences between the present small animal imaging approach and previous
608 clinical imaging studies on sepsis is the use of anesthesia to prevent movement at the level of
609 the brain. While the effects of isoflurane anesthesia on rodent regional cerebral blood flow is
610 relatively well-known (Sicard et al., 2003), to the best of our knowledge no comparison of
611 white matter diffusion MRI parameters between isoflurane anesthetized and awake rodents
612 has been done to date. Future work will need to address this matter and determine to what
613 extent the present findings are translationally relevant. If the effects of anesthesia turn out to
614 be negligible, then longitudinal MRI studies of experimental SAE can be envisioned.

615 In conclusion, our findings confirm the existence of brain dysfunction and increased T2-
616 weighted contrast at the base of the brain 24 h after CLP in rodents. In addition, they expand
617 previous findings in showing increased T2-weighted contrast in the striatum and cortex,
618 decreased perfusion distribution to the cortex and increased axial water diffusion in the
619 corpus callosum. Importantly, these imaging findings were accompanied by lower numbers
620 of Iba-1- and GFAP-immunoreactive fragments in the corpus callosum, decreased neuronal
621 COX-2 and AQP4 expression in the cortex, but not by widespread perivascular IgG diffusion
622 indicating breakdown of the BBB. Collectively, these findings indicate that early SAE is
623 related to changes in cerebral blood flow and white matter microstructure, but not to BBB
624 breakdown.

625

626 **Acknowledgements**

627 This work was supported by public grants from the French Agence Nationale de la Recherche
628 within the context of the Investments for the Future program referenced ANR-10-LABX-57
629 named TRAIL (project HR-dMRI).

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894 **Figure and table legends**

895

896 *Figure 1*

897 Placement of different Region Of Interest 1: hemisphere; 2: corpus callosum; 3 external
898 capsule; 4 cortex and 5: striatum. (ROIs; A) used to analyze perfusion-weighted (example in
899 B), T2-weighted (example in C) and diffusion-weighted images (example in D).

900

901 *Figure 2*

902 Food intake relative to body weight (A) during 24h after CLP or sham surgery. Group size
903 Sham: n=9; CLP: n=11-12. Reflex scores: Pinna (B), corneal (C) and righting (D) reflexes at
904 24h, 12h, and 1h prior to surgery then 4h, 8h and 24h later. Group size Sham: n=8-9; CLP:
905 n=8-12.

906

907 *Figure 3*

908 Changes in T2-weighted intensity at the base of the brain (A), in the cortex (B), striatum (C)
909 and the corpus callosum (D). Graphs represent maximum intensities in the VOI relative to
910 that of the muscle 24h after laparotomy or cecal ligation and puncture. *: $p < 0.05$. Group
911 sizes Sham: n=8; CLP: n=11.

912

913 *Figure 4*

914 Perfusion distribution to rat cortex (A), striatum (B), corpus callosum (C) and external
915 capsule (D). Graphs represent perfusion relative to both hemispheres for all structures except
916 for the corpus callosum that was expressed relative to global brain 24h after surgery. *: $p <$
917 0.05 ; Group sizes Sham: n=8; CLP: n=12.

918

919 *Figure 5*

920 Changes in corpus callosum axial water diffusion at bregma -0.51mm (A) and left external
921 capsule fractional anisotropy the at bregma -1.08mm (B) 24h after laparotomy or cecal
922 ligation and puncture. *: $p < 0.05$. Group sizes Sham: n=7-8; CLP: n=12.

923

924 *Figure 6*

925 Photomicrographs illustrating the distribution of rat IgG-immunoreactivity in ventral
926 hippocampal commissure (A,B), the corpus callosum (C,D), external capsule (E,F),
927 dorsolateral striatum (G,H) and hippocampus (I,J), 24 h after laparotomy (A, C, E, G, I) or
928 cecal ligation and puncture (B, D, F, H, J) in rats. cc: corpus callosum; ec: external capsule;
929 vhc: ventral hippocampal comissure; SFO: subfornical organ. Arrow heads > and < indicate
930 perivascular diffuse cloud-like labeling Scale bar = 100 μ m.

931

932 *Figure 7*

933 Photomicrographs illustrating the distribution of COX-2-immunoreactivity in the
934 ventromedial preoptic area (A, B), caudate putamen (C, D), external capsule (E, F),
935 hippocampus (G, H) and cortex (I, J) 24h after laparotomy (A, C, E, G, I) or cecal ligation
936 and puncture in rats (B, D, F, H, J). och: optic chiasm; ec: external capsule. Arrow heads >
937 and < indicate labeling. Scale bar = 100 μ m. Quantification of cortical COX-2-
938 immunoreactive cells (K) and surface (L) 24h after cecal ligation and puncture or laparotomy.
939 i.r.: immunoreactivity *: $p < 0.05$. Group sizes Sham: n=6; CLP: n=7.

940

941 *Figure 8*

942 Photomicrographs illustrating Iba1-ir microglia in the corpus callosum after laparotomy (A)
943 or cecal ligation and puncture (B) in rats. Quantitative Fraclac-based analysis of microglial

944 height (C) and width (D) in the corpus callosum. i.r.: immunoreactivity *: $p < 0.05$. Group
945 sizes Sham: $n=9$; CLP: $n=11$. Scale bar = $100\mu\text{m}$.

946

947 *Figure 9*

948 Photomicrographs illustrating GFAP-ir astrocytes in the corpus callosum after laparotomy
949 (A) or cecal ligation and puncture (B) in rats. Quantitative analysis of astrocyte height
950 (Fraclac; C) and the relative surface occupied by GFAP-ir fragments (D) in the corpus
951 callosum. i.r.: immunoreactivity ***: $p < 0.001$. Group sizes Sham: $n=9$; CLP: $n=11$. Scale
952 bar = $100\mu\text{m}$.

953

954 *Figure 10*

955 Photomicrographs illustrating AQP4-ir astrocytes end-feet in the cortex after laparotomy (A)
956 or cecal ligation and puncture (B) in rats. Quantitative analysis of counts (C) and the relative
957 surface occupied (D) by AQP4-ir fragments in the cortex. i.r.: immunoreactivity ***: $p <$
958 0.001 . Group sizes and the surface occupied by AQP-4. Sham: $n=7$; CLP: $n=9$. Scale bar =
959 $50\mu\text{m}$.

