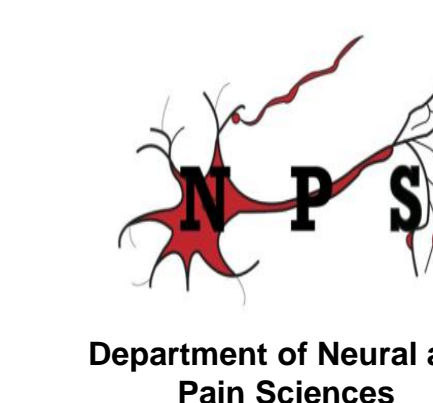


# Effects of Environmental Enrichment on a Mouse Model of Chronic Overlapping Conditions

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## Introduction

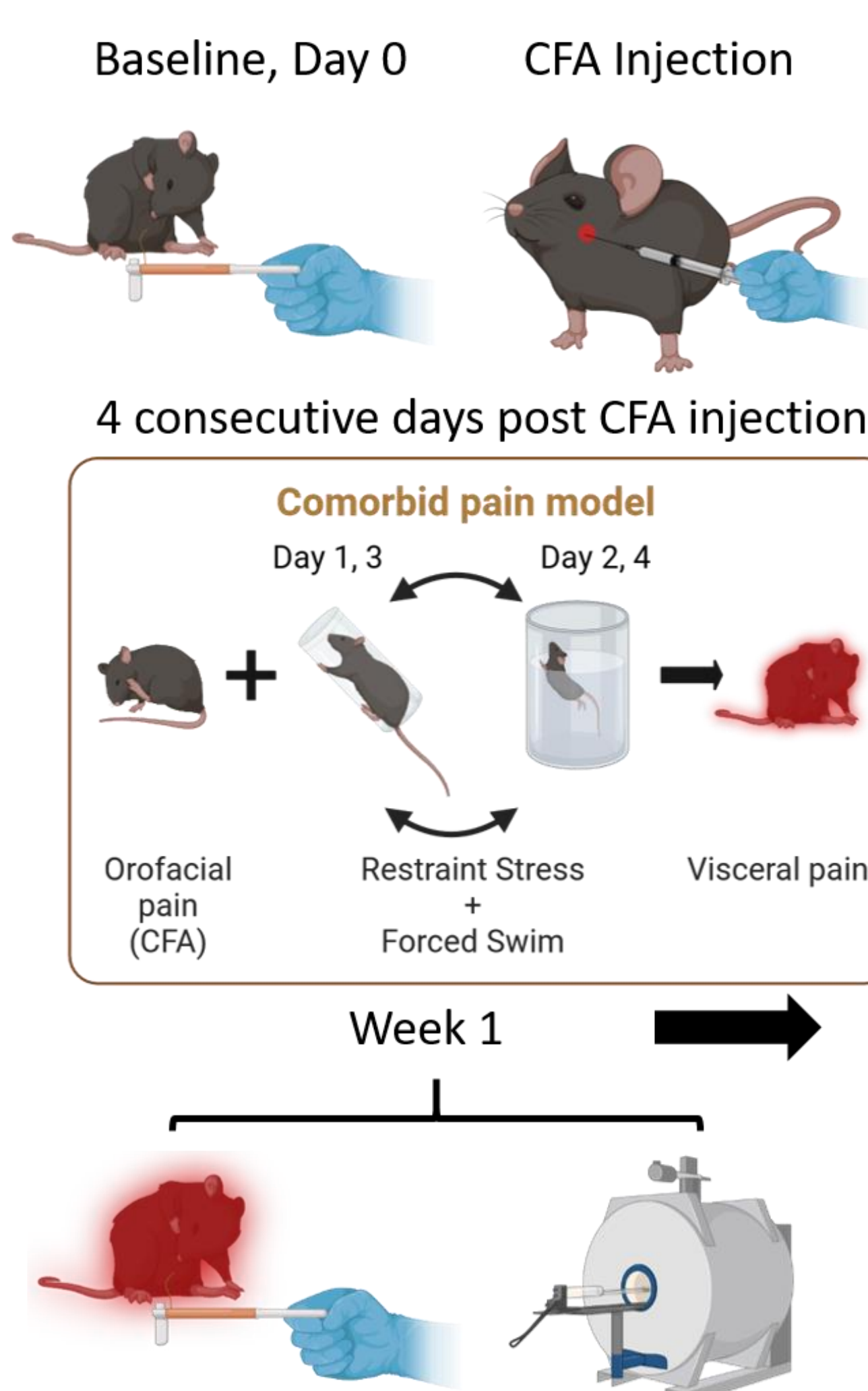
- Temporomandibular disorder (TMD) and irritable bowel syndrome (IBS) are two chronic overlapping pain conditions (COPCs) that present with significant comorbidity.
- Both conditions are more prevalent in women and are exacerbated by stress.
- COPCs may be the result of dysfunctional CNS pain processing caused by altered connectivity among several brain regions, including the insula.
- Nonpharmacological therapies such as Environmental Enrichment (EE) have been shown to reduce pain- and anxiety-like behaviors, enhance learning and memory, and induce neural plasticity.
- We have developed a mouse model of comorbid pain hypersensitivity (CPH: stress during preexisting orofacial pain, Complete Freund's Adjuvant (CFA) induced masseter muscle inflammation) and examined the effects of EE on behavioral and brain connectivity changes induced by this model.

