Early Control of Systemic HMGB1 Activity Prevents Synaptic Dysfunction in Burn and Hindlimb Unloaded Rats

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Introduction

- Severe burn injuries cause both physical and psychological changes, with higher rates of mental-health-related emergency care than the general population. *– Jeschke MG et al. Ann Surg. 2008; Mason SA et al. J Am Coll Surg. 2017*
- Prolonged immobilization is an unavoidable clinical need but contributes to neurocognitive disturbances. *–Bohmer AB et al. Crit Care. 2014;Parry SM et al. Extrem Physiol Med. 2015*
- Synaptic plasticity, involving both activity-dependent synaptic potentiation and structural reorganization, plays a central role in maintaining hippocampal learning, memory, and adaptive cognitive function. *-Hagena H et al. Philos Trans R Soc Lond B Biol Sci. (2024)*
- High mobility group box 1 (HMGB1) functions as a key inflammatory mediator for post-burn hyperinflammation and hypermetabolism. Targeting HMGB1 treatment reveals multiple outcomes from burn and trauma.*- Song J et al. Sci Rp. 2023; Preeti JM et al. J Exp Orthop. 2022*
- **Aim**: Investigate whether anti-HMGB1 neutralizing antibody treatment preserves neural synaptic plasticity in burn rats with a prolonged immobilization.

Methods

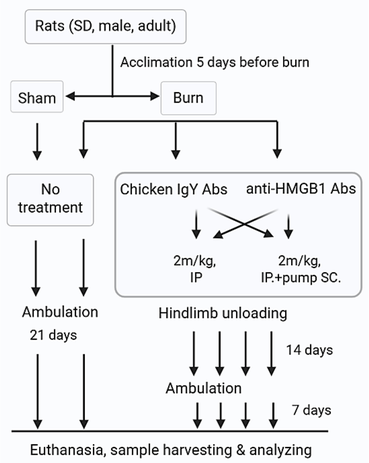


Figure 1. Experiment flow chart. 47 rats were randomly grouped: Sham (n=10) and Burn (n=12) without treatment and kept ambulatory for 21 days. Burn and hindlimb unloaded rats (BH) were administered chicken IgY (control) antibody by IP injection (BHV, n=10) or by IP and SC inserted pump (BHVP, n=3). BH groups were treated with IP delivery of anti-HMGB1 antibody (BHH, n=6) or with IP and SC inserted pump (BHHP, n=6). All treated rats were released from the tail suspension system at 14 days and allowed to be ambulatory for additional 7 days to meet the end point. Systemic inflammatory and immune responses were assessed by Bio-Plex analysis and flow cytometry. Hippocampal synaptic integrity was evaluated using field electrophysiological recordings at Schaffer collateral synapses.

Results

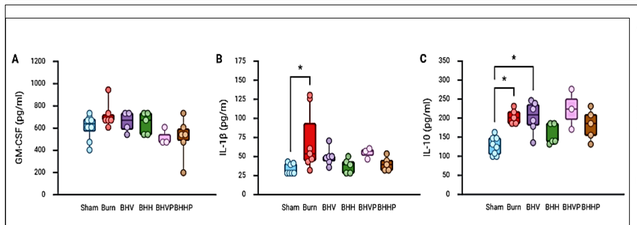


Figure 2. Systemic biomarkers of inflammation in burn rats with and without treatment. A panel of 10 cytokines was analyzed using the Milliplex MAP Rat Cytokine/Chemokine Magnetic Bead Panel. Shown are (**A**) GM-CSF, (**B**) IL-β, and (**C**) IL-10 levels. Data were collected from sham (n=8) and burn (n=6) rats without treatment; BHV (n=6) and BHVP (n=3) rats with burn/hindlimb unloading and vehicle Ab delivery by IP or IP/pump, respectively; and BHH (n=6) and BHHP (n=6) rats with burn/hindlimb unloading and anti-HMGB1 Ab delivery by IP or IP/pump, respectively. Statistical analysis was performed using a Kruskal-Wallis test with Dunn's post-hoc test (*p<0.05).