960

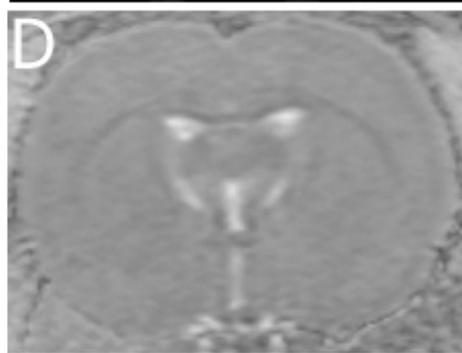
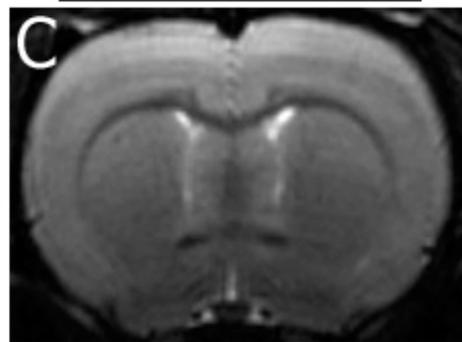
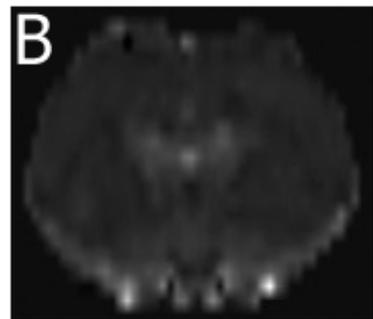
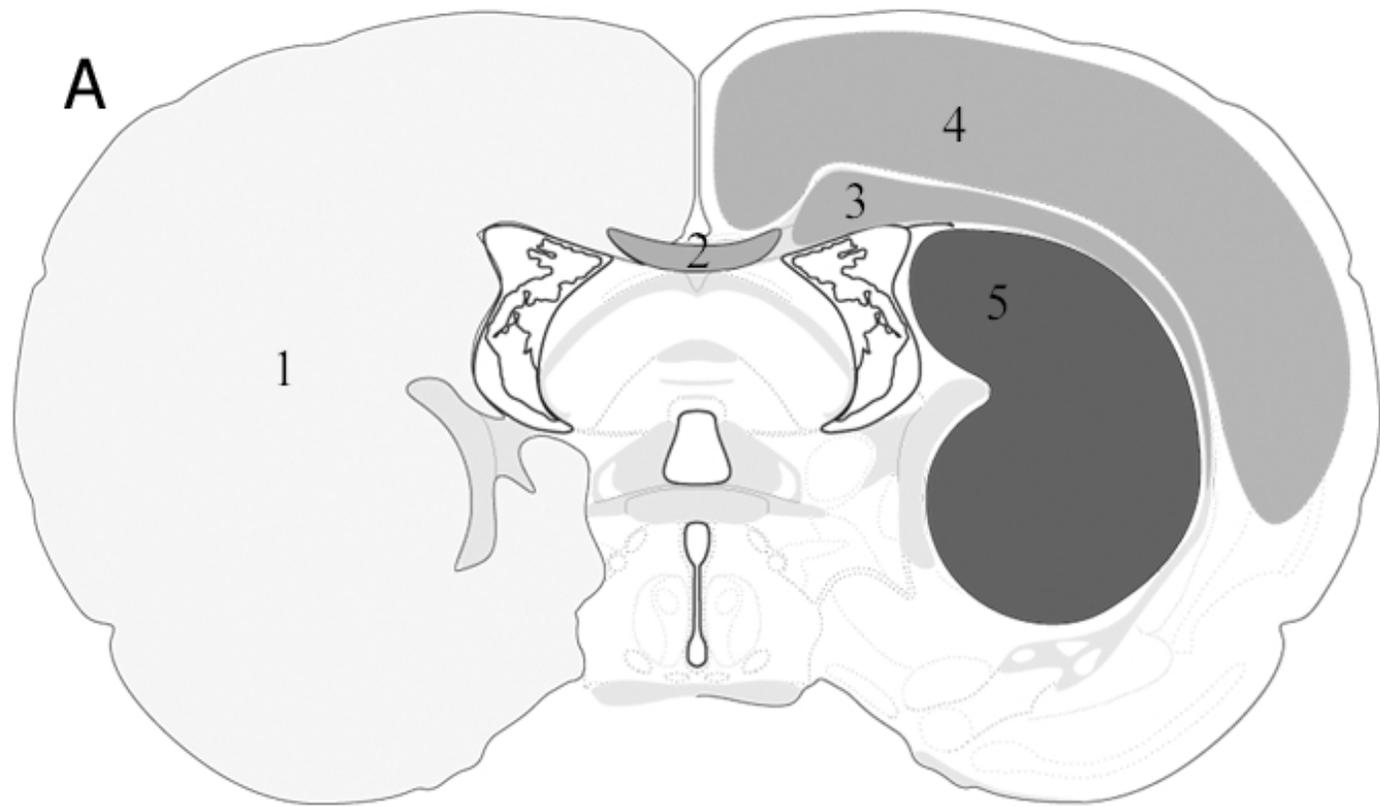
961 *Supplementary figure 1*

962 Location of differences in fractional anisotropy indicated by arrows as found in the left
963 striatum using Voxel-based analysis using tract-based spatial statistics (TBSS) with 5000
964 permutations in the horizontal (A), sagittal (B) and coronal plane (C).

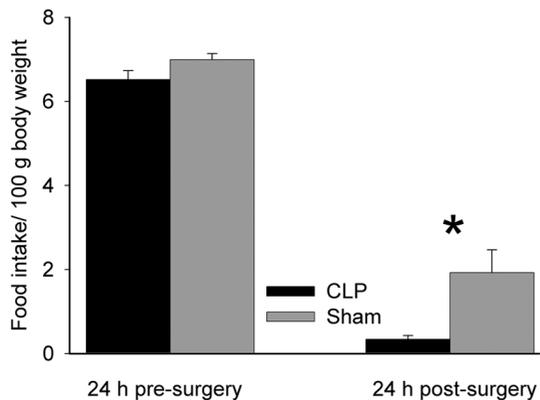
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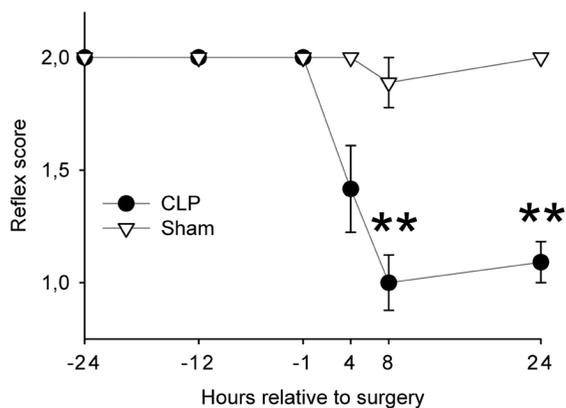
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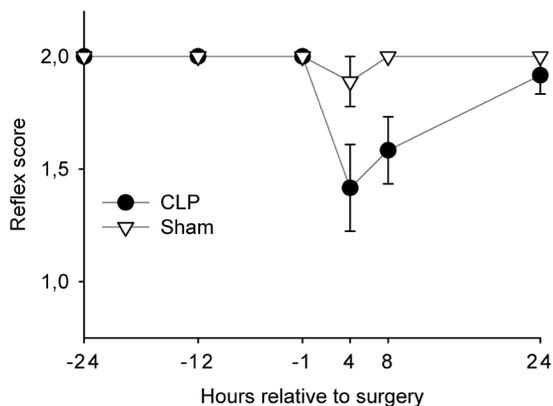
A Food intake per 100 g body weight



