ALTERATIONS IN GLIAL MORPHOLOGY IN THE CHRONIC PAIN HIPPOCAMPUS

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INTRODUCTION

Why study chronic pain (CP)?

- CP → estimated to affect around 1.5 billion people globally.
- CP comorbidities → individual suffering and distress, psychiatric mood disorders, and cognitive deficiencies
- Pain centralization → confers resistance to classical treatments for chronic pain

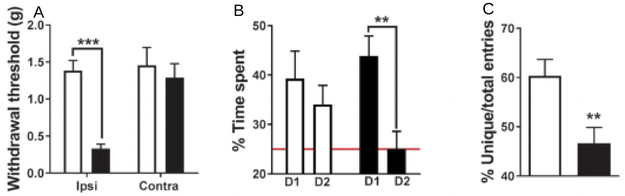


FIGURE 1. A Seven weeks post-peripheral injury, mice reveal a reduction in mechanical thresholds on the ipsilateral hind paw (two-way ANOVA, post hoc Holm–Sidak test for multiple comparisons, n = 16–17 mice/group). **B** Injured mice found to be deficient in location memory recall 1 day post-exposure to a female mouse (two-way ANOVA, post hoc Bonferroni test for multiple comparisons, n = 7–9 mice/ group). **C** Similarly, mice present deficits in spatial working memory in the Y maze, demonstrated by the percentage of unique triad combinations of consecutive arm entries (Student's t test, n = 8–9 mice/ group). Adapted from Tajerian et. al., 2018.

HYPOTHESIS

Peripheral injury results in neuroinflammation and morphological changes in astrocytes and glia in the hippocampus

METHODS

- Following the random allocation to the control or fracture/cast group, mice are anesthetized with 1.5% isoflurane while undergoing a distal tibial fracture in the right leg.
- A hemostat is used to make a closed fracture of the right tibia immediately distal to the midsection of the tibia, and the hindlimb is wrapped in casting tape.
- Nociceptive indices (von-Frey), measures of memory (y-maze), and anxiety tests (open field, zero-maze), were carried out at acute, chronic, and resolved timepoints.
- Microglial and astrocytic activation measurements gathered using immunohistochemistry (Iba1, GFAP respectively), and morphological analysis conducted with ImageJ.

CHRONIC PAIN AND GLIAL MORPHOLOGY

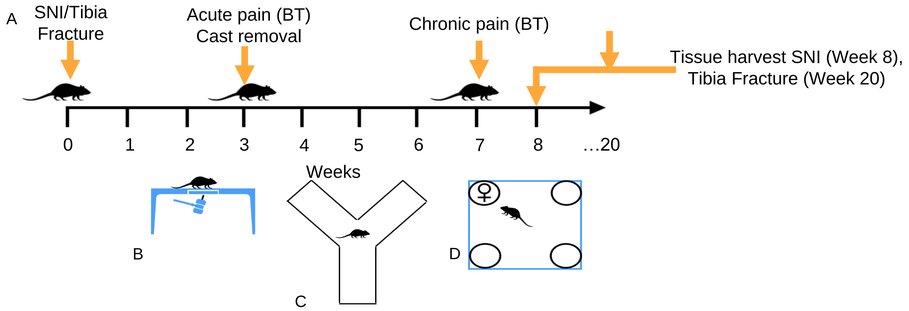


FIGURE 2. Experimental timeline: C57BL mice undergo tibia fracture. Three weeks post-injury, behavioral tests were conducted while in the acute stage of pain, as well as seven weeks post-surgery once the chronic pain stage was reached. At the 20-week mark, mice reach the resolved state; subjects are sacrificed, and tissue is collected **(A)**. Behavioral testing (BT) for nociceptive indices (von Frey mechanical allodynia, Y maze, social memory) **(B-D)**

