April 2017 progress report for the PRF for 2016 Research Grant to Andreas Goebel.

This research has progressed well and results are being written up now for submission. The results are shown in these figures

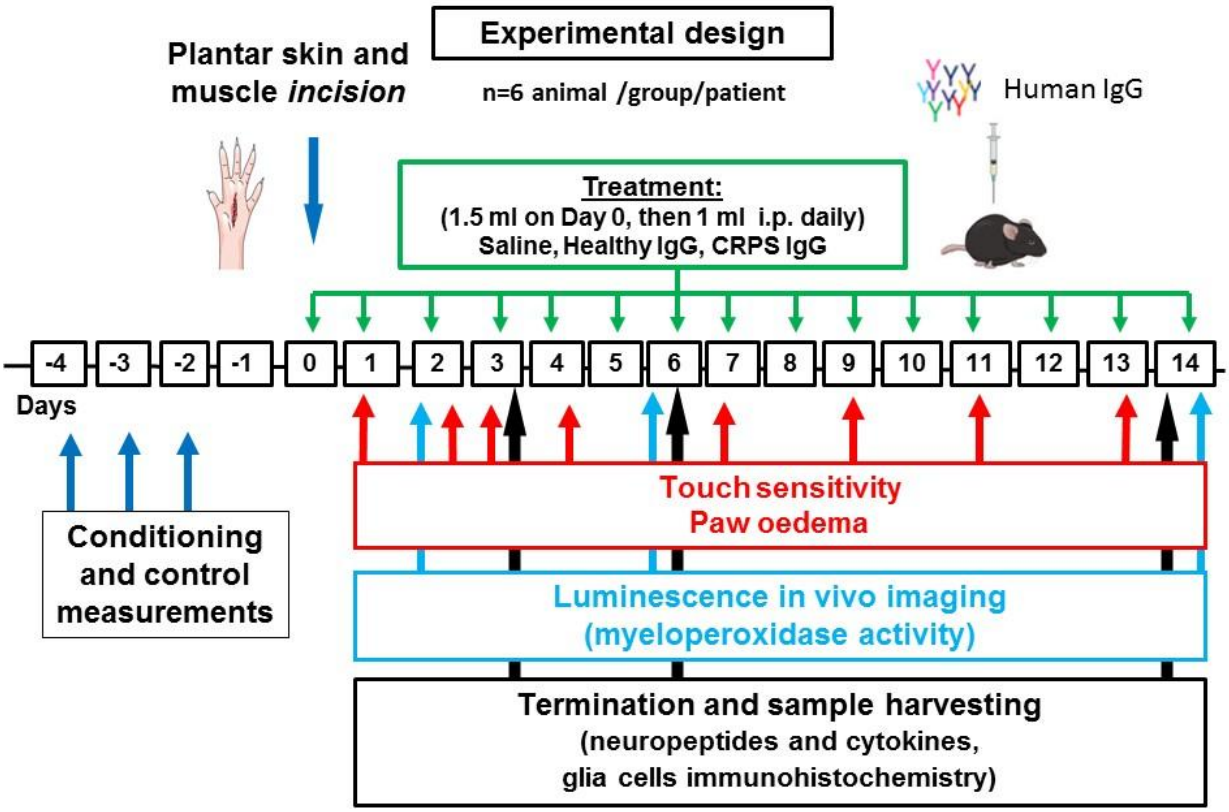


Fig.1. Scheme of the experimental paradigms and investigational techniques. The black arrow shows the time of termination; i.p. intraperitoneally