B Righting reflex (complex postural somatomotor function)



C Pinna reflex (simple non-postural somatomotor function)



D Corneal reflex (simple non-postural somatomotor function)

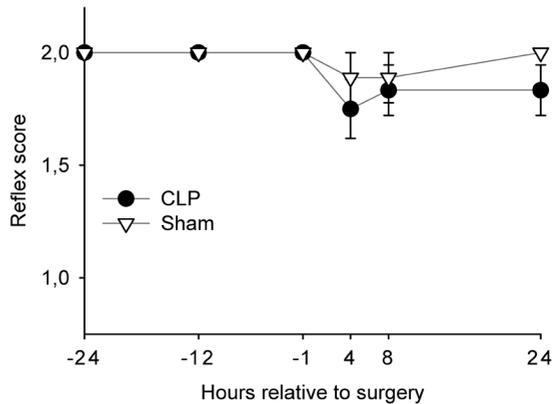


Fig. 2, Griton et al.

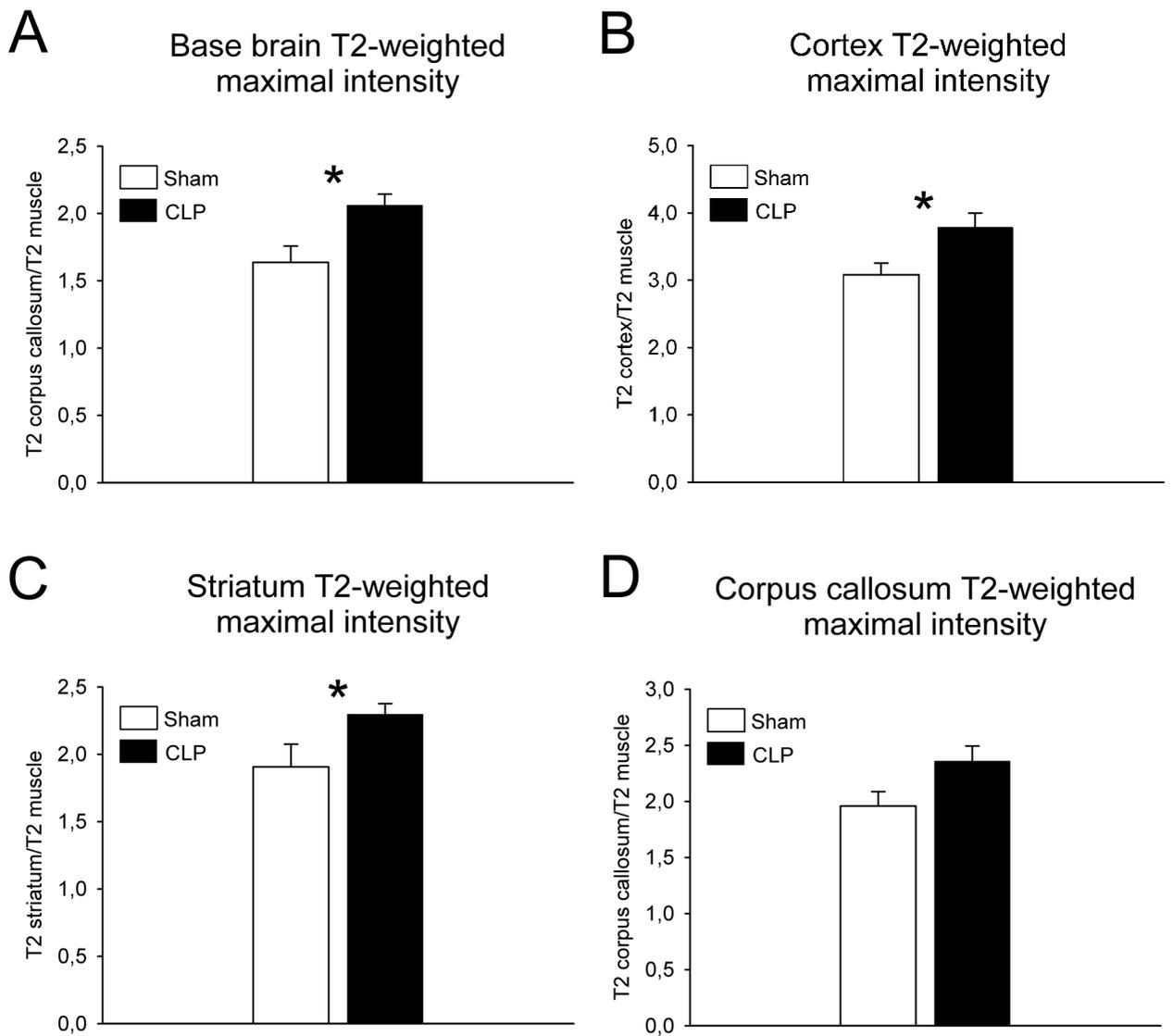
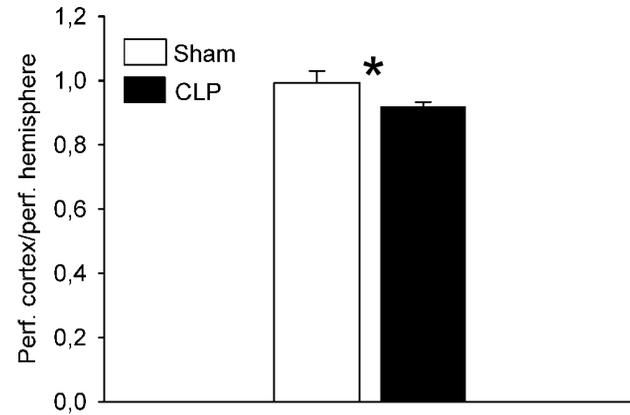
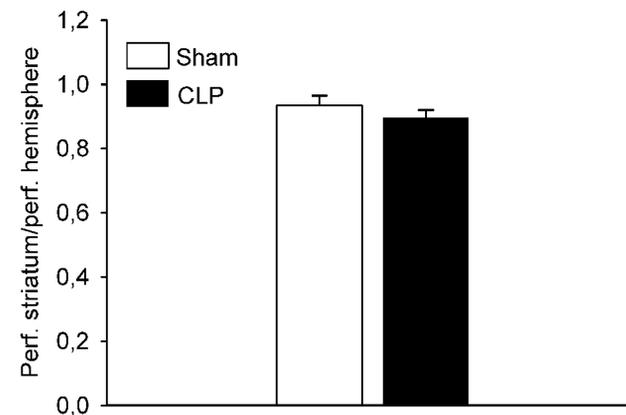


Fig. 3, Griton et al.

