SREBP1c-Dependent Lipid Modification in the Insular Cortex Regulates Chronic Overlapping Pain

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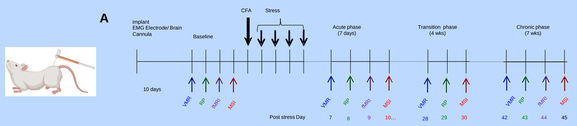
Abstract

Chronic pain, impacting 21% of adults in the U.S., remains a critical medical challenge with few effective therapies. Chronic overlapping pain conditions (COPCs), such as temporomandibular disorder (TMD), irritable bowel syndrome (IBS), and fibromyalgia, frequently co-occur, predominantly affect women, and are often worsened by stress. While peripheral mechanisms may underlie individual pain conditions, central nervous system dysregulation appears to play a key role in COPCs. Current therapies are insufficient, highlighting the need to target CNS pathways for effective intervention. This study seeks to identify novel, non-addictive pharmacological targets to alleviate COPC-associated pain using a rat model of comorbid pain hypersensitivity (CPH), in which orofacial pain and stress lead to chronic visceral hypersensitivity. We investigated brain activity and connectivity changes using fMRI and analyzed lipid and metabolite alterations by spatial lipidomics using mass spectrometry imaging (MSI) to identify molecular mechanisms underlying pain. Preliminary results revealed heightened visceral hypersensitivity in CPH rats, with fMRI indicating increased activity and disrupted connectivity in the insula cortex (IC), mPFC, and amygdala. MSI analysis revealed altered lipid profiles in the IC, particularly changes in fatty acid elongation and desaturation patterns. Since SREBP1c regulates key enzymes mediating fatty acid elongation and desaturation, such as ELOVL2, ELOVL6, and SCD, we silenced SREBP1c using siRNA in the IC. This intervention reduced referred pain by ~60%, establishing a mechanistic link between lipid metabolism and pain modulation. Our findings identify the IC and modulation of lipid populations as promising therapeutic targets for effective chronic pain intervention.

Experimental Design and behavioral assessment



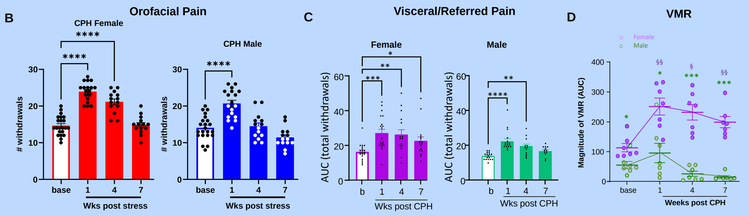


Figure 1. Overview of experimental design and behavioral assessment of COPC. A) The timeline for the experimental procedure for each rat is shown at the top left. B) The area under the curve of orofacial pain measured by von Frey filaments at baseline and 1, 4 and 7 weeks post-CPH in female and male rats. **** p<0.0001 vs. same sex baseline. C) The area under the curve of referred pain (mechanosensitivity measured at the base of the tail) at baseline and 1, 4 and 7 weeks post-CPH. *,**,***,**** p<0.05, 0.01, 0.001, 0.0001 vs baseline. D) The area under the curve of VMR data at baseline and 1, 4 and 7 weeks post-CPH in female and male rats. *, ***p< 0.05, 0.001 vs male at the same time point. §, §§ p< 0.05, 0.01 vs same sex baseline. Comorbid pain hypersensitivity persisted longer in females compared to males by several measures.