## Methods

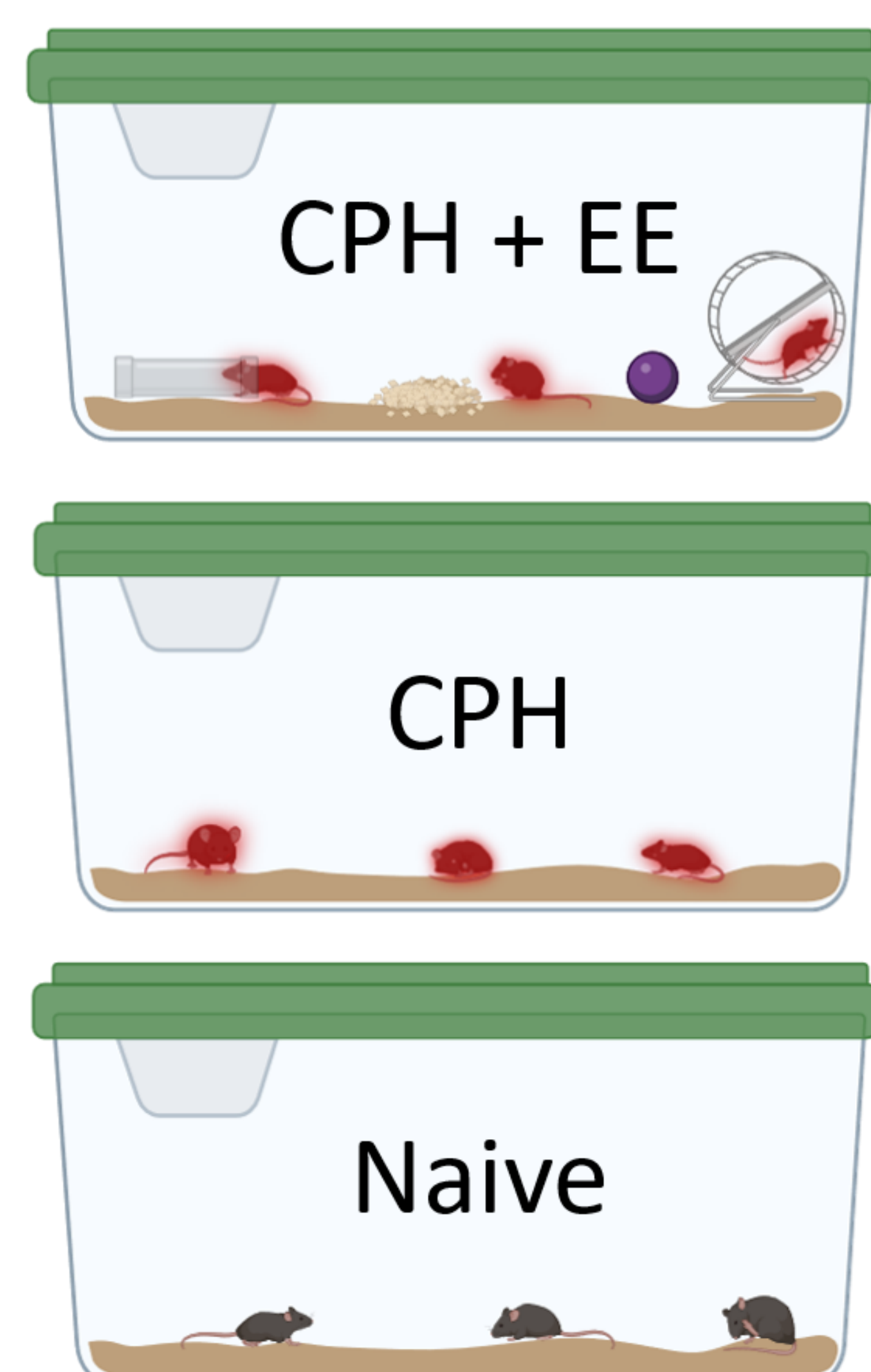
- **Animals and groups:** Eighteen female mice underwent resting-state fMRI scans. The 18 C57BL/6 mice were divided into 3 groups: 6 CPH + EE, 6 CPH only, and 6 naive mice.
- **Comorbid pain hypersensitivity (CPH model):** Masseter muscles were injected with Complete Freund's Adjuvant (CFA, 10  $\mu$ l, 1:1 in saline) one day prior to initiating the stress protocol (2 days of restraint stress for 2 hours in confining tubes alternating with 2 days 20 min forced swim stress). The day following the last stress session was designated as day 1. Baseline data were collected prior to the CFA injection/restraint stress.
- **EE housing** started 3 weeks before the stress paradigm to give sufficient time for a therapeutic effect. During EE, objects (marble, pvc tunnel, running wheel) were placed in the cage for unrestricted use. Control for the EE were housed in a cage with normal bedding.
- **Referred pain (visceral pain correlate)** was measured as the increase in responsiveness to von Frey stimulation of the lower abdomen above baseline mechanosensitivity following inflammation and stress.