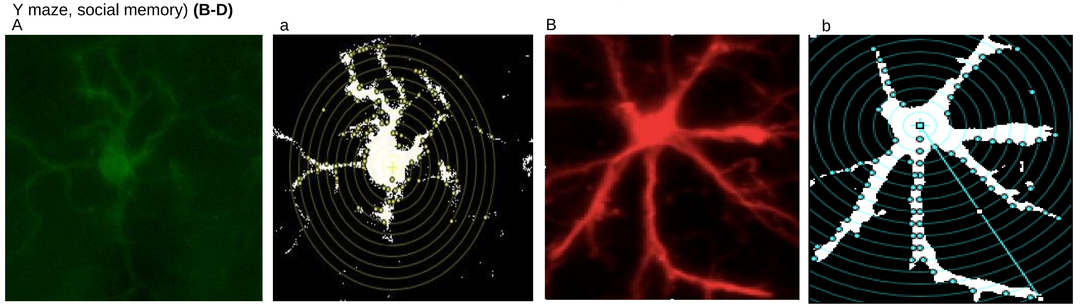


FIGURE 3. Hippocampal microglia stained with anti-Iba1 **(A)**, and astrocyte stained with anti-GFAP (**B**). Scholl analysis of dendritic arborization and soma diameter (ImageJ) of microglia (**a**), and astrocyte (**b**).

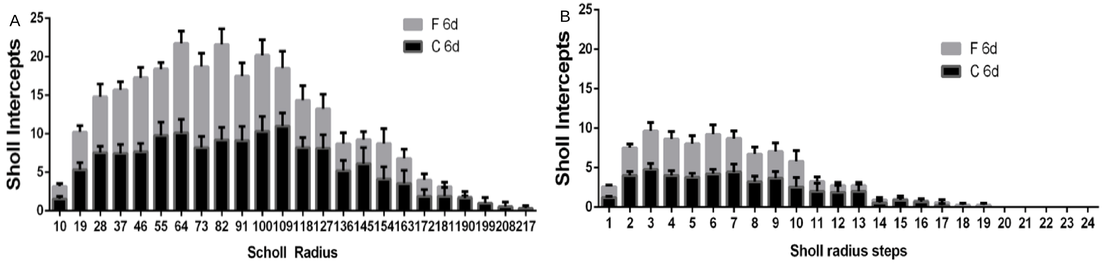


FIGURE 4. Scholl analysis revealed decreased dendritic complexity, as well as increased dendritic length was evidenced in microglia (**A**) and astrocytes **(B)** of injured mice (F) compared to control subjects (C). Sample size 6-8/group, error bars are S.E.M.

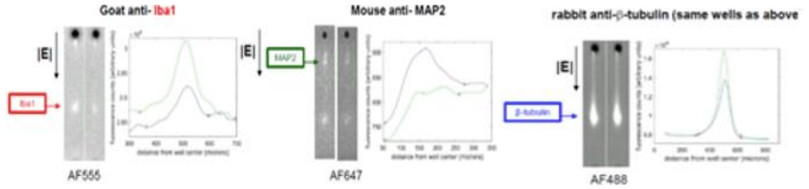
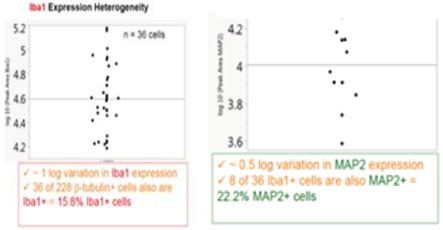


FIGURE 5. Quantification of Neural Engulfment by Glia. Through a novel in vitro assay, we can quantify the engulfment of MAP2+ distal dendrites by Iba1+ microglia on a single-cell level. 8-weeks post-op, animals were sacrificed, the hippocampus was extracted, and cells were suspended on a slide separating each individually. Cells were probed for antibodies with markers for neurons and glia. Sample analysis show that 22% of hippocampal glia cells engulfed neuronal dendrites.



FUTURE DIRECTIONS

How does altered glial morphology relate to glial function?

- We propose a reductionist approach of the mechanisms by which the ECM modifies neurophysiology, which will enable us to pinpoint the exact ECM changes that can regulate pain-related plasticity

CONCLUSIONS

- This study will allow for a better understanding of brain plasticity as a result of peripheral injury
- Possible novel therapeutic approaches for targeted treatment options that modulate alterations present in chronic pain

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