Sex Difference in Brain activity in CPH rats

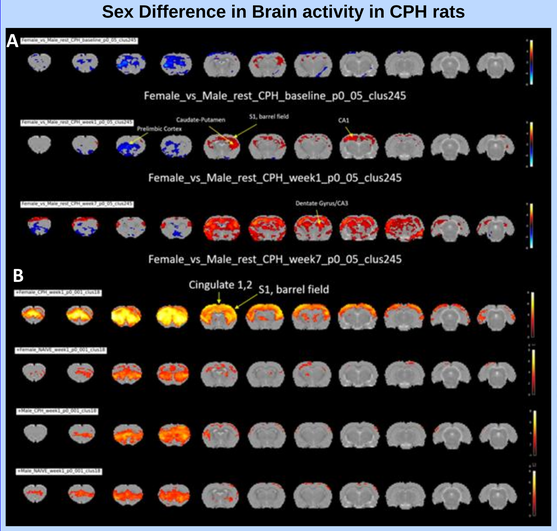


Figure 2A. Sex differences in Insula Functional Connectivity. Female CPH vs Male CPH, independent t-test contrasts at baseline, 1- and 7-weeks post stress, p < 0.05 cluster-size corrected, min cluster size = 245 voxels. Male CPH rats showed greater Insula functional connectivity than females in the Prelimbic Cortex during week 1. Female CPH group showing consistently greater Insula functional connectivity in regions involved in pain perception such as Caudate-Putamen, S1 barrel field, and CA1 during week 1 and increased during week 7. Additionally, Female CPH rats increased their functional connectivity with the Insula compared to males in the Dentate Gyrus and CA3 regions. Hot colors = Significant brain regions where Female CPH rats show greater functional connectivity than Males to the Insula. Cold colors = Significant Brain regions where Male CPH rats show greater functional connectivity than Females to the Insula. **B:** Group and Sex differences in Insula Functional Connectivity. Results depicting group and sex differences in Insula Seed functional connectivity. Group one-sample t-test maps for CPH and Naive groups at week 1 for both Males and Females, p < 0.001 cluster-size corrected, min cluster size = 18 voxels. Female CPH rats showed significant Insula functional connectivity with regions involved in pain perception such as Cingulate 1,2 and S1 barrel field while the effect was negligible for Female Naive, Male CPH, and Naive rats.

FosB expression is increased in the IC of CPH rats

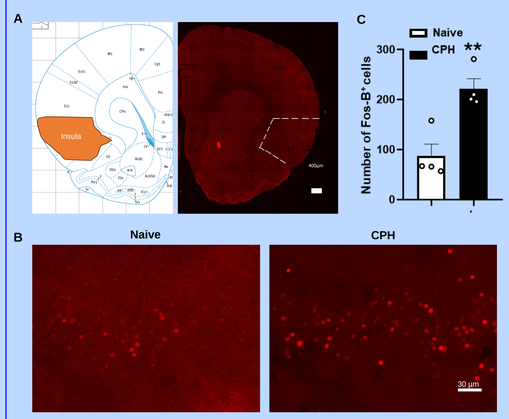


Figure 3: The effects of Fos B in Naïve and CPH rats 1 week after stress. A) Graphical representation of insular cortex (bregma + 2.65-2.52mm; representative micrograph of FosB positive cells (red) in the IC. FosB is a marker that labels neurons persistently activated by chronic noxious stimuli, indicating long-lasting changes in neuronal activity associated with persistent pain conditions. B) Representative FosB+ immunoreactivity at higher magnification showing higher expression in CPH rats compared to Naïve rats one week after stress. C) Quantification of FosB+ cells in naïve and CPH rats indicates significant difference in CPH rats 1 week after stress. Data were expressed as mean ± SEM, *n* = 4 in each group.