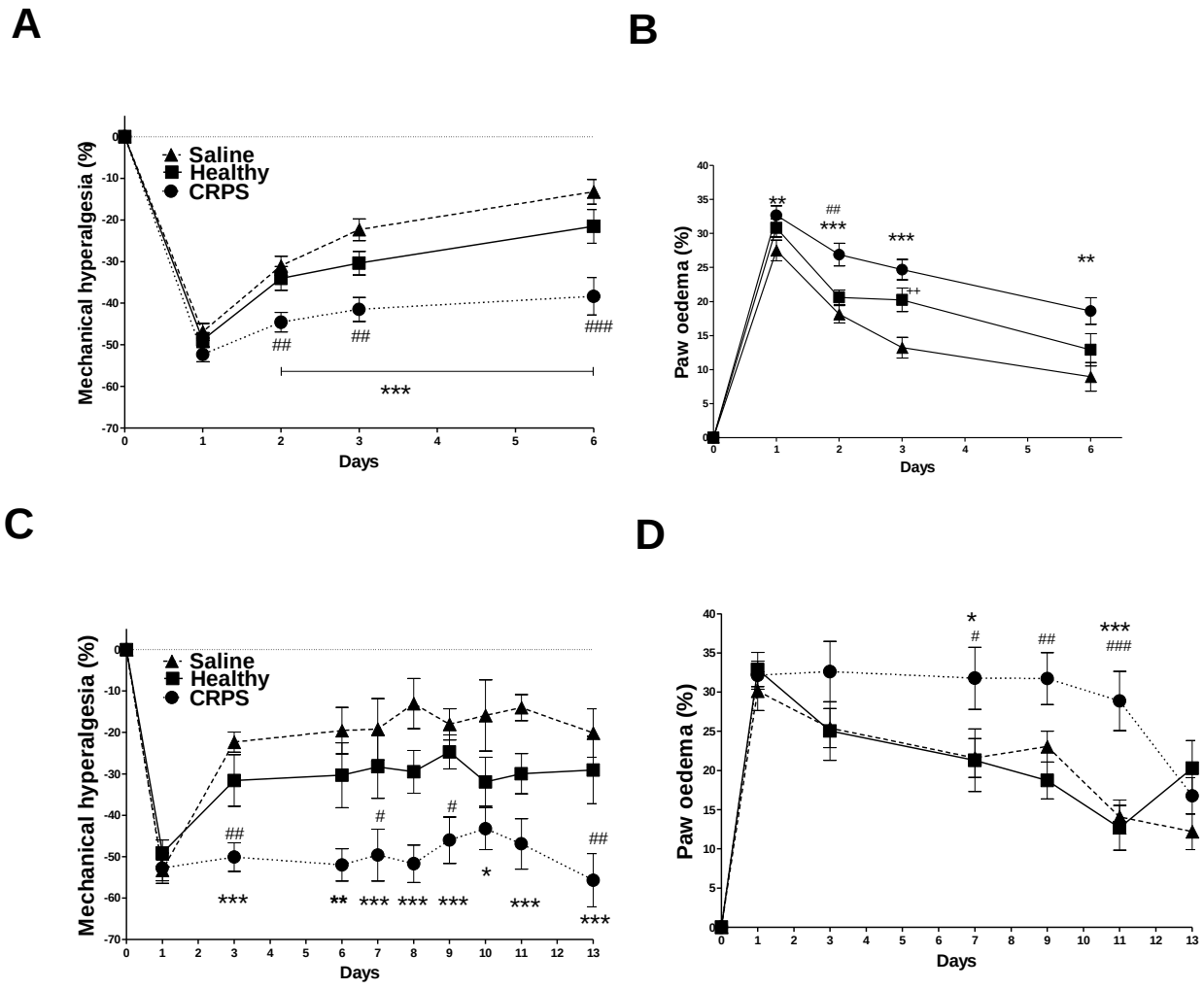
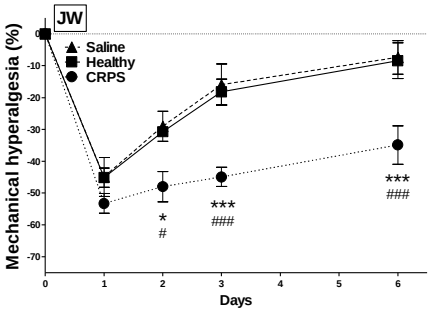
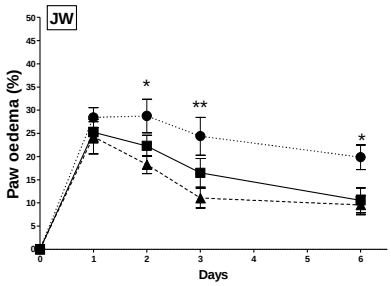


Fig.2. Effect of serum IgG derived from complex regional pain syndrome (CRPS) patients or healthy controls, or saline on plantar incision-induced **mechanical hyperalgesia (A, C) and swelling (B, D)** of the injured mouse hind paw. A volume of 1.5 mL/mouse on the first day, and 1 mL/mouse on subsequent days were administered intraperitoneally. Pooled results from short experiments up to day 6 (**A, B),** and of 14-day experiments (**C**, **D),** demonstrate development of stable hyperalgesia, whereas paw swelling reduces over time. Data are shown as means + SEM, *p < 0.05, **p < 0.01, ***p < 0.001 (vs. saline-treated control mice), #p < 0.05, ###p < 0.001 (vs. healthy IgG-treated mice); two-way ANOVA followed by Bonferroni's multiple comparison test.