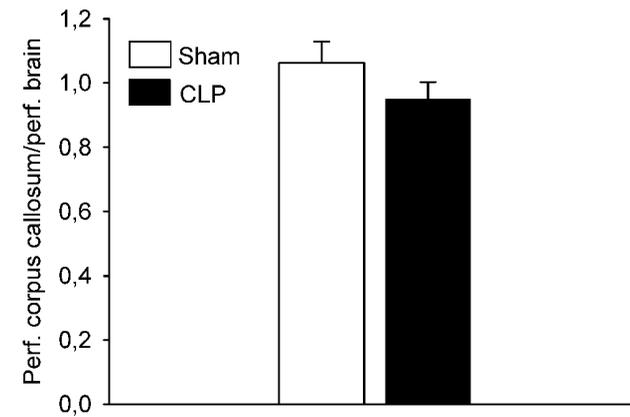
A Perfusion distribution to cortex



B Perfusion distribution to striatum



C Perfusion distribution to corpus callosum



D Perfusion distribution to external capsule

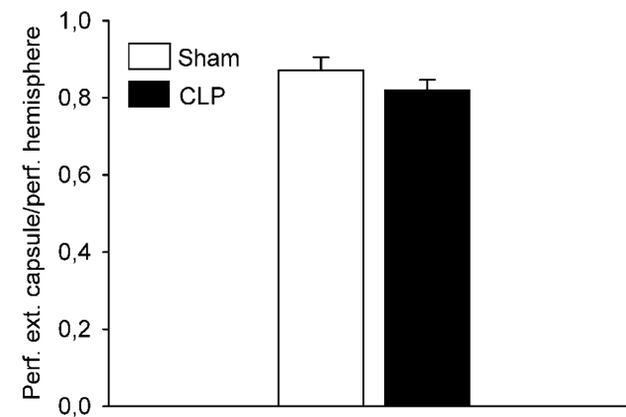
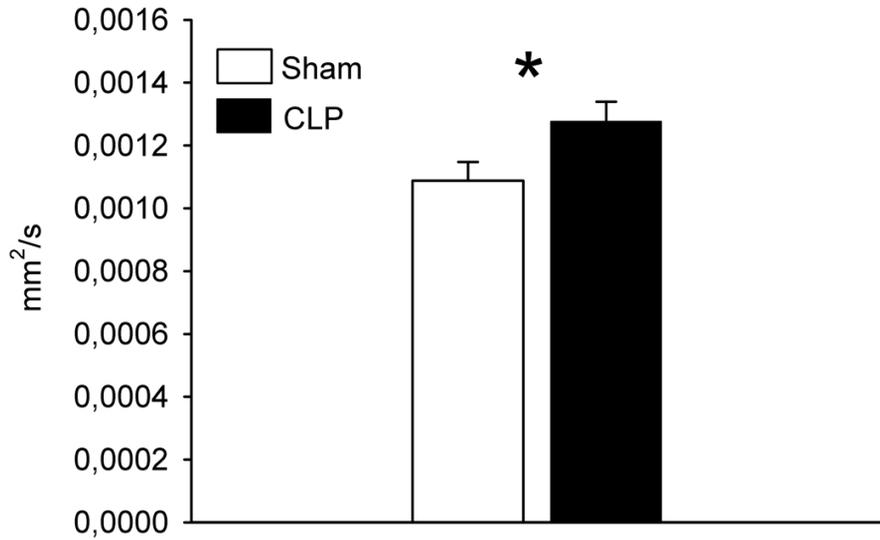


Fig. 4, Griton et al.

