Revealing microbiome-gut-brain interactions in opioid dependence

Anna M.W. Taylor[1], Kevin Lee[1], Helen Vuong[2], David Nusbaum[2], Elaine Hsiao[2], Christopher Evans[1]

[1]Department of Psychiatry, University of California, Los Angeles; [2]Department of Integrated Biology and Physiology, University of California, Los Angeles

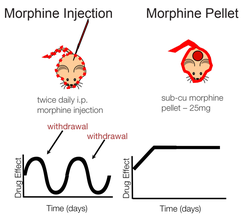
Introduction

- Opioids are highly effective analgesics for acute pain, but long term use is limited by adverse side effects such as constipation, hyperalgesia, and negative affect.
- Opioid-induced neuroinflammation contributes to these adverse effects, such as hyperalgesia and negative affect (Ferrini et al., 2013;Taylor et al. , 2016).
- Commensal bacteria that form the gut microbiome contribute to the development and function of microglia in the brain, and perturbations in microbiota are associated with abnormal microglial reactivity (Erny et al. , 2015, Sampson et al. , 2016).
- Changes in the gut microbiome are also associated with many psychological disorders, including depression and anxiety (Zheng et al., 2016). Changes in gut motility are known to influence the gut microbiome (Rhee et al. , 2009).
- Chronic opioid treatment has profound effects on gut motility during drug use and withdrawal, and initial empirical evidence suggests that opioids impact the gut microbiome (Banerjee et al. , 2016).

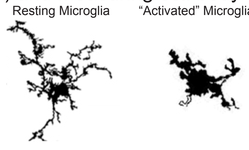
We hypothesize that opioid-induced changes in the gut microbiome contribute to the inflammation-driven hyperalgesia and negative affect associated with opioid withdrawal.

Methods

- Adult male C57Bl/6J mice were treated with either subcutaneous morphine pellet (25mg) or intermittent morphine injection (10-40mg/kg, i.p.) for 4 days.



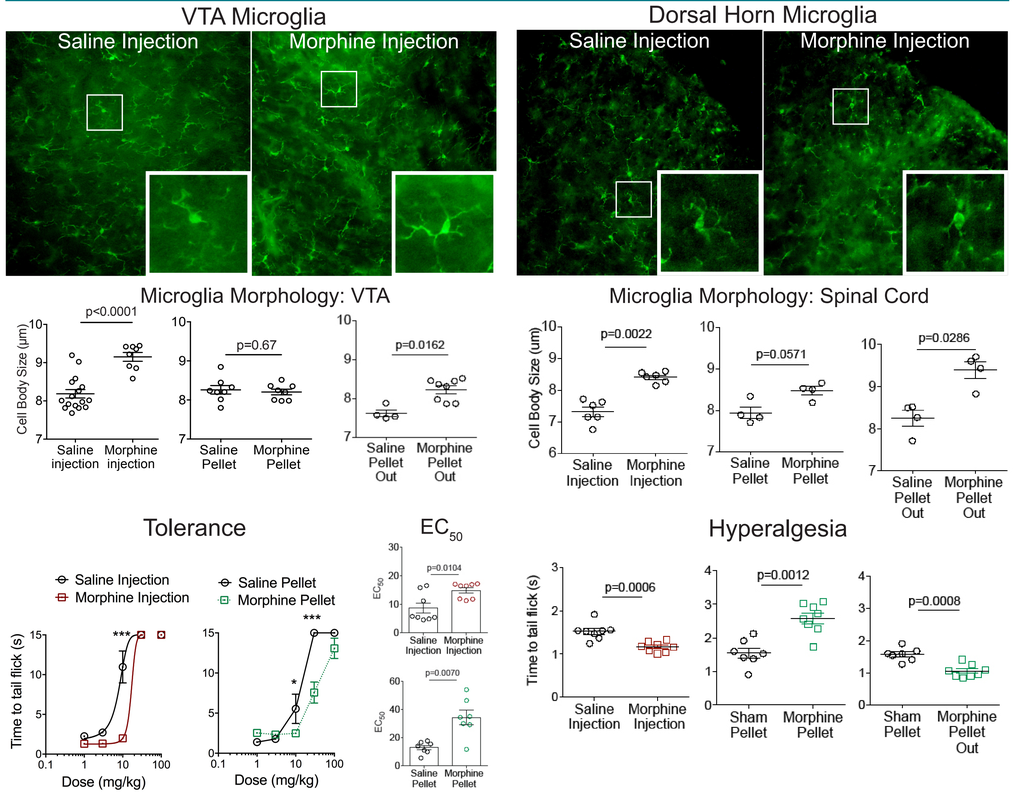
- Changes in microglia morphology were measured by immunostaining IBA1 in the ventral tegmental area (VTA) and measuring cell body size.