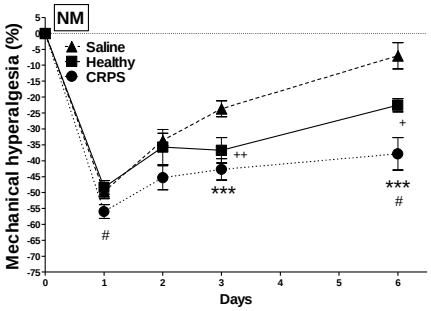
A


JW
Saline
Healthy
CRPS
Mechanical hyperalgesia (%)
Days

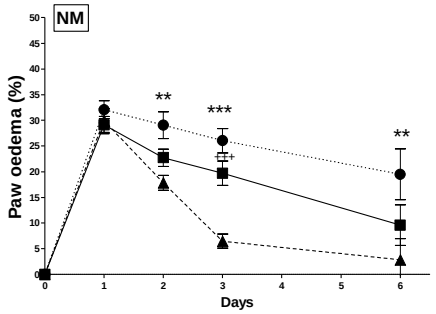
B


JW
Paw oedema (%)
Days

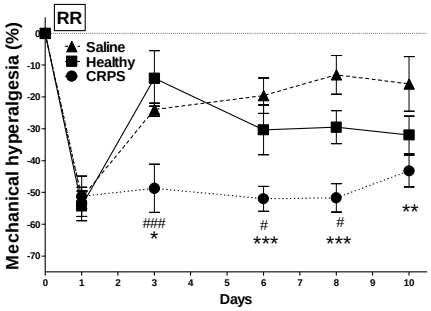
C


NM
Saline
Healthy
CRPS
Mechanical hyperalgesia (%)
Days

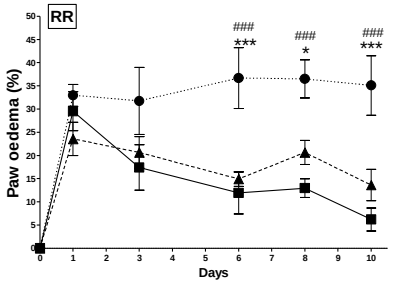
D


NM
Paw oedema (%)
Days

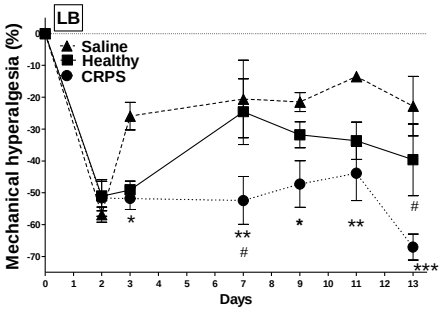
E


RR
Saline
Healthy
CRPS
Mechanical hyperalgesia (%)
Days

F


RR
Paw oedema (%)
Days

G


LB
Saline
Healthy
CRPS
Mechanical hyperalgesia (%)
Days

H

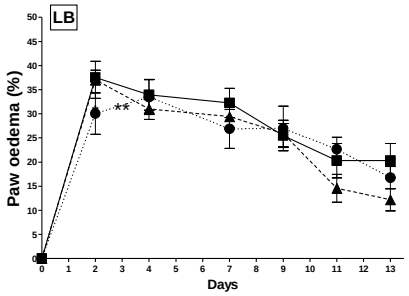

LB
Paw oedema (%)
Days

Fig. 3. Individual functional data for the 4 patients represents the effect of serum IgG derived from complex regional pain syndrome (CRPS) patients serum IgGs from healthy controls and saline on plantar incision-induced **mechanical hyperalgesia (A, C, E, G) and swelling (B, D, F, H)** of the mouse paw. Panels show all short-and long-termed experiment pooled from the same patient, demonstrating the patient/preparation-dependent variations and the independent changes between oedema (inflammation) and hyperalgesia. Data are shown as means + SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (vs. saline-treated control mice), #$p < 0.05$, ###$p < 0.001$ (vs. healthy IgG-treated mice), +$p < 0.05$, +++$p < 0.001$ (vs. saline-treated mice); two-way ANOVA followed by Bonferroni's multiple comparison test.

