



The Immunomodulating Effects of Morphine Dependence and Withdrawal in a Rat Model

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Introduction

The opioid crisis continues to pose a significant health burden, as evidenced by an increase in opioid overdose reports during the COVID-19 pandemic. Coinciding with the ongoing opioid epidemic and global pandemic is the increased prevalence of ultra-potent fentanyl, a trend responsible for more than 80,000 overdose deaths in 2021. Opioids can modulate the immune response following acute and chronic administration; however, the effect of opioid withdrawal on immune function is limited, with variable results.

Materials and Methods

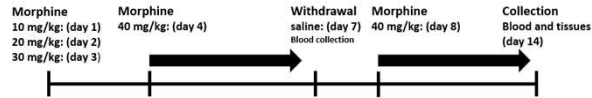


Figure 1. Rat addiction model
Administration of opioid dose to Wistar Rats, withdrawal at day 7, and collection at day 14.

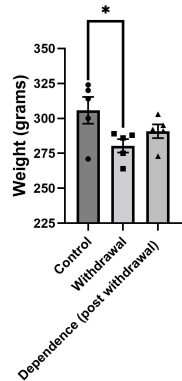
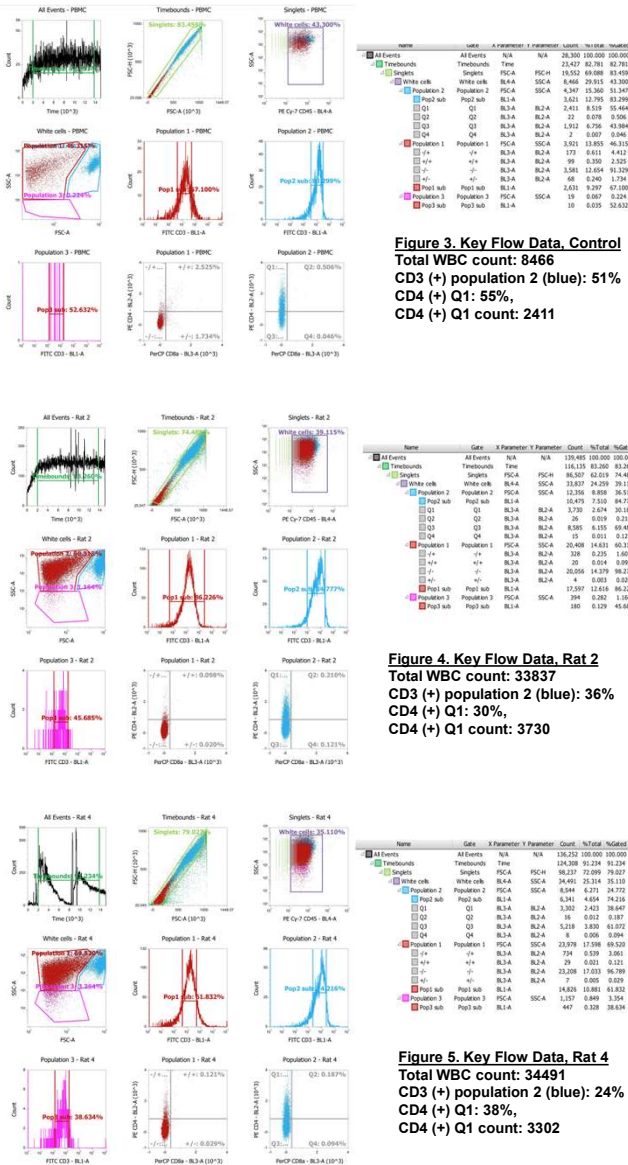


Figure 2. Rat weight
Rat weight measured in saline or morphine. Perhaps indicative of distress and unique underlying immuno-profiles.

Whole blood RBCs were lysed, cell pelleted and washed. The pellet was suspended in staining buffer. Fluorochromes include: FITC-CD3, PE-CD4, PerCP-CD8, and PE-Cy7-CD45. Samples were then run on an Attune NXT(BR) ThermoFisher Flow cytometer and data was analyzed.

Results



Gating Strategy and Control

Primary gating includes all events shown on a time histogram and selecting for events to avoid early carryover. Doublet events were excluded using an FSC-area:FSC-height dot plot. Singlets were defined as when fluorescent area most nearly approximated fluorescent height. A CD45:SSC-A gate excludes non-leukocyte cells. This gate can begin to demonstrate differences in white cells. Lymphocyte cells were plotted on FSC:SSC dot plot. The CD3 histogram demonstrates rat lymphocytes. CD4:CD8 quadrant plot divides lymphocytes into their roles as Cytotoxic T cells and Helper T cells. The PerCP-CD8 fluorochrome did not demonstrate any qualitative or quantitative brightness – which we have confirmed as an instrument anomaly; therefore, only CD4 counts were analyzed for a subset of rats.

Significant Findings and Conclusions

Rats in withdrawal increased absolute counts and lower CD4 relative lymphocyte counts. These findings indicate that morphine withdrawal alters the total number of cells and immunological profile of circulating leukocytes to a greater extent than the immunological profile during the morphine dependent state.

Opioid withdrawal may vary in onset, duration, and recovery. As such, the implications of immunoregulatory dysfunction in opioid dependent persons extend beyond treatment for opioid dependence and must include immunosurveillance in the context of infection.

Future Directions

Analysis of additional drugs and how they effect the immunoprofile is needed to elucidate immune function during withdrawal. Expand the flow cytometry panels and increase the overall number of animals.

| | Tube 1 | Tube 2 |
|-----------------------------------|-----------------|------------|
| Blue Laser | FITC | CD3 (5uL) |
| | PE | CD4 (5uL) |
| | PERCP-Cy5.5 | CD8 (5uL) |
| Red Laser | PE-Cy7 | CD45 (5uL) |
| | APC | CD45 (5uL) |
| | AlexaFluor700 | CD19 (5uL) |
| | APC-Cy7 | CD56 (5uL) |
| | Washed Rat WBCs | 50uL |
| | 50uL | 50uL (5uL) |
| Total Reaction volume before wash | 70uL | 70uL |

Figure 6. Reaction tube set up
Flow cytometry fluorochromes on a 2 laser 7 color ThermoFisher Attune NXT. Each reaction tube allows 3 additional fluorochromes to be added for further immunoprofile.