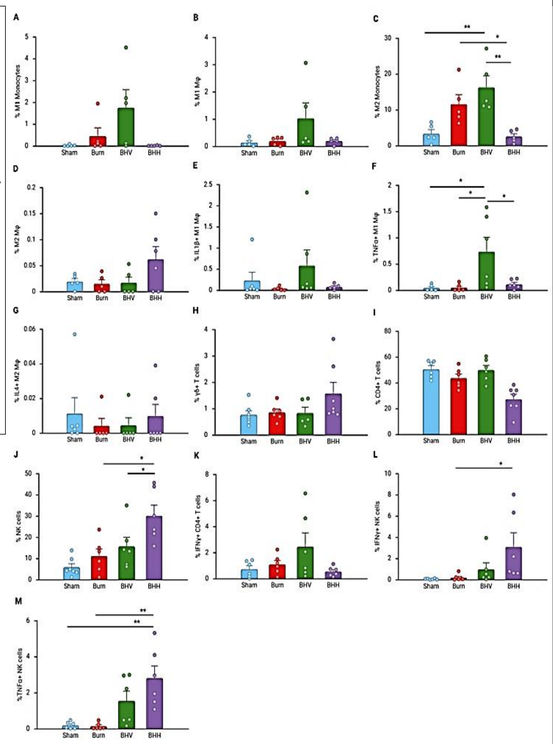


Figure 3. Treatment effect on immune cell profile in burn and hindlimb unloaded rats. Single cell suspensions of peripheral blood mononuclear cells (PBMC) were labeled with fluorescent-conjugated antibodies and analyzed by flow cytometry. Percentages of (**A**) M1 monocyte, (**B**) M1 macrophages, (**C**) M2 monocytes, (**D**) M2 macrophages, (**E**) IL-1β+ M1 macrophages, (**F**) TNFα+ M1 macrophages, (**G**) IL4+ M2 macrophages, (H) gamma-delta T cells, (I) CD4+ T cells, (**J**) NK cells, (**K**) IFNγ+ CD4+T cells, (**L**) IFNγ+ NK cells, and (**M**) TNFα+ NK cells of all groups are shown. Data are derived from n ≥ 6 rats per group and plotted as mean values ± SEM. Significance was calculated by 1-way ANOVA/Tukey's post-hoc test or Kruskal–Wallis H/Dunn's post-hoc test. The p-values of ≤ 0.05 and ≤ 0.01 are plotted with * and ** respectively. Horizontal bar shows the compared groups.

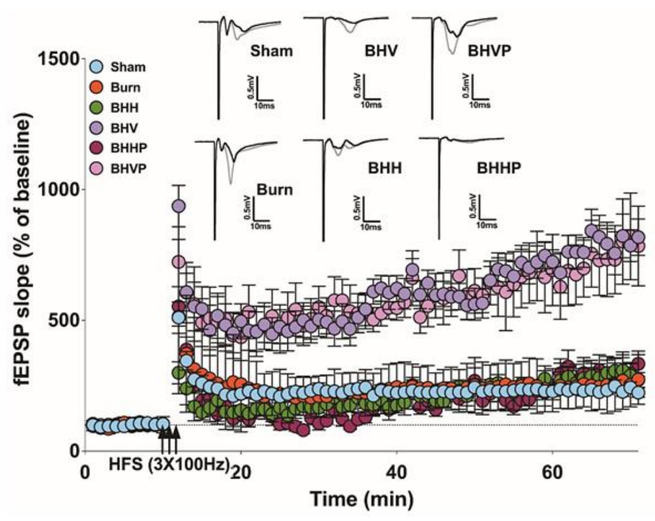
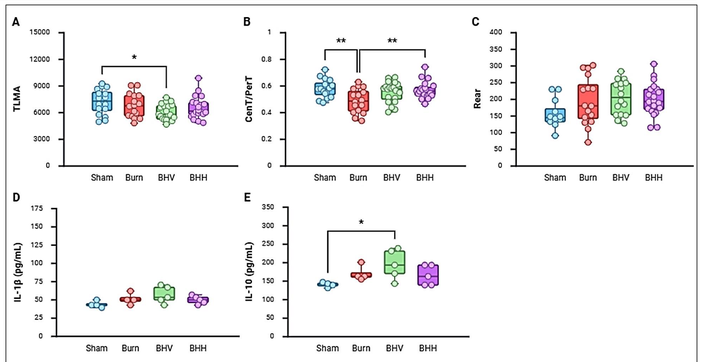