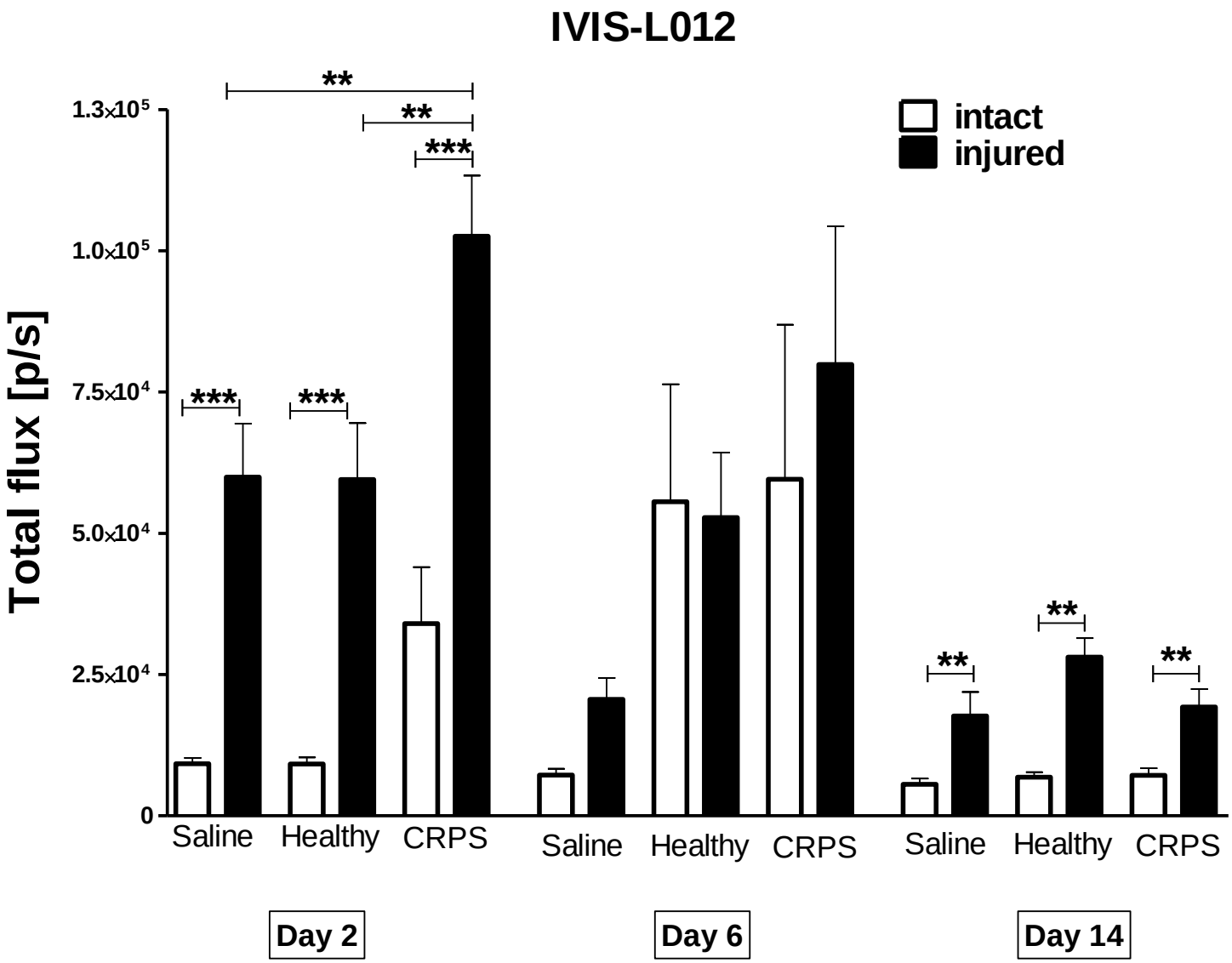


Fig. 4. *Reactive oxygen species in injured hindpaws of CRPS-IgG treated animals, and controls.* In vivo bioluminescent images of the injured hind paw were obtained during general anaesthesia on days 2, 6 and 14 after paw incision. Reactive oxygen species - induced oxidation of intraperoneally-injected L-012 was measured by quantification of L-012¯derived bioluminescence. Data are from at least 2 experiments conducted at each time point, with two different CRPS-IgG preparations, and depict means + SEM of n= 7 mice/group *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (vs. saline-treated control mice), #$p < 0.05$, ###$p < 0.001$ (vs. healthy IgG-treated mice); two-way ANOVA followed by Bonferroni's multiple comparison test.