A Corpus callosum axial diffusion



B Left external capsule fractional anisotropy

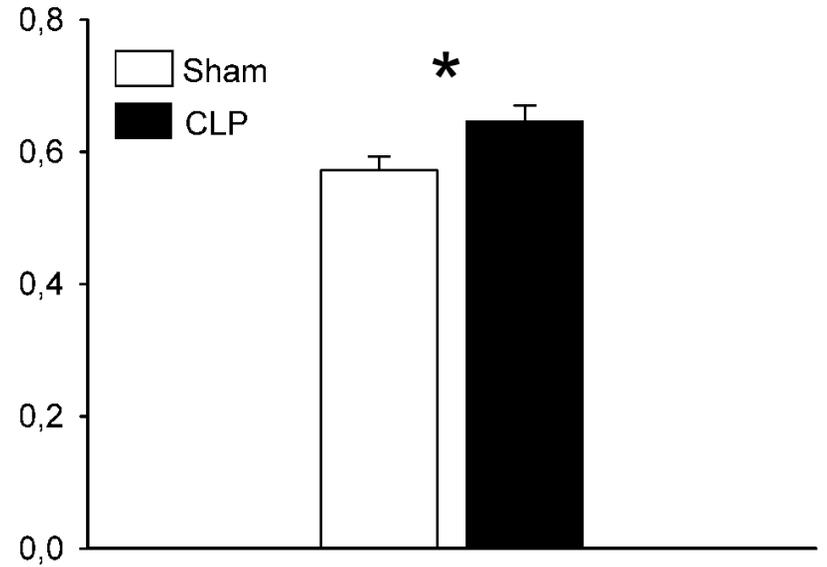
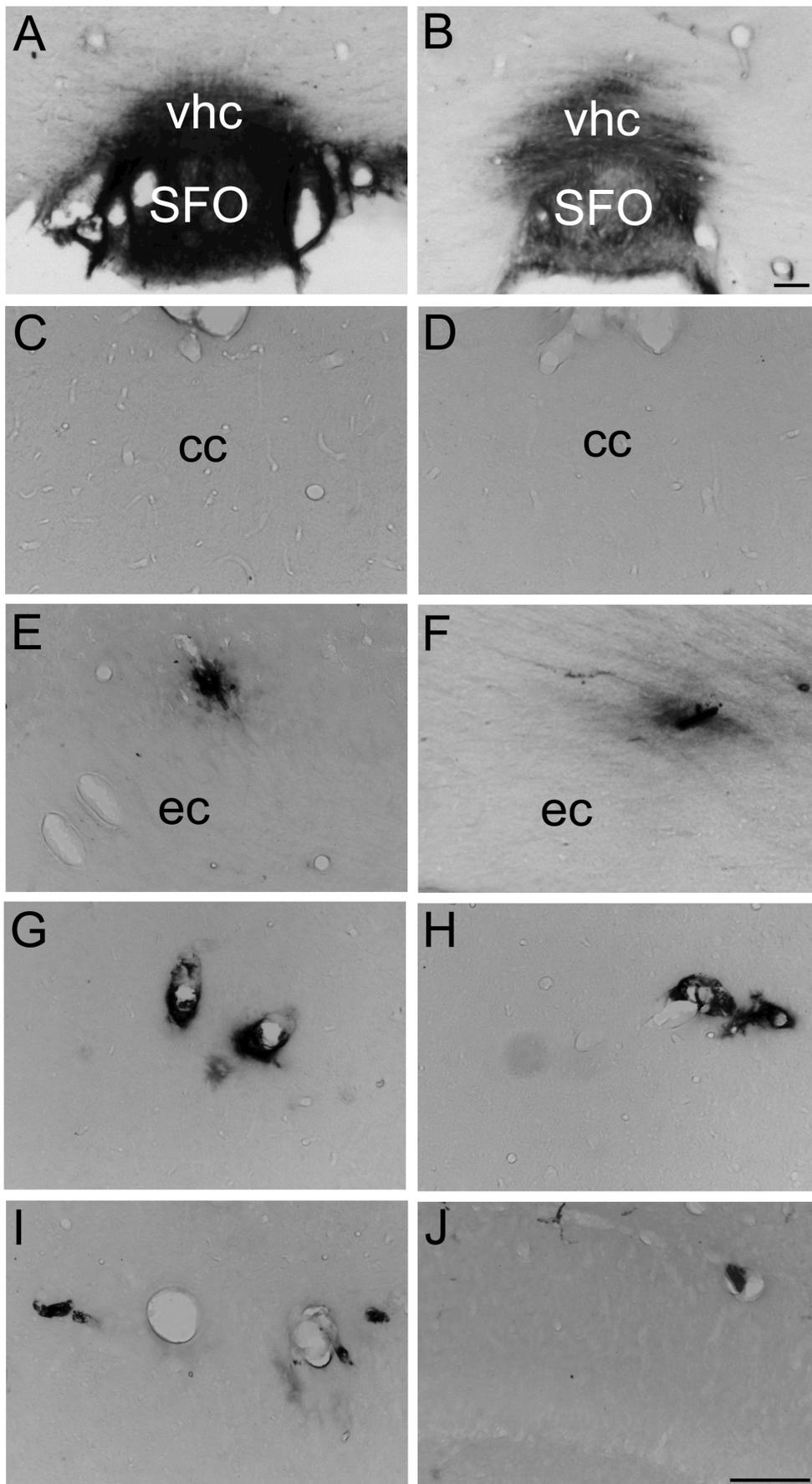
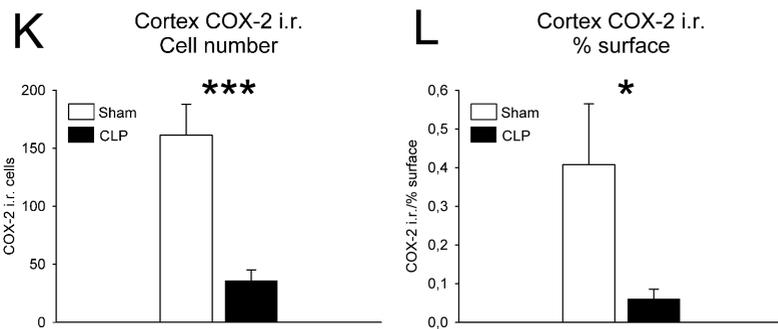
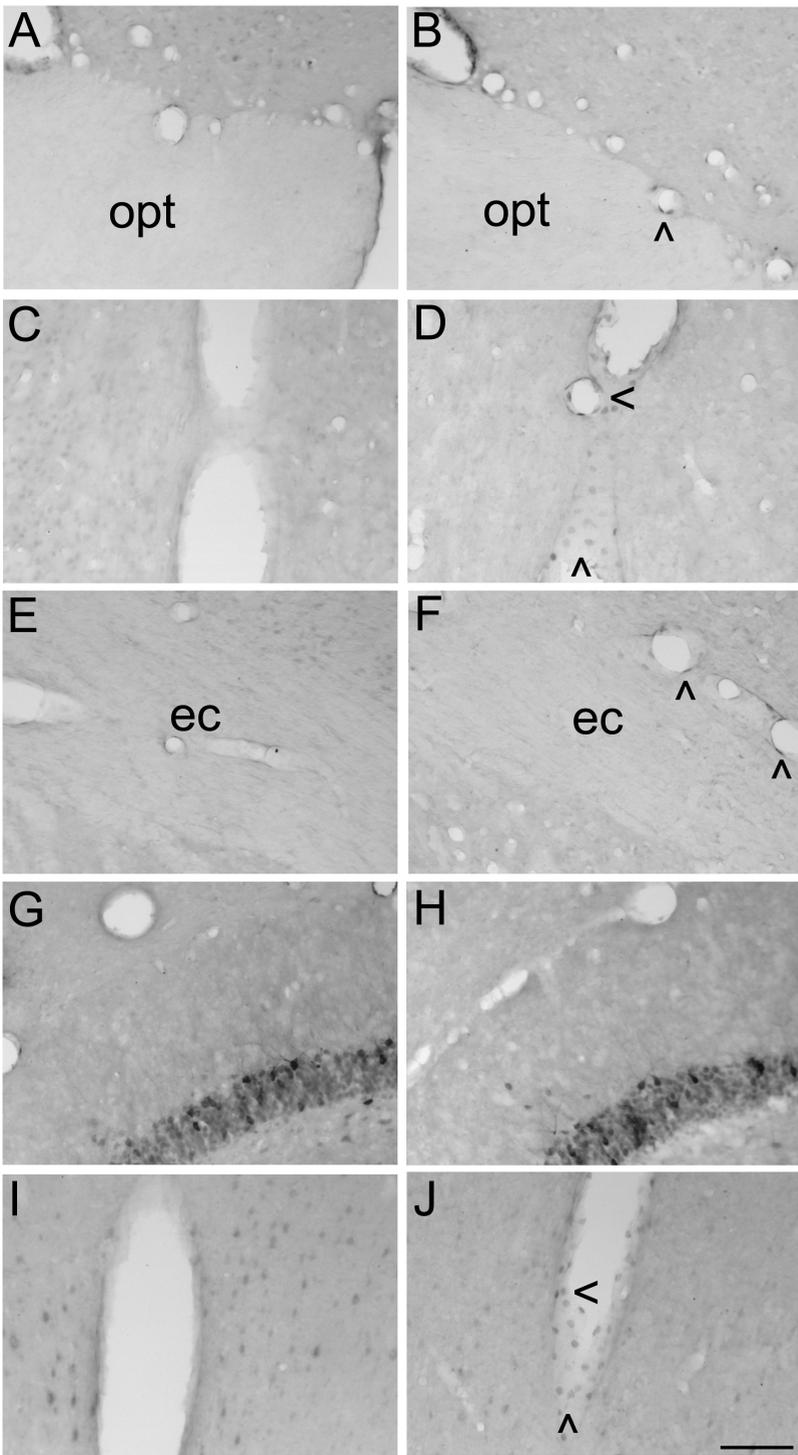