## Design

### Timeline



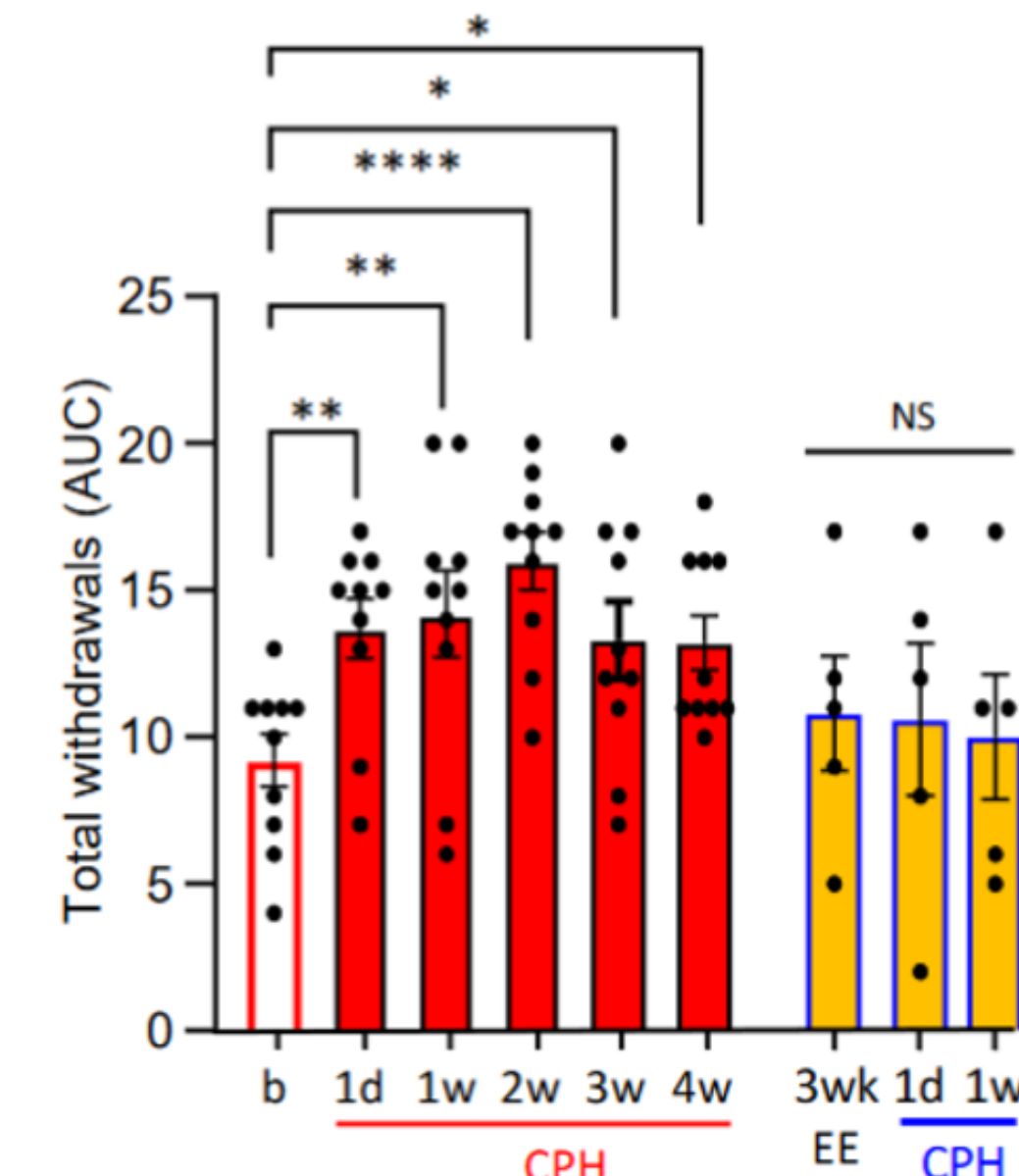
### Housing



## fMRI Analysis

- Data was preprocessed using AFNI, (Cox, 1996) and ANTS (Avants et al., 2011) software packages. Each mouse's functional images were slice-time corrected, despiked, motion corrected (AFNI) and coregistered to its T2 image (ANTS). T2 and functional scans were then spatially normalized to the Allen Institute for Brain Science template (0.15mm x 15mm x 0.15mm resolution), Common Coordinate Framework space, CCF v3 (Lein et al., 2007). The template was downloaded from Scalable Brain Atlas (Bakker et al., 2015). Functional images were then bandpass filtered (0.009–0.2 Hz) and spatially smoothed (0.3mm, FWHM).
- Network analysis determined ROI-to-ROI functional connectivity differences across the 3 groups,  $p < 0.05$ , uncorrected. A seed-based correlation analysis was conducted using the Insula as a seed. Second-level independent sample t-tests compared EE vs Comorbid groups and Comorbid vs Naive groups. Group contrast maps were thresholded at  $p < 0.05$ , uncorrected.