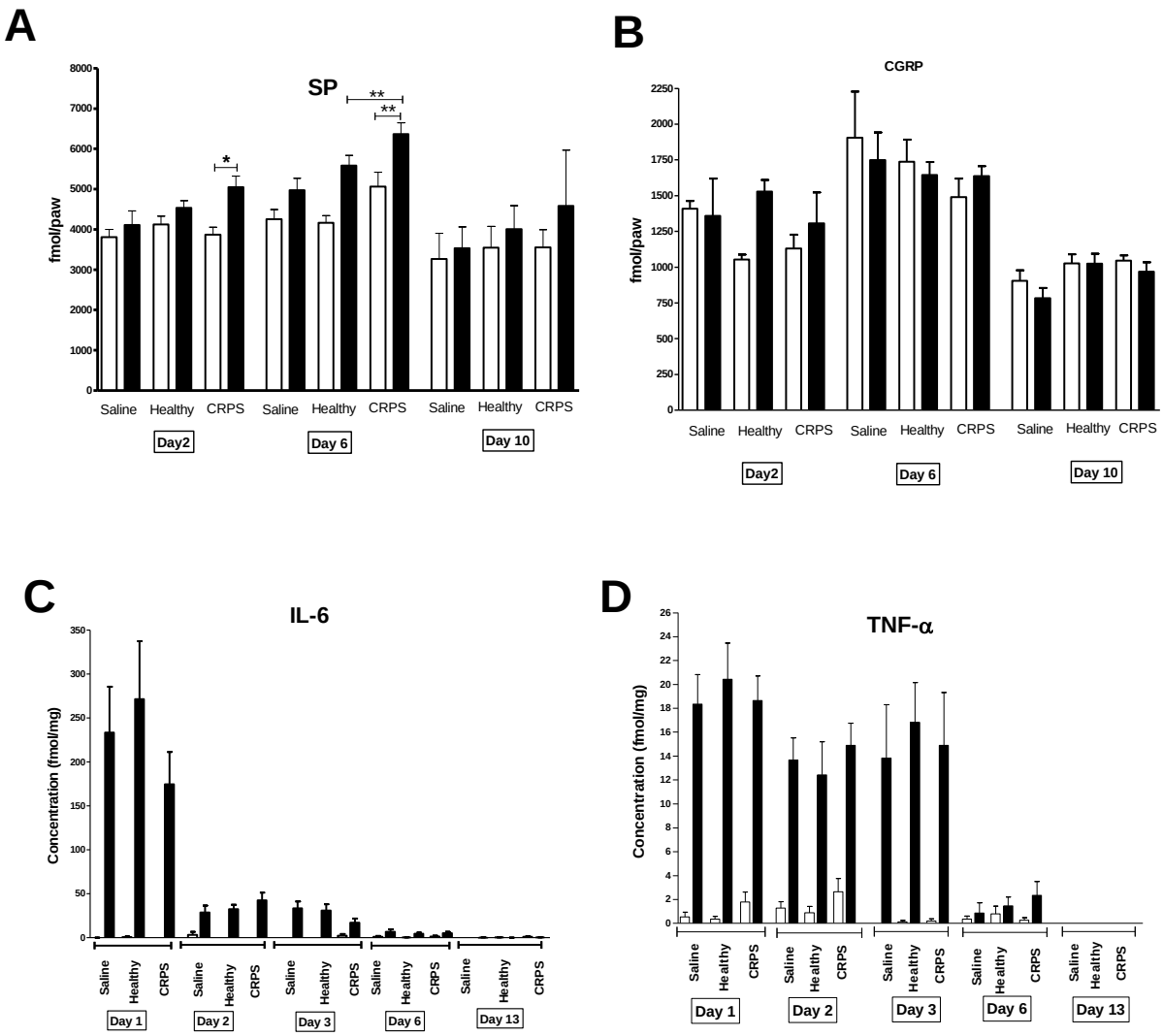


TABLE for cytokines data

Fig. 5. Effects of human IgG treatments on sensory neuropeptide and inflammatory cytokine concentrations in the hind paws. Concentrations of (A) substance P (SP) and (B) calcitonin gene-related peptide (CGRP) were measured by radioimmunoassay in hind paw homogenates excised after sacrifice. Concentrations of (C) interleukin 6 (IL-6) and (D) tumour necrosis factor alpha (TNF-alpha) were measured by Luminex and/or enzyme-linked immunospecific assay from the same samples. Columns show the means ± SEM of 30–37 mice per group (SP, CGRP, TNF-a). *P < 0.05, **P < 0.01, ***P < 0.001 vs respective intact limbs; (one-way analysis of variance followed by Bonferroni's modified post hoc test).