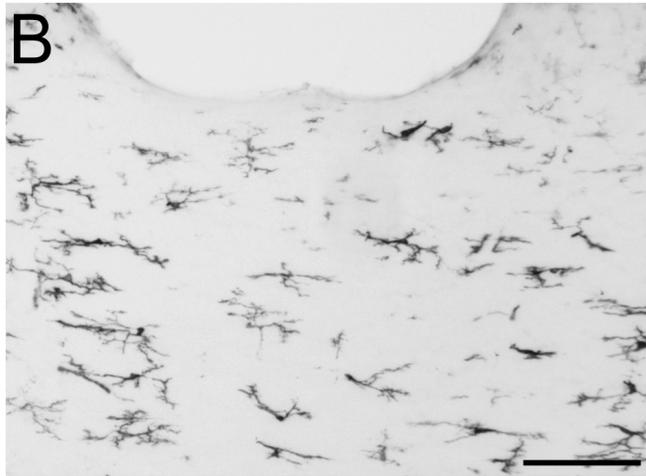
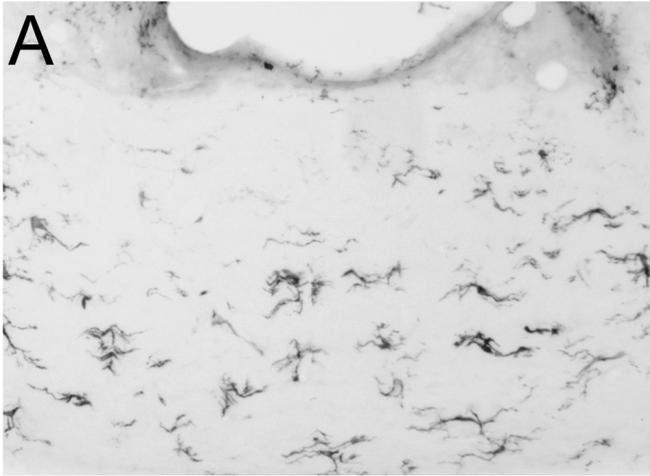
Fig. 5, Griton et al.



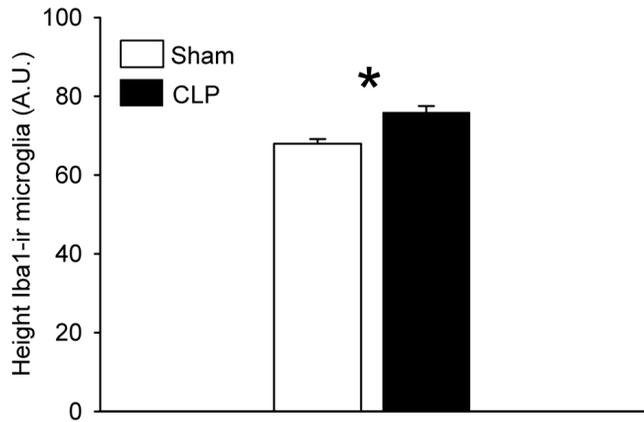
Griton et al., Fig. 6



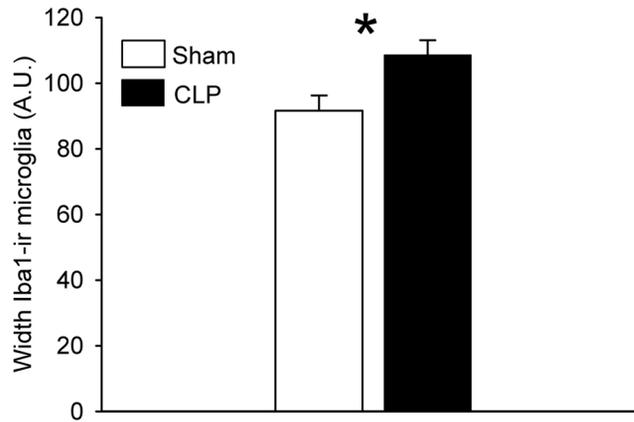
Griton et al., Fig. 7

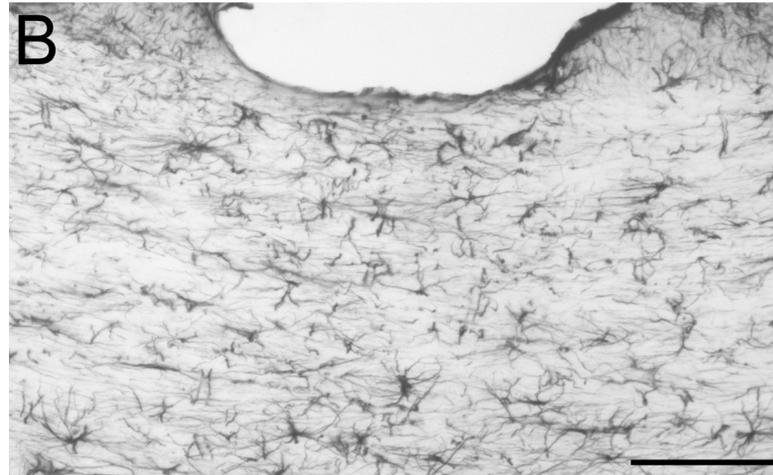
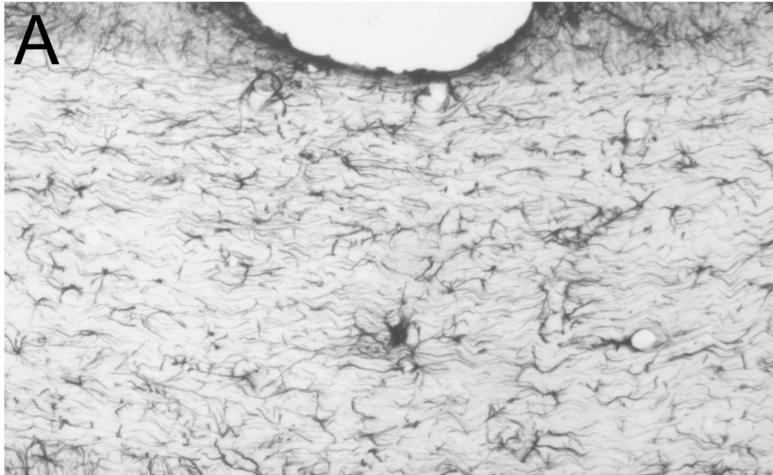