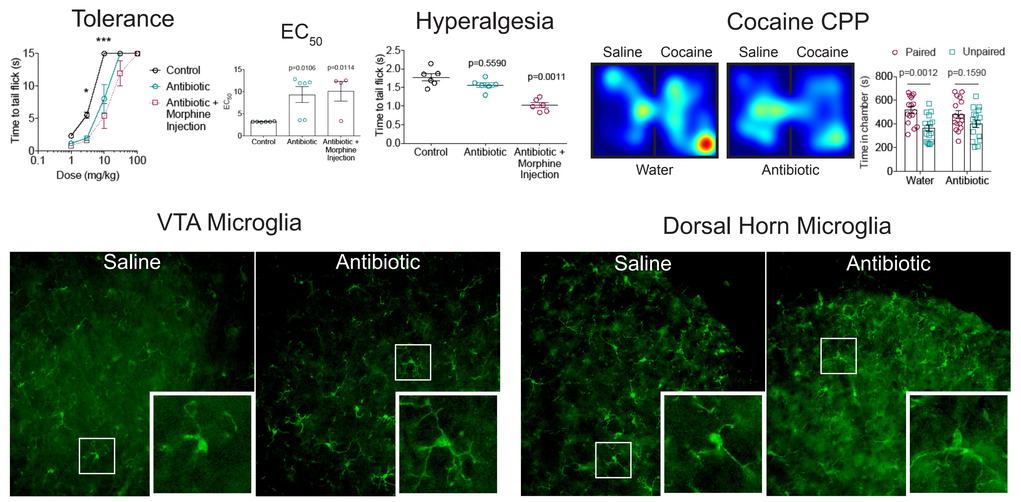
- Reward behavior was assessed in a balanced two-chamber conditioned place preference (CPP) apparatus, using cocaine (10mg/kg) as the conditioning stimulus.
- Opioid induced hyperalgesia was assessed using the tail flick assay (49°C water) in a drug free state. Morphine tolerance was assessed by measuring the analgesic potency of escalating doses of morphine (1, 3, 10, 30, 100 mg/kg, i.p.) in the tail flick assay.
- Gut microbiome was sampled by isolating fecal pellets collected from awake control and morphine dependent animals. Metagenomic DNA was extracted with Powersoil DNA Isolation Kit, and the V4 region of the 16s rRNA gene was amplified using barcoded primers. Sequencing was performed using an Illumina MiSeq. Operational Taxonomic Units (OTUs) were picked using the USearch pipeline with a similarity index of 97%, then assigned taxonomic classification using the basic local assignment search tool (BLAST)
- For microbiome depletion, animals were provided ampicillin (1g/L), vancomycin (0.5g/L), and neomycin (100mg/L) in sterile drinking water for at least 4 days.
- For gut microbiome recolonization, fecal pellets isolated from four mice treated with either escalating doses of morphine (10-40mg/kg, i.p.) or saline were isolated 12 hours after the last morphine injection, and homogenized in 0.1M PBS to create a fecal slurry for oral gavage, administered to drug naive mice pre-treated with oral antibiotics.

Results

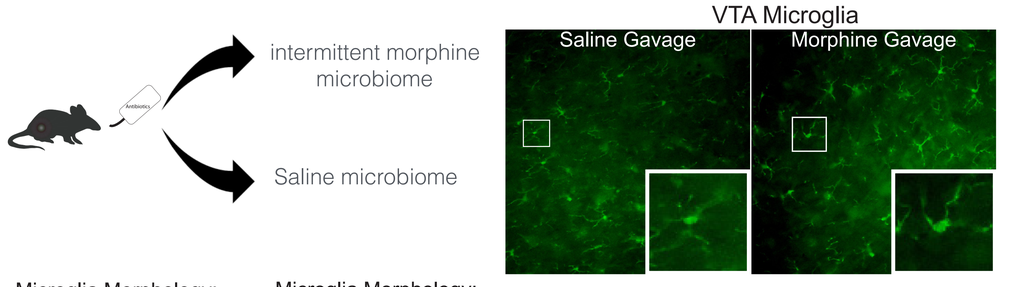
Intermittent morphine, but not sustained morphine, leads to changes in microglial cell body size, hyperalgesia, and negative impairments in reward behavior