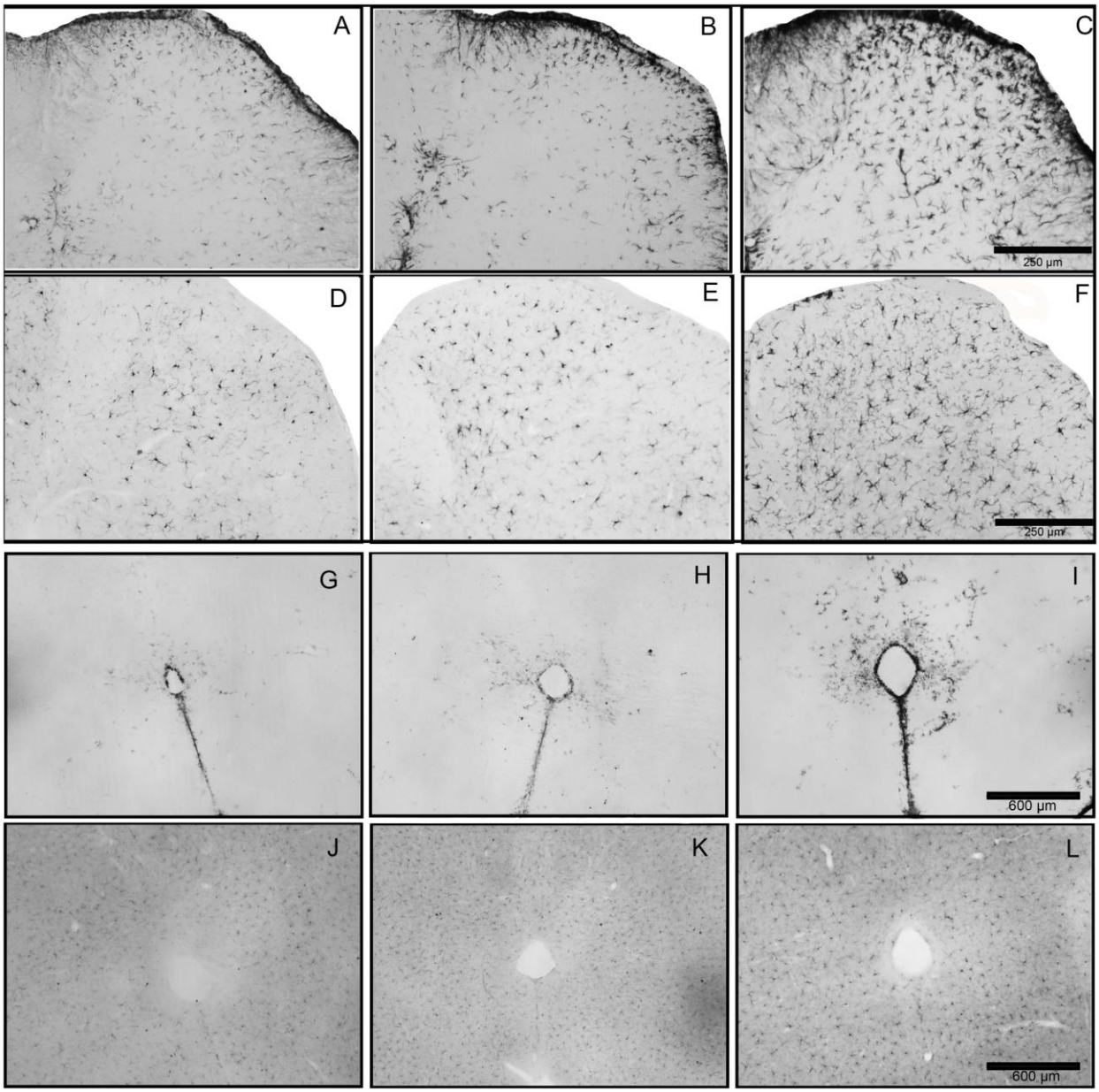


Fig. 6. *Representative photomicrographs of GFAP or Iba1 staining, of ipsilateral L5 spinal cord dorsal horn and periaqueductal gray matter*. Panels **A-C** and **G-I** show GFAP immunopositivity indicating astrocytes, and panels **D-F** and **J-L** panels show Iba1 immunopositivity indicating microglia, in dorsal horn or periaqueductal gray respectively. GFAP immunopositive sections are derived from day 6-, and Iba1 sections from day 13 **?14** after paw incision (Figure 7). Magnifications are 10x on panels A-F and 4x on panels G-L.