C Corpus callosum Iba1-ir microglia: height

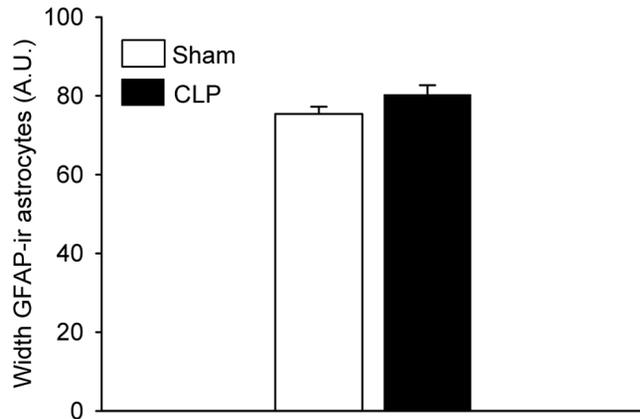


D Corpus callosum Iba1-ir microglia: width

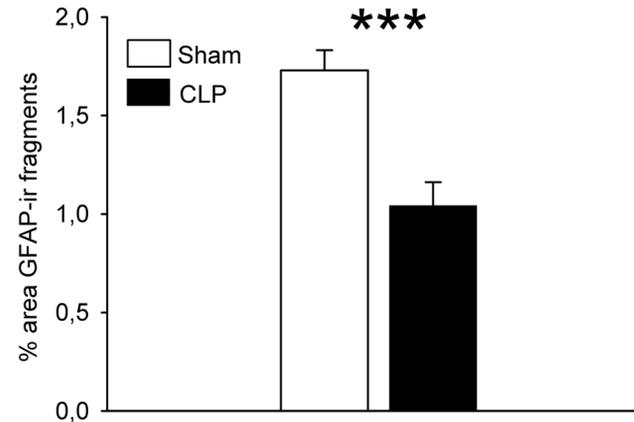


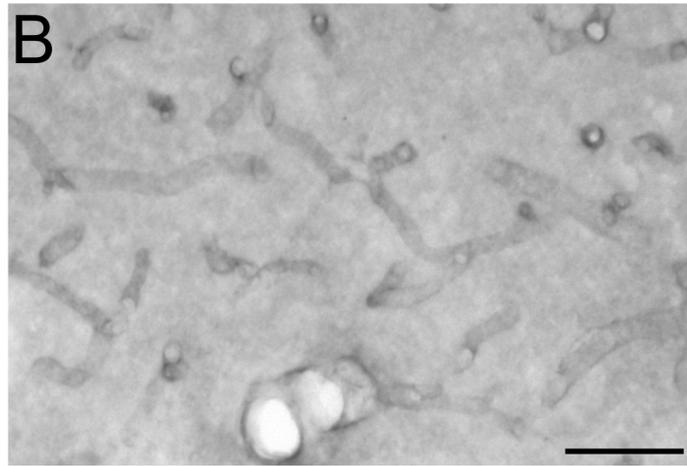
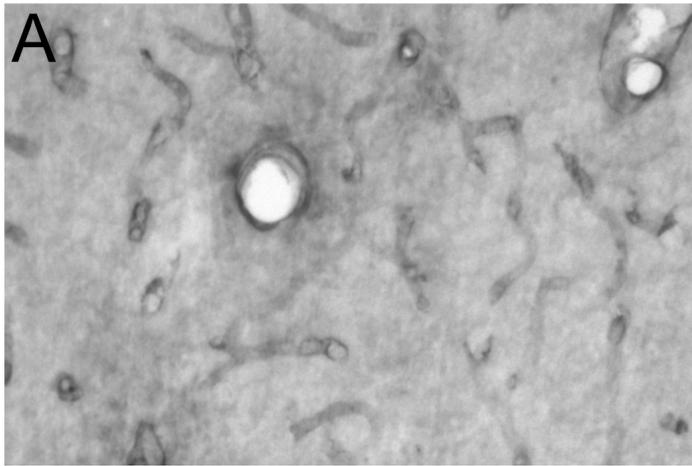