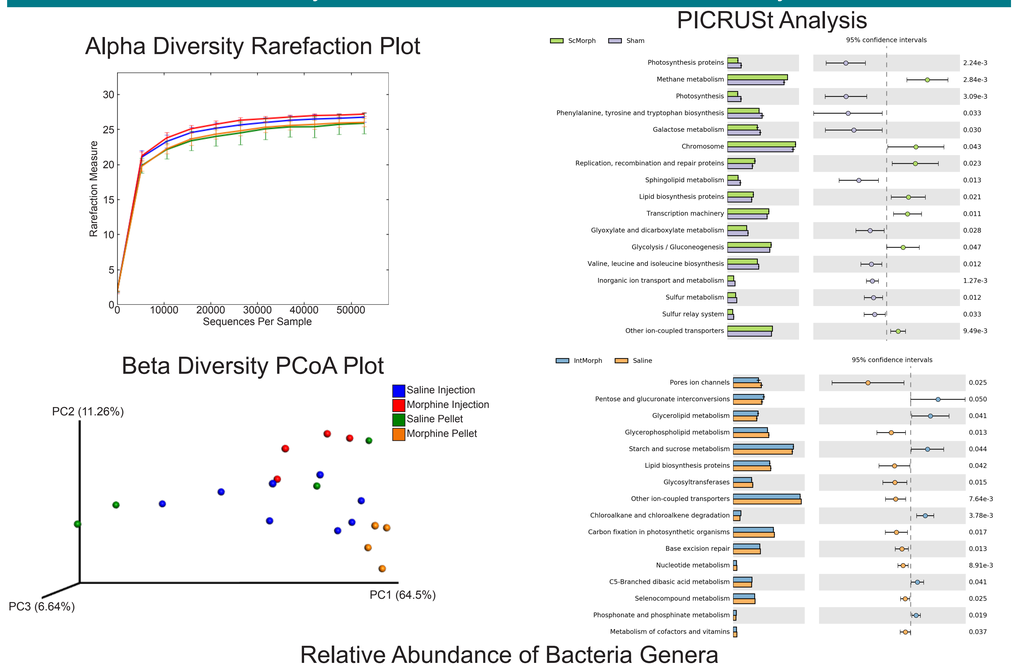
Depletion of the gut microbiome leads to changes in microglial cell body size, hyperalgesia, and negative impairments in reward behavior



Gut microbiome recolonization from drug-naive donors, but not morphine-dependent donors, restores normal reward behavior and decreases microglial cell body size

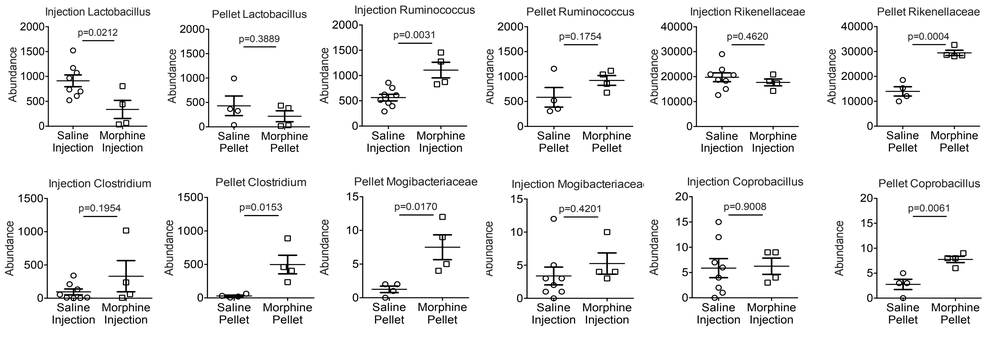


Morphine injection and morphine pellet mice show comparable levels of alpha diversity and distinct differences in beta diversity



Relative Abundance of Bacteria Genera

Relative abundance of bacteria genera in fecal samples islolated from sustained and intermittent morphine treated mice



Conclusions

- Opioid-induced perturbations in the gut microbiome causally relate to central inflammation, negative affect, and hyperalgesia.
- Repeated morphine withdrawa exacerbates microglial activation in the VTA and alters reward circuitry
- Therapies that restore healthy gut microbiome composition may help alleviate the side effects of opioid usage.

Acknowledgements

Funding provided by the NIH K99DA040016, The Shirley and Stefan Hatos Center for Neuropharmacology, and The American Pain Society.