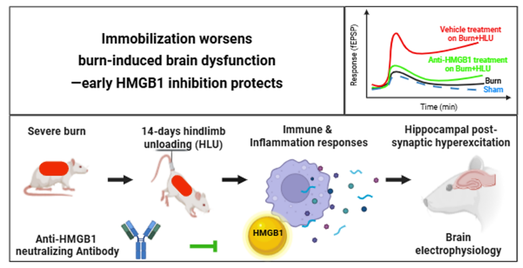
Figure 4. Targeting HMGB1 treatment restores long-term potentiation (LTP) at Schaffer collateral synapses following burn and hindlimb unloading. The field excitatory postsynaptic potential (fEPSP) slope is plotted as a percentage of the initial 10-minute baseline over 60 minutes total time where the effects of burn injury and hindlimb unloading, with or without HMGB1 treatment, on synaptic plasticity. The data show that while the combined injury (burn and hindlimb unloading) impairs the maintenance of LTP, treatment with HMGB1, administered either directly or via a pump, rescues this deficit, restoring synaptic potentiation to levels comparable to sham controls. High-frequency stimulation (HFS; 3 trains of 100 Hz), indicated by the black arrows, was delivered at the 10-minute mark to induce LTP at the Schaffer collateral-CA1 synapses. Three slices were recorded for each animal and averaged. n represents the number of animals. Each data point represents the mean ± SEM for each experimental group. Sham (blue, n= 5), Burn (red, n= 5), BHV (purple, n=3, Burn and hindlimb unloaded rats treated with vehicle, BHH (green, n= 3, Burn and hindlimb unloaded rats treated with HMGB1), BHVP (pink, n=2, Burn and hindlimb unloaded rats with an implanted pump delivering vehicle) and BHHP (brown, n=3, Burn and hindlimb unloaded rats with an implanted pump delivering HMGB1 Ab). Inset panels show representative traces for each experimental group, providing a snapshot of synaptic strength before and after the induction of LTP. The traces visually correspond to the time-course data presented in the previous figures. Notably, the potentiation in animals subjected to burn injury appears qualitatively larger, potentially indicating a state of neuronal hyperexcitability. Each panel displays two overlaid fEPSP traces from a single representative slice. The black trace represents the average baseline synaptic response before HFS, and the grey trace represents the potentiated response 60 minutes after HFS. Calibration bars represent 0.5 mV and 10 ms.



Supplemental Figure. **Cognitive impairment and inflammation in burn rats over 3 months.** Eighteen rats were randomly grouped into sham (n=4), Burn (n=4), Burn/Hind-limb unloading/vehicle only (BHV, n=5), and Burn/Hind-limb unloading/HMGB1 Ab treatment (BHH, n=5) groups. Open field test was conducted on 4 consecutive days at 3 months after burn. All test data were pooled into each group. (**A**) Total Locomotor Activity (TLMA) was calculated as the total distance traveled along 60 minutes of experiment period. (**B**) The ratio of time spent in the center vs. periphery space (CenT/ PerT), and (**C**) The frequency of standing (Rearing) was calculated for animal anxiety and stress estimation. (**D-E**) Plasma levels of IL-1β and IL-10 are shown. Statistical significance was analyzed by one-way ANOVA with Tukey's multiple comparisons test (*p<0.05, **p<0.01).

Summary & Discussion

- **Severe burns combined with prolonged immobilization** cause hyperinflammation and hippocampal synaptic dysfunction, characterized by pre- and post-synaptic hyperexcitability and impaired long-term potentiation (LTP). These deficits persisted despite 7 days of ambulation recovery, highlighting the need for additional exercise or therapeutic interventions.
- **HMGB1-mediated inflammation** is amplified by hindlimb unloading, increasing burn wound size, inflammatory cytokines (IL-1β, IL-10), and immune activation, thereby worsening peripheral and central outcomes. Our previous findings also identified a shared, aggravated coagulation pathway in burn and hindlimb unloading conditions, suggesting a novel mechanism linking HMGB1, burn injury, and immobilization.
- **Anti-HMGB1 neutralizing antibody treatment** reduces systemic inflammation and preserves hippocampal function. Future studies should distinguish its effects on immobilization alone from those on burn-induced pathology.



The presentation has been published and is accessible via QR code below:

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