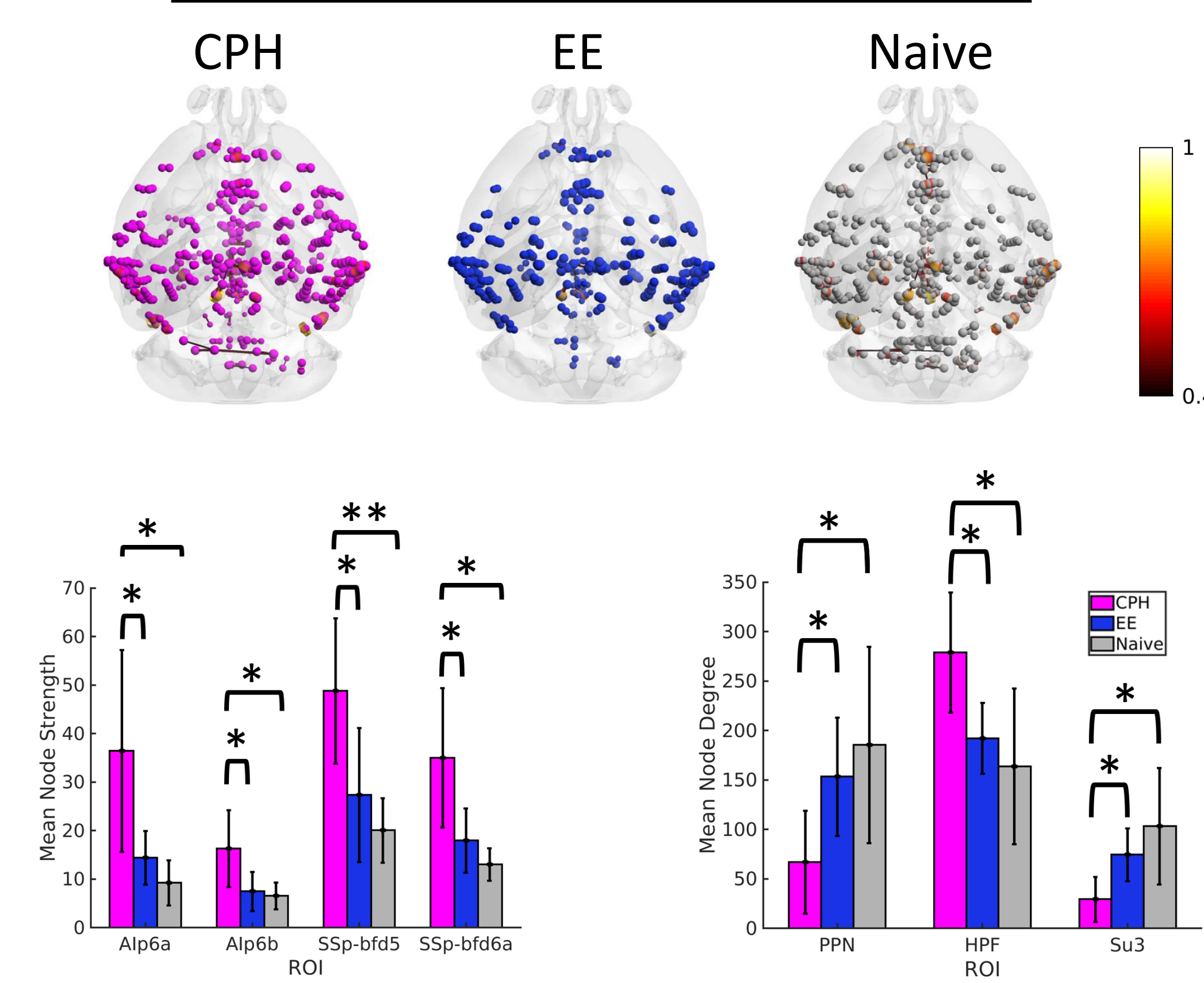
## Behavior Results



**Figure 1. Referred pain in mice.** Time course of referred pain in CPH female mice (red bars). Three weeks of EE prior to inducing CPH blocked its development (orange bars).