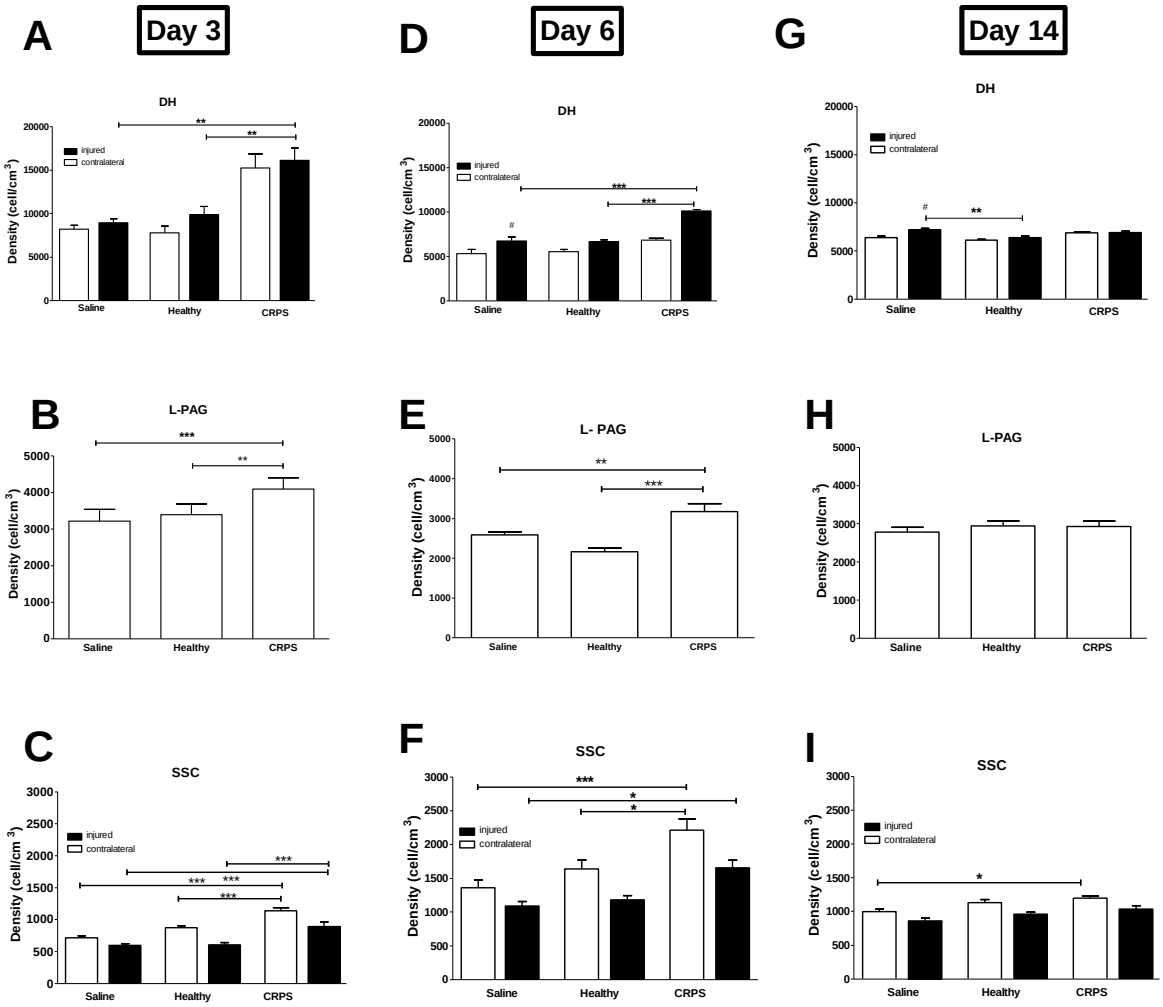


Fig. 7. *GFAP immunofluorescence staining of human IgG and saline treated animals*. Quantification of astrocyte staining in lamina I-II dorsal horn of the L4-L6 spinal cord ('DH', A, D, G), lateral periaqueductal grey ('L-PAG', B, E, H), and somatosensory cortex ('SSC', C, F, I) at 3, 6, and 14 days post paw incision. Shown are means ± SEM of 6-7 mice per group *p< 0.05, **p< 0.01, ***p< 0.001; one-way ANOVA followed by Bonferroni's modified post hoc test.