C Corpus callosum GFAP-ir astrocytes:
height

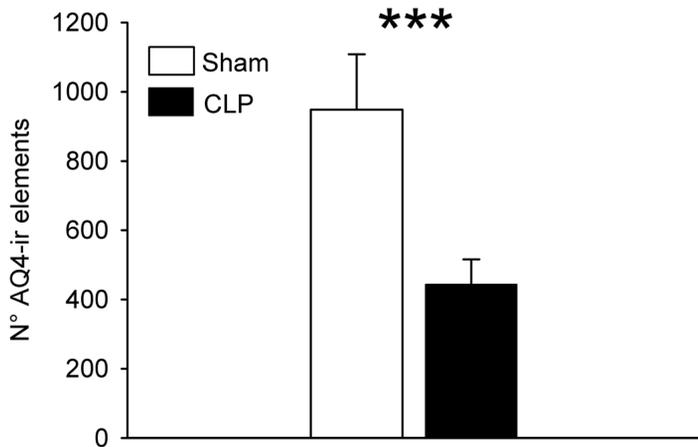


D Corpus callosum GFAP-ir fragments:
% area

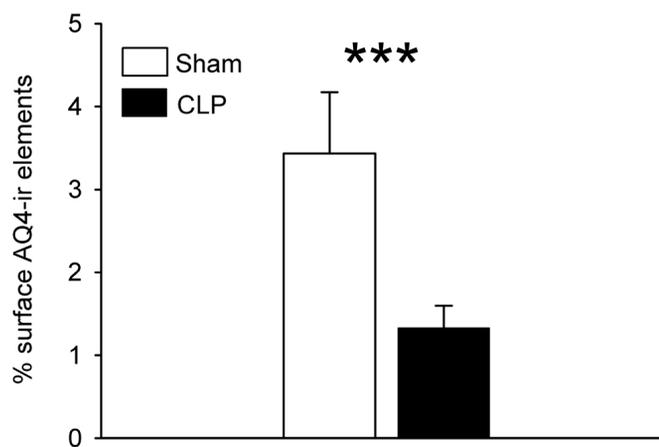




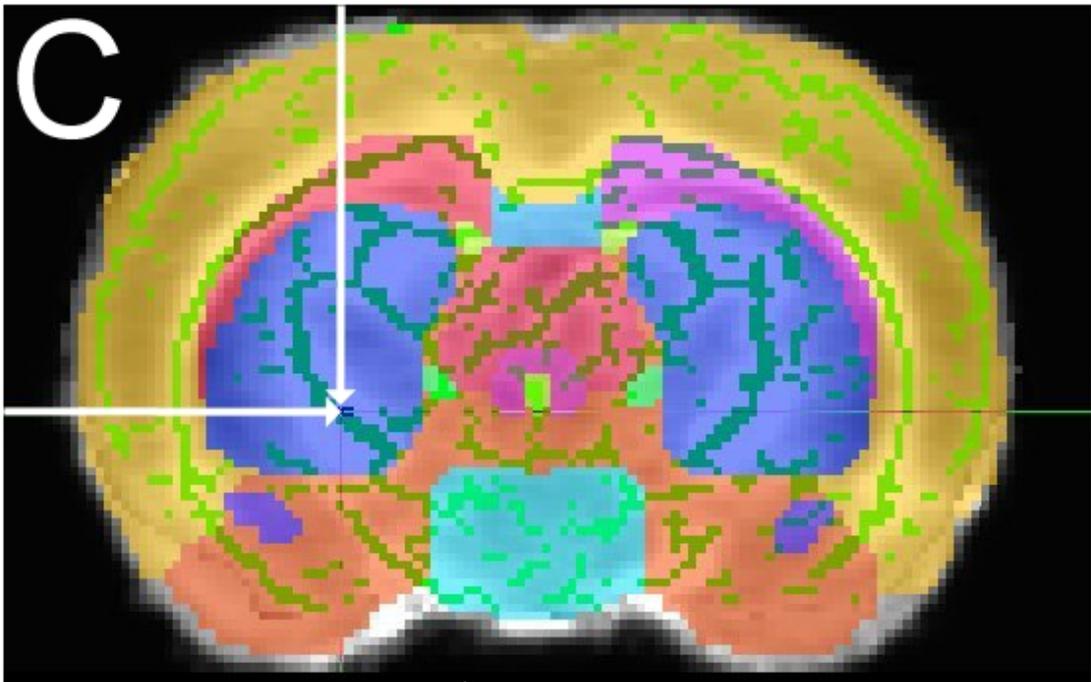
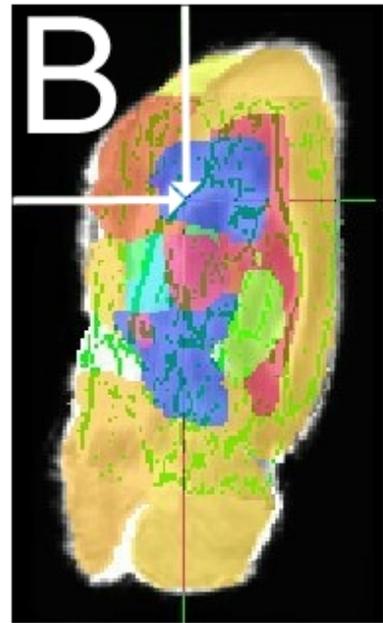
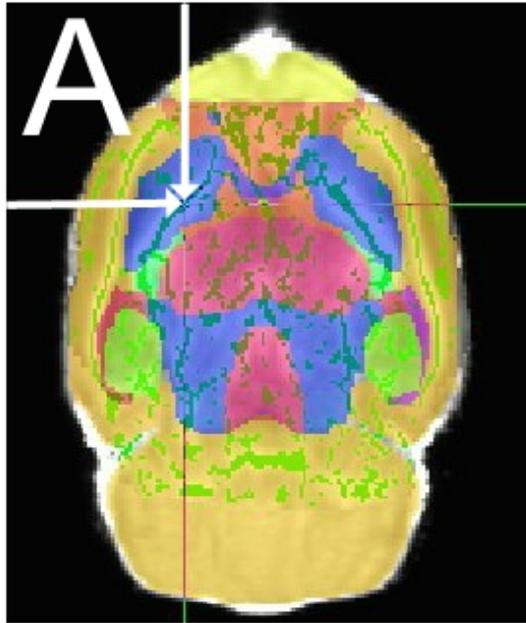
C Cortex Aquaporin-4



D Cortex Aquaporin-4



Griton et al., Fig. 10



Griton et al.,
Suppl. Fig. 1

Supplementary figure 1

Location of differences in fractional anisotropy indicated by arrows as found in the left striatum using Voxel-based analysis using tract-based spatial statistics (TBSS) with 5000 permutations in the horizontal (A), sagittal (B) and coronal plane (C).

Supplementary materials and methods

Magnetic Resonance Imaging

- T1-weighted MRI

T1-weighted images were obtained with a FLASH (Fast Low Angle Shot) pulse sequence with the following parameters: repetition time [TR]/echo time [TE]: 15/2.5ms; flip angle: 30°; voxel size: 117×117×234 μm^3 ; Field of View (FOV): 30×30×30mm³; acquisition time: 6 min.

- T2-weighted MRI

T2-weighted images were acquired using a Rapid Acquisition with Relaxation Enhancement (RARE) sequence with the following parameters: repetition time: 3.64s, echo time: 70ms, flip angle: 90°, RARE factor: 16, number adjacent (non-contiguous) axial slices: 20, slice thickness: 0.7mm, image matrix size: 196×196 pixels, FOV: 25×25mm² and in plan spatial resolution of 128 μm , 8 averages were acquired giving a total acquisition time of 4min51s.

- Arterial Spin Labeling (ASL)

Arterial Spin Labeling data were acquired from a single 1.5 mm-thick axial slice, with a FOV of 35×35 mm² using a Flow-sensitive Alternating Inversion Recovery–Echo Planar Imaging) pulse sequence (FAIR-EPI) (Kim and Tsekos, 1997). The acquisition was performed twice, first with selective inversion of the slice and then with a global inversion recovery technique (Kober et al., 2004). For each of these acquisitions, and based on previous publications (Carr et al., 2007), the following parameters were used: TR/TE 16000/15ms; flip angle: 90°; number of averages: 6; inversion times: 0.2, 0.6, 0.8, 0.9, 1.0, 1.1, 1.2, 1.5, 2.0, and 2.5s; acquisition duration: 14min25s.

- Diffusion-weighted MRI (dMRI)

A Stejskal-Tanner Echo Planar Imaging (EPI) pulses sequence was used to collect diffusion-

weighted images (Stejskal and Tanner, 1965). A 3D multi-shot EPI dMRI pulse sequence was used to increase the sensitivity of diffusion-weighted MRI (Crombe et al., 2018; Tounekti et al., 2018). Twenty-one diffusion-weighted images were acquired with b-value =1000s/mm² applied along 21 non-collinear diffusion encoding directions (B1000 images) with a Stejskal-Tanner diffusion-sensitized acquisition scheme (Stejskal and Tanner, 1965). One other DW image was acquired with a b-value of 0s/mm² (B0 image). DW-images were recorded with the following parameters: TR/TE=1200/36.86ms; voxel size: 153×148×333μm³; FOV: 30×22×16mm³; flip angle: 90°; acquisition duration: 22min4 s.

References

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