Intensity plots for 2 differential ions shown from IC fROI

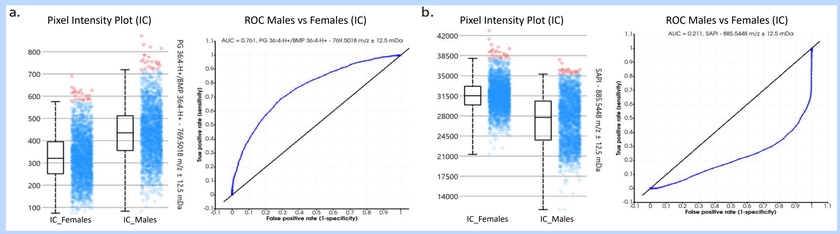


Figure 6: Intensity plots for 2 differential ions shown from IC fROI. a.) Pixel intensities from IC fROI for females and males (n=3), from individual ion images. Top: *m/z* 769.5018 +/- 12.5 mDa - phosphatidylglycerol (PG) 36:4 $[M\text{-}H^+]^-$. Receiver-operator characteristic (ROC) for the same ion in IC fROI showing robust sex discrimination by area under the curve (AUC) x>0.75 (top) or (1-x)>0.75. b.) *m/z* 885.5448 +/- 12.4mDa, annotation – phosphatidylinositol 38:4 $[M\text{-}H^+]^-$ and ROC as in a. Normalized (total ion current, TIC). Representative of 11 differential ions, 8 with tentative or known annotations.

Lidocaine microinjection in the Insula Cortex attenuates comorbid pain in the rat

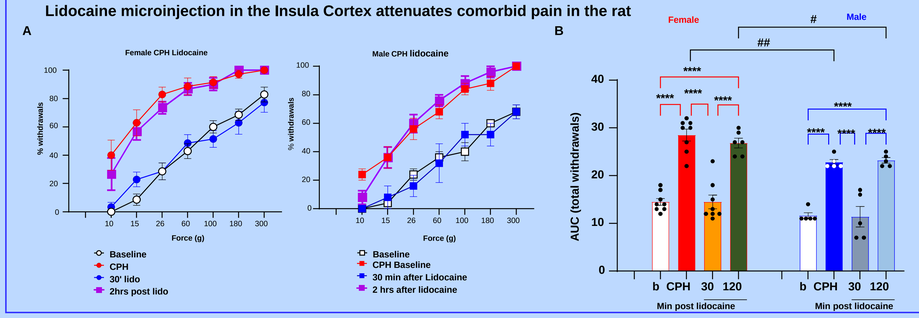


Figure 6. Intra-insular cortex lidocaine infusion attenuates referred pain. A) Stimulus response curves for referred pain at baseline, 30 min and 120 min post lidocaine injection in female (left) and male (right) CPH rats. C) The area under the curve of referred pain at baseline, before (CPH) and 30 min and 120 min post lidocaine injection of male and female CPH rats. **** p<0.0001 vs same sex baseline. #, ## p<0.05, 0.01 vs same condition, other sex. The effect of lidocaine resolved by 120 min.

Biomarker Discovery for Stress-Induced Pain

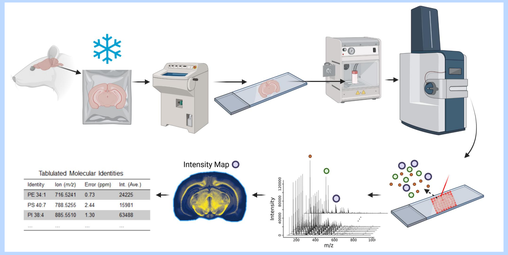


Figure 4: Post-fMRI, rat brains were removed, segmented using a custom brain matrix, and sections collected in functional regions of interest (fROIs). Mass spectrometry imaging (MSI) data collected for lipids at 50 µm spatial resolution and fROIs interrogated for differential expression between males and females.

Identification of lipid molecules and enzymes by MSI

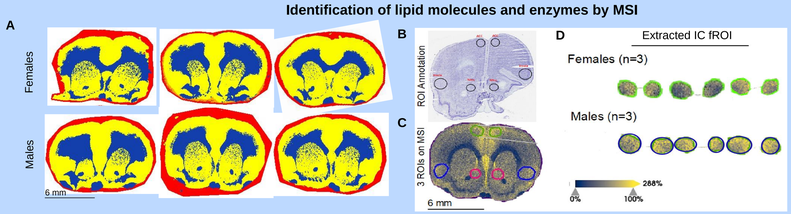


Figure 5: A) Unsupervised clustering of lipid MSI recapitulates histology and isolates the on-tissue segments of the data for further analysis. Image segmentation from lipid data (false-colored, arbitrary). Differential lipid signatures from fROIs point to differential and regional lipid homeostasis. B) Representative section depth (containing fROIs: ACC - greens, NAcc - pinks, and Insula - blues) shown, not reference for b-c. C) Ion image *(m/z* 769.5018 +/- 12.5 mDa), phosphatidylglycerol (PG) 36:4 $[M\text{-}H^+]^-$, MALDI-TOF, negative ion mode, 50 µm spatial. D) IC fROI extracted (L, R) and analyzed for differential lipid features, ion in b. shown. Biological triplicates show for females, males.