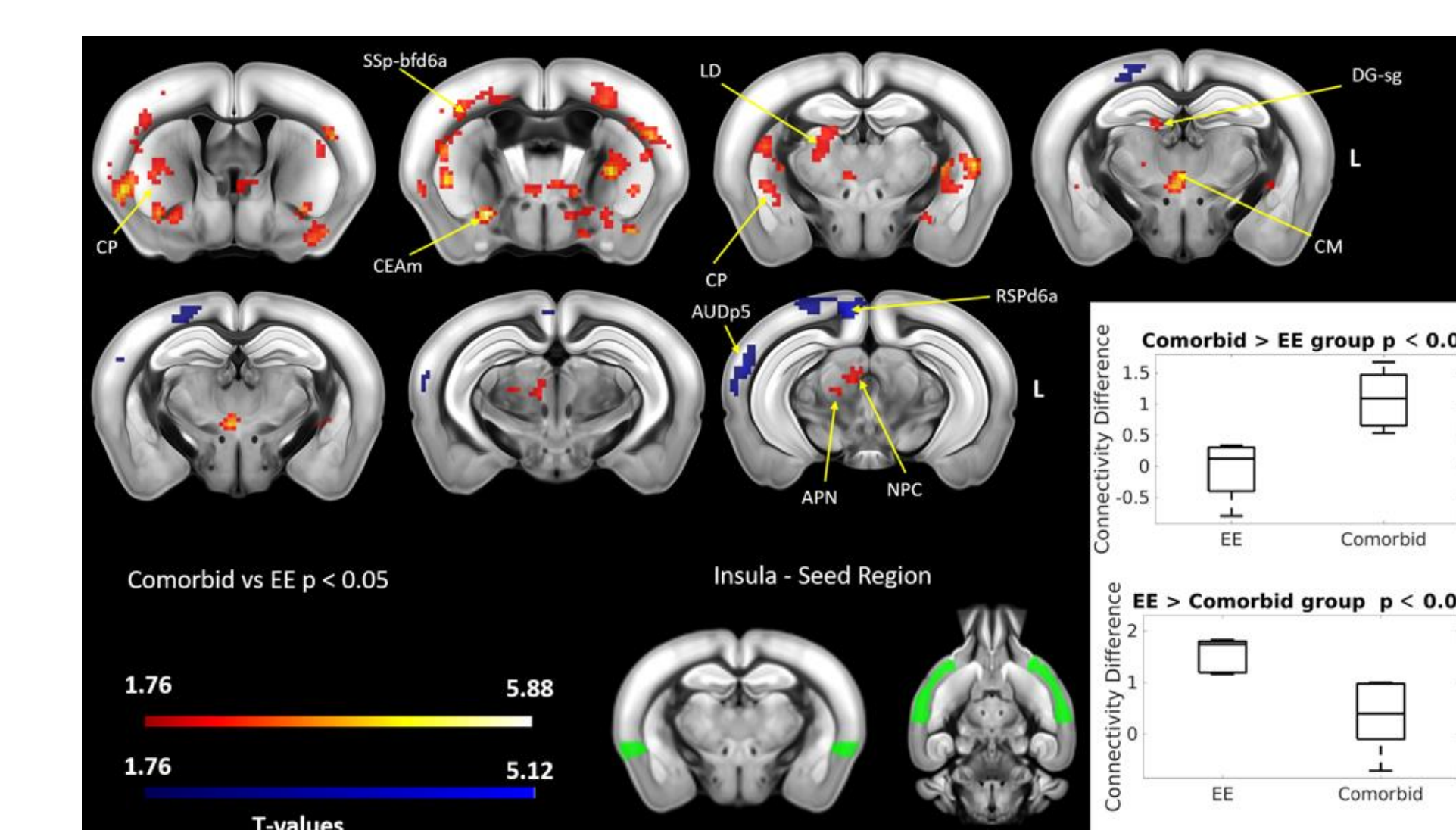
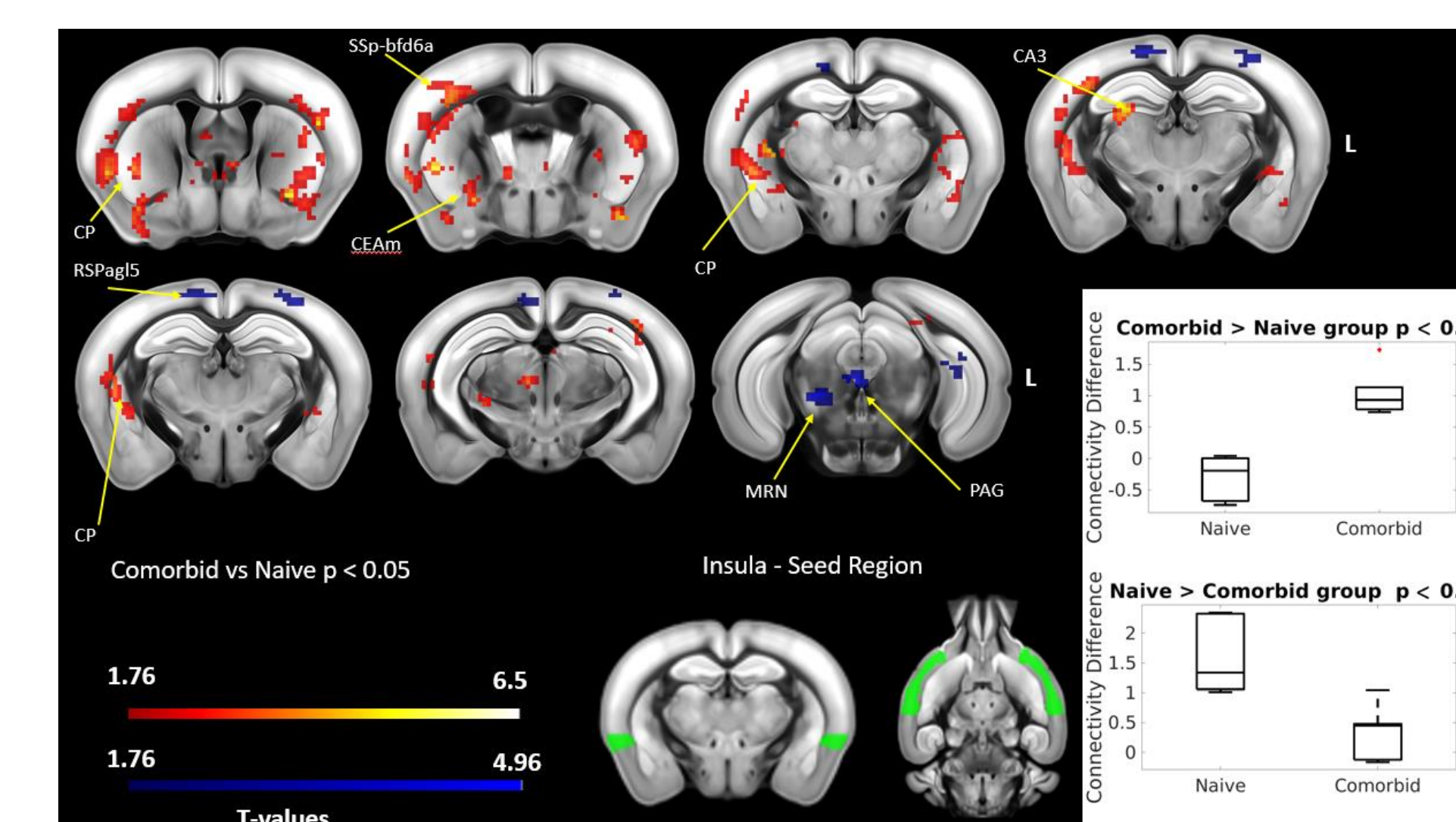
\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.005$ , \*\*\*\* $p < 0.0005$ , NS

