Supplementary information

Maternal immune activation facilitates epilepsy in the offspring

Eduardo Pineda, Don Shin, Su Jeon You, Stephane Auvin, Raman Sankar, Andrey Mazarati

Selection of doses and optimization of treatment regimens (Table 1S). Starting point for dose selection was based on prior publications[1-3]. When optimizing doses for our experiments, we considered survival and the extent of behavioral changes in the offspring. Additionally, the preference was given to a lower dose of recombinant cytokine or antibody, if there were no differences in effects between higher and lower doses. Finally, levels of cytokine levels observed in plasma after recombinant cytokine administration as compared with those produced by PIC, were also considered (see Fig. 1S). Table 1S shows various treatment regimens used; those selected for final analyses are in bold. Since IL-1β itself had no effects on both seizures and behavior, the selected doses of rIL-1β and IL-1β antibodies were congruent with those for rIL-6 and IL-6 antibodies respectively. Furthermore, considering the need to stay within the age of interest, and high volume of the studies, not all survivors were used for final studies; instead, animals were picked randomly from larger groups. In some cases, larger sample sizes were used at the beginning of the study; however, some animals were eliminated due to the loss of electrode implants and/or general health impairments related to surgery.

In addition, in order to verify that recombinant cytokines did not show bell-shaped dose response effects, rIL-6 and rIL-1β were adminisetered separately at 10 μg/kg (i.e. at the dose chosen for their combined administration), and the offspring underwent the sociability test followed by the measurement of baseline affterdischarge properties. Table 1S shows that the effect of rIL-6 at 10 μg/kg on behavior was similar to the one observed after rIL-6 injected at a dose 20 μg/kg, or rIL-6+rIL-1β given at 10 μg/kg each (compare with data on Fig. 2B). rIL-1β (10 μg/kg) did not modify animals' behavior. After rIL-6, afterdishcarge threshold was 536±3.2 μA

and afterdischarge duration- 11.8±1.3 s (n=7, p>0.05 vs. Saline). After rIL-1β, afterdischarge threshold was 566±2.4 μA, and afterdisahcrge duration – 12.3±2.2 s (n=6; p>0.05 vs. Saline)

Table 1S. Pilot experiments - dose selection.

Treatment, dose	Number of pregnant mice	Number of offspring mice survived at P7	Sociability index (n = number of mice tested)
Saline	**3**	**17**	**29.1±3.5 (n=10)**
PIC, 5 mg/kg E12	3	3	Not tested
PIC, 5 mg/kg E16	4	1	Not tested
PIC, 5 mg/kg E12-E16	3	0	Not tested
PIC, 1 mg/kg E12-E16	3	14	33.9±4.5 (n= 7; p>0.05 vs. Saline)
PIC, 2.5 mg/kg E12-E16	**3**	**15**	**6.3±4.1** **(n=10; p<0.05 vs. Saline)**
PIC 2.5 mg/kg + IL-6 AB, 50 μg/kg	2	9	8.9±3.7 (n=9, p>0.05 vs. PIC 2.5 mg/kg)
PIC 2.5 mg/kg + IL-6 AB, 250 μg/kg	**3**	**16**	**30.7±5.1** **(n=8; p<0.05 vs. PIC; p>0.05 vs. Saline)**
PIC 2.5 mg/kg + IL-6 AB, 1 mg/kg	1	5	27.1±3.1 (n=5; p>0.05 vs. PIC+IL-6 AB at 250 μg/kg)
rIL-6, 2.0 μg/kg	1	7	31.3±5.6 (n=7; p>0.05 vs. Saline)
rIL-6, 5.0 μg/kg	2	13	17.4±4.2 (n=8; p<0.05 vs. Saline)
rIL-6, 10 μg/kg	2	11	3.1±2.2 (n=7; p<0.05 vs. Saline and vs. rIL-6, 5 μg/kg)
rIL-6, 20 μg/kg	**3**	**13**	**-2.3±3.3** **(n=9; p<0.05 vs. Saline)**
rIL-6, 50 μg/kg	1	5	-1.28±7.2