SREBP-1 siRNA microinjection in the Insula Cortex attenuates comorbid pain in the rat

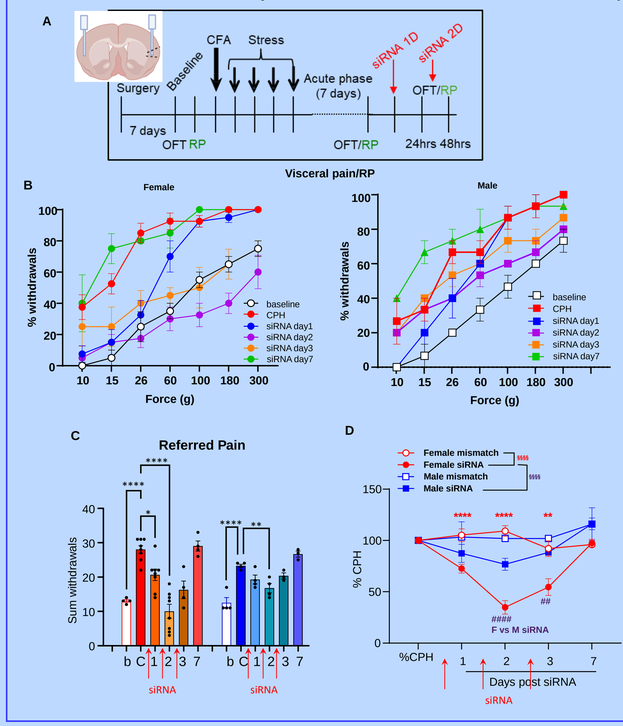
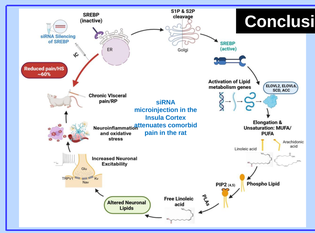


Figure 7. Inhibition of SREBP activities in the IC reduces referred pain.

Experimental design for silencing of SREBP function in insular cortex by microinjection of siRNA followed by RP assessment. B) Stimulus response curves for referred pain at baseline, post CPH and 1, 2, 3 and 7 days following siRNA injections in female (left) and male (right) CPH rats. siRNA was injected up to 3 times; 24hr prior to testing on day1, day2 and day3. C) The area under the curve derived from the SRFs in B. *,**,**** p<0.05, 0.01, 0.0001 vs. same sex CPH (C). D) referred pain response normalized to the comorbid pain condition (CPH) for each sex. **,**** p<0.01, 0.0001 vs. same sex CPH. ##, #### p<0.01, 0.0001 vs. male siRNA. §§§§, p<0.0001.



Conclusion:

- Through behavioral assessments, fMRI, immune, pharmacological studies, and MSI data, we identified heightened activity in the insula cortex (IC) under comorbid conditions, with this effect being more pronounced in female rats compared to males.
- Sterol Regulatory Element-Binding Proteins (SREBPs) are key transcription factors that regulate lipid metabolism by controlling the expression of genes involved in fatty acid and cholesterol biosynthesis. Emerging research suggests that dysregulated lipid metabolism, particularly in the central nervous system (CNS), plays a crucial role in chronic pain conditions by affecting neuronal excitability and inflammation.
- In a rat model of chronic overlapping pain conditions (COPCs), silencing SREBP1c using siRNA in the insula cortex significantly reduced pain hypersensitivity (~60%).
- Restoring lipid homeostasis through SREBP inhibition reduces neuronal hyperexcitability and inflammation, suggesting a promising non-opioid therapeutic approach for chronic pain management.

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