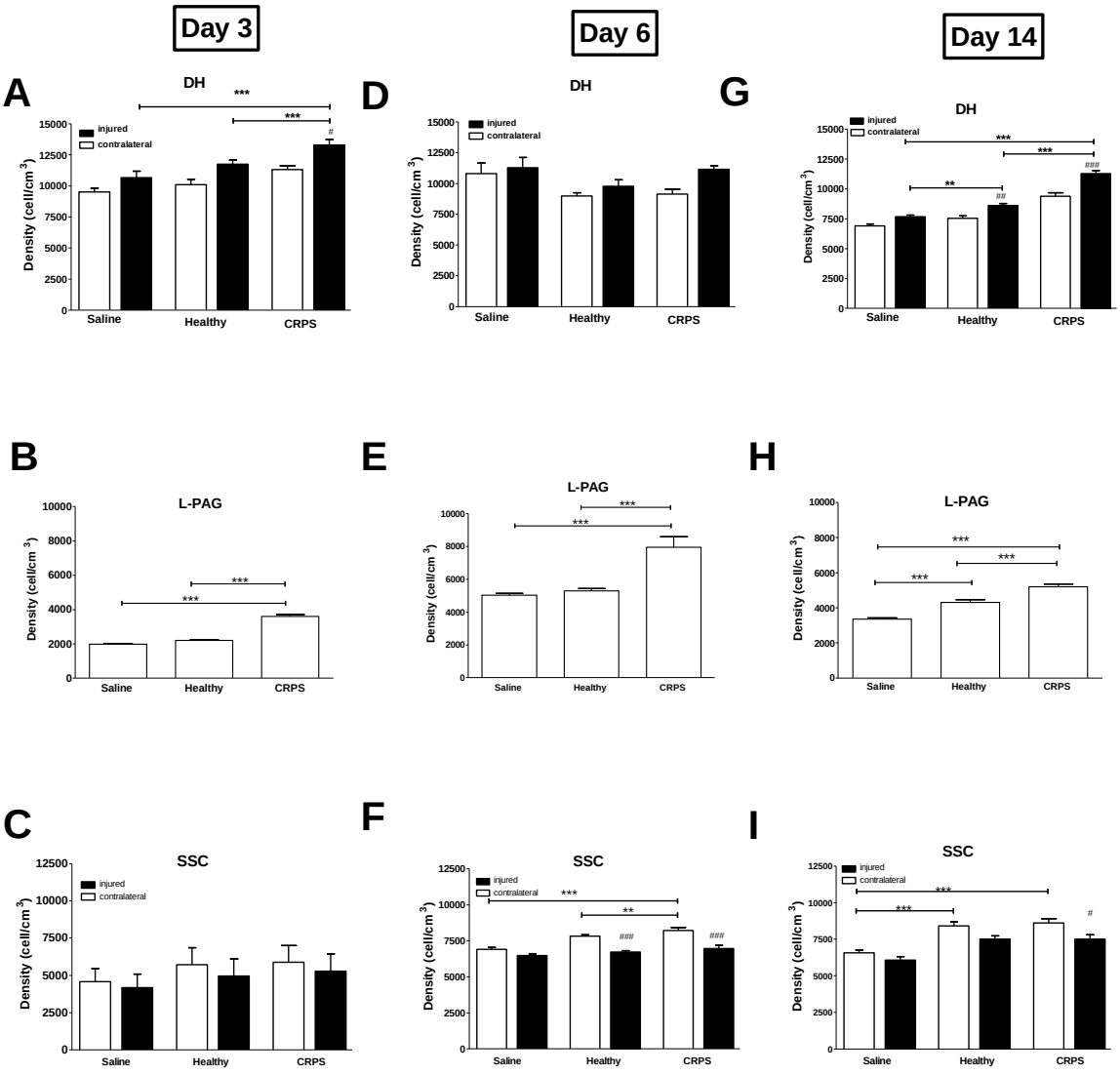


Fig. 8. *Iba-1 immunofluorescence staining of human IgG and saline treated animals*. Quantification of microglia staining in lamina I-II dorsal horn of the L4-L6 spinal cord ('DH', A, D, G), lateral periaqueductal grey ('L-PAG', B, E, H), and somatosensory cortex ('SSC', C, F, I) in samples derived on day 3, 6, and 14 post paw incision. Shown are means ± SEM of 6-7 mice per group *p< 0.05, **p< 0.01, ***p< 0.001; one-way ANOVA followed by Bonferroni's modified post hoc test.

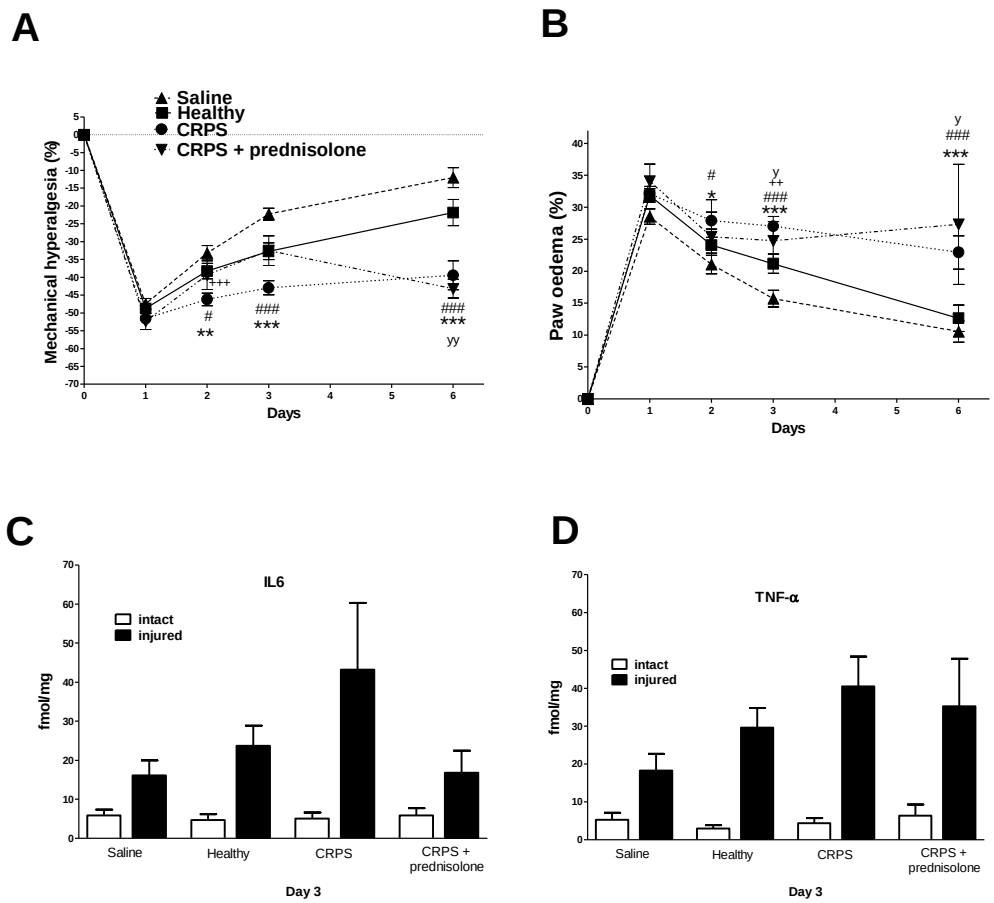


Fig. 9. Effects of steroid treatment on passive-transfer trauma murine model induced by serum IgG derived from complex regional pain syndrome (CRPS) patients serum IgGs from healthy controls, and saline on plantar incision-induced **mechanical hyperalgesia (A) and swelling (B)** of the mouse paw. Panels **C and D** show the concentrations of (C) interleukin 6 (IL-6) and (D) tumour necrosis factor alpha (TNF-alpha) were measured by a multiple enzyme-linked immunoassay from the same samples.