			(n=5; p>0.05 vs. rIL-6, 20 μg/kg)
rIL-6, 100 μg/kg	1	0	Not tested
rIL-1β, 10 μg/kg	2	12	33.9±5.2 (n=6; p>0.05 vs. Saline)
rIL-1β, 20 μg/kg	**3**	**16**	**28.8±4.2** **(n=7; p>0.05 vs. Saline)**
rIL-1β, 100 μg/kg	1	3	Not tested

Core temperature and serum cytokine assay. Separate female adult mice were injected with saline (n=6), PIC (n=6) or rIL-6+rIL-1β (n=4) as described above. Core temperature was measured daily 30 and 60 min after injections, as well as 24 hrs after the last injection. After the last temperature measurement, whole blood (400 μl) was collected via cardiac puncture. Plasma levels of IL-1β and IL-6 were measured using enzyme linked immunoabsorbent assay kits (IL-6: KMC0061; IL-β: KMC0011C; Life Technologies, Grand Island, NY). The described studies were performed in separate mice (i.e. not used to generate the offspring for further experiments), as measuring core temperature disrupts pregnancy, and the amount of blood needed for the assay requires exsanguination.

Repeated injections of PIC resulted in a significant increase of both IL-6 and IL-1β levels in plasma. Twenty four hours after the last injection of recombinant cytokines, they were detected in plasma at levels compatible with those observed after the PIC administration (Fig. 1S). Neither PIC, nor cytokine treatment affected core temperature as compared with saline administration (Saline- 36.85±0.1 Cº, PIC- 36.82±0.17 Cº, rIL6+rIL1β- 36.93±0.083 Cº, $p<0.05$, One-way ANOVA).

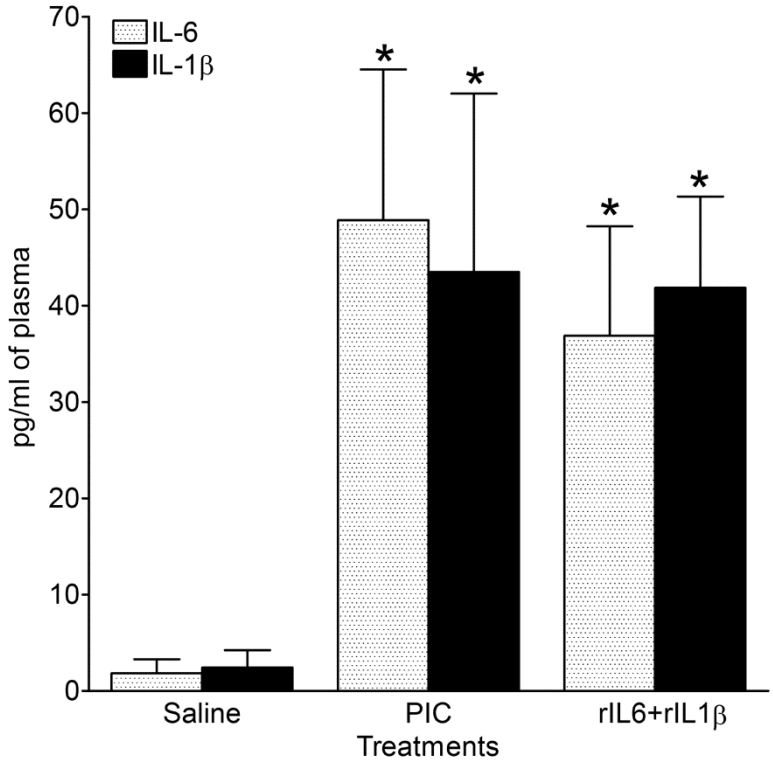

Fig. 1S. Plasma concentration of IL-6 and IL-1β. Plasma samples were taken 24 hrs after the last administration of saline (n=6), PIC (n=6), or a combination of rIL-6 and rIL-1β (n=4). Data are presented as Mean±SEM. *- $p<0.05$ vs. Saline (Two-way ANOVA + Bonferroni posttests).