## Network Results



**Figure 2. Network results.** Global Network Connectivity (brain images),  $z < 0.4$ . Differences in Node Strength and Node Degree (bar graphs). \* $p < 0.05$ , \*\* $p < 0.01$ . PPN, Pedunclopontine Nucleus; HPF, Hippocampal Formation; Su3, Supraoculomotor PAG. Alp6a, 6b, Agranular Insular area, posterior part, layer 6a, 6b. SSp-bfd5, 6a; Primary somatosensory area, layer 5, 6a.

## Seed-Based Results



**Figure 3. Insula Seed-Based correlation results.** Top: Brain regions with increased connectivity to insula in CPH females (hot colors) and naive females (cold colors). Box plots show values of connectivity differences between groups. Bottom: brain regions with increased connectivity to insula in CPH females (hot colors) and CPH females with EE (cold colors). Box plots show values of connectivity differences between groups.  $p < 0.05$

## Conclusions

These results demonstrate that EE can reduce referred pain and functional connectivity of areas involved in pain and stress processing, and may be strengthening the impaired endogenous pain inhibitory system seen in comorbid pain conditions. Further experiments are currently being done to fully investigate the effects of EE on comorbid pain conditions and potential sex differences.



We acknowledge the support of the Department of Neural and Pain Sciences (NPS), School of Dentistry; Center for Translational Research In Imaging (CTRIM); University of Maryland Baltimore; NIDCR.