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Alginate Attenuates Reactive Microglia *In Vivo*

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GENERAL INFORMATION

- The present dataset aimed to evaluate effects of the marine polysaccharide alginate (ALG) on immune cells *in vivo* in 2 animal models:
  - ✓ ibotenic acid (IBO)-induced brain lesion model [IBO-lesioned rats]
  - ✓ pilocarpine model of mesial temporal lobe epilepsy [epileptic lesioned rats]
- For experiments on IBO-lesioned (non-epileptic) rats, 1 week after animals' arrival, rats underwent IBO intracerebral injection through 26s-gauge needles inserted bilaterally to reach the targeted area of the ventral CA3. The second injection of saline (SAL) or alginate (ALG), when needed, was performed 4 days later. Sham animals underwent the same surgical procedure, with infusion needles simply being inserted and removed after 5 min. Naïve rats did not receive any procedure.
  Experimental groups: IBO, IBO-ALG, IBO-SAL, naïve, sham
- For experiments on epileptic lesioned rats, 1 week after the animals' arrival, rats were injected with pilocarpine to induce *status epilepticus* (SE). After two weeks of recovery, rats underwent stereotaxic surgery for bilateral cannula implantation to perform 10-14 days later multiple injections of IBO followed by alginate (ALG), neural stem cells (NSCs), or ALG+NSCs. Control group did not receive any injection (SE)
  Experimental groups: IBO, IBO-ALG, IBO-ALG-NSC, IBO-NSC, SE
- Rats were intracardially perfused and brains were extracted. Brains were fixed with 4% PFA via transcardial perfusion. They were then kept in PFA for 24 hours, followed by a few days in a 30% sucrose solution. Finally, they were quickly frozen with dry ice and stored at -80 °C.
- Slices were cut using a cryostat at a thickness of 30 µm and stored in an anti-freeze solution at -20 °C.
- Immunofluorescent and immunohistochemical stainings were performed on free-floating sections. Histological stainings (Hematoxylin&Eosin and Toluidine blue) were conducted on sections previously mounted onto glass slides.
- Slices were captured using the ECLIPSE Ci Microscope (Nikon Corporation, Japan; 4x, 10x, 20x images), or EVOS M5000 (Thermo Fisher Scientific, USA; 100x images), and analysed offline with NIS-elements BR Software (Nikon Corporation, Japan).
- The filters used in fluorescence were: FITC [488 nm], TRITC [547 nm] and DAPI [350 nm] channels.
- Images of Hematoxylin & Eosin (HE) and Toluidine Blue (TB) were used to visualize and analyze lesion induced by injection of ibotenic acid (IBO).
- Immunofluorescence (IF) and Immunohistochemistry (IHC) were used to visualize and analyze astrocytosis (GFAP mean intensity and glial scar thickness) or microglial cells states (Iba1 mean intensity, microglial cells density and cell body size, surveillant cells count over total microglial cells).

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DATASET FORMAT

The experiments folders are compressed in *.zip format.
Below summarized the file formats and the software with which they can be opened:

File format	Required software
.nd2 **.jp2** **.tif**	ImageJ/Fiji, NIS-elements BR Software (Nikon Corporation, Japan), and other major imaging software (e.g. Adobe Photoshop)

DATASET STRUCTURE

- Main folders [staining type_model]:
  - GFAP IF_epileptic lesioned rats
    Groups: IBO
    IBO-ALG
    IBO-NSC
    IBO-ALG-NSC
  - GFAP IF_IBO-lesioned rats
    Groups: NAÏVE
    SHAM
    IBO
    IBO-SAL
    IBO-ALG
  - Iba1 IHC_epileptic lesioned rats
    Groups: SE
    IBO
    IBO-ALG
    IBO-NSC
    IBO-ALG-NSC
  - Iba1 IF_IBO-lesioned rats
    Groups: NAÏVE
    SHAM
    IBO
    IBO-SAL
    IBO-ALG
  - HE_IBO-lesioned rats
    Groups: NAÏVE
    SHAM
    IBO 0.3 µl

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IBO 2 µl
IBO-SAL
IBO-ALG

- TB_IBO-lesioned rats
  Groups: SHAM
  IBO 2 µl

FILE NAMING STRUCTURE

Rat ID [X]_staining/target [XXXXX]_magnification [XXx]_hemisphere [SX/DX]_slice number [slice XX]_quadrant [QX]_slide date [YYYYMMDD]

PLEASE NOTE: only the information necessary to distinguish slices is included in the file name, depending on the immunoreaction.

- e.g.: Rat 1_GFAP lesion_10x_SX_20230727.jp2: Slice of rat 1, GFAP staining at the lesion site, 10x magnification, left hemisphere, slide dated July 27th 2023 (slide date for identification purposes).
- e.g.: Rat 3_GFAP lesion_10x_SX.jp2: Slice of rat 1, GFAP staining at the lesion site, 10x magnification, left hemisphere (no slide date).
- e.g.: Rat 2_Iba1_40x_DX_slice 2_Q1.tif: Slice of rat 2, Iba1 staining, 40x magnification, right hemisphere, slice number 2, quadrant 1.

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