Cresyl violet, glial fibrillary acidic protein (GFAP), CD11b and doublecortin (DCX) staining. Animals were generated as described in the Methods section, and were processed at P40 (Saline n=11, PIC n=9, rIL-6+rIL-1β n=12). At P40, under Pentobarbital anesthesia, the animals were transcardially perfused with paraformaldehyde. For cresyl violet staining, brains were embedded in Paraffin; for immunohistochemistry, brains were cryoprotected in 30% sucrose. Staining was performed in coronal sections of the hippocampus (mounted 10 micron-thick for cresyl violet; free floating 20 micron-thick for immunohistochemistry). Cresyl violet staining was performed using 0.1% cresyl violet acetate. For immunohistochemistry, the following antibodies were used: glial fibrillary acidic protein (GFAP) to examine the extent of astroglial presence[4] (primary: rabbit polyclonal antibodies, 1:10,000, Thermo Scientific, Rockford, IL; secondary: biotinylated goat anti-rabbit, 1:200); CD11b to examine the extent of microglial activation[4] (primary: goat rat monoclonal antibodies, 1:5,000, Abcam, Cambridge,

MA; secondary: biotinylated goat anti-rat, 1:200), and doublecortin (DCX) to examine neurogenesis in the dentate gyrus [5] (primary: rabbit monoclonal antibodies, 1:2,500,Cell Signaling, Danvers, MA; secondary: biotinylated goat anti-rabbit, 1:200). Immunostaining was visualized using 3,3-diaminobenzidine (Sigma) with nickel intensification.

Image analysis.

Cresyl violet. Using an eyepiece graticule with an indexed grid under 20X magnification on a Leica DLM microscope (McBain Instruments, Simi Valley, CA), cells were counted in three consecutive coronal sections of CA1 area of the hippocampus at the level 2 mm posterior from Bregma [6](Fig. 2S, E). For each animal, data across all sections were averaged.

Immunohistochemistry. Six consecutive digital images of left hippocampus were captured using Leica DLM microscope with the attached digital camera (Panasonic) at the level 2 mm posterior from Bregma [6] (Fig. 2S, E). Images were analyzed using Adobe Photoshop software. Images were converted into the grey scale. GFAP, CD11b and DCX were analyzed in the areas outlined in Fig. 2S, E; for DCX, area included subgranular zone to account for cell bodies, as well as granule cell layer to account for the abundance of processes (Fig. 2S, B). First, grey values were detected in the outlined areas. Next, background was detected in 50 square pixel standard areas (dark squares in Fig. 2S, E- 5 areas for GFAP and CD11b and 2 areas for DCX). Signal strength was calculated using the following formula: [SC= (1-S/B)/1], where SC- signal coefficient, S- signal, B- background averaged over all areas for each section. On the resulting 0 to 1 scale, 0 corresponds to complete absence of signal, and 1- to an uninterrupted presence of the signal. For each animal, the data from all sections were averaged.

No measurable differences were observed between control animals (i.e. prenatal saline exposure) and animals of either PIC or rIL-6+rIL-1β groups (Fig. 2S, F). Because of lack of effects of the mentioned treatments, data obtained following single cytokine exposure, or antibody injections were not analyzed.

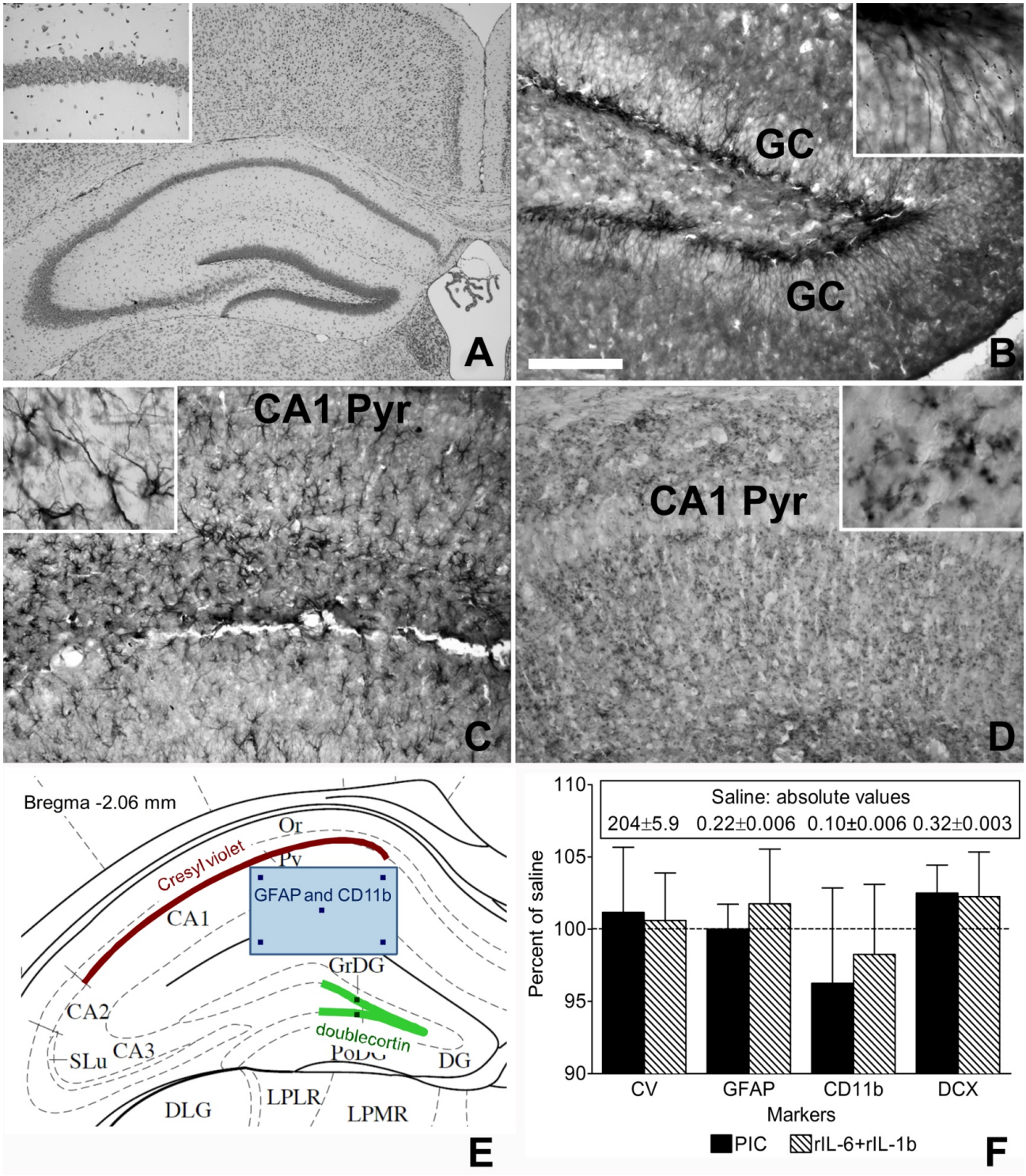

Fig. 2S. Cresyl violet (CV), GFAP, CD11b and DCX staining in the hippocampus of P40 offspring of mice subjected to PIC and rIL-6+rIL-1β injections. A-D: representative staining with CV (A), DCX (B), GFAP (C), CD11b(D). Scale bar: A: main 500 μm, inset 190 μm; B-D: Main 120 μm, inset 40 μm. Abbreviations. CA1 Pyr- Pyramidal cell layer of CA1, GC- granule

cell layer of dentate gyrus. E: Panel from the mouse brain atlas [6], showing areas used for the quantification. Dark squares in GFAP/CD11b and DXC areas indicate sites used as background. F: Image analysis. Neither PIC, nor cytokine administration modified any of the examined markers ($p>0.05$, One-Way ANOVA; for the quantification, absolute values were used). Data are presented as percent of values from animals of Saline group (Mean+SEM). Numbers on the top show absolute values in the Saline group.

References.

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