

NEUROINFLAMMATORY MODULATION OF NICOTINE DEPENDENCE

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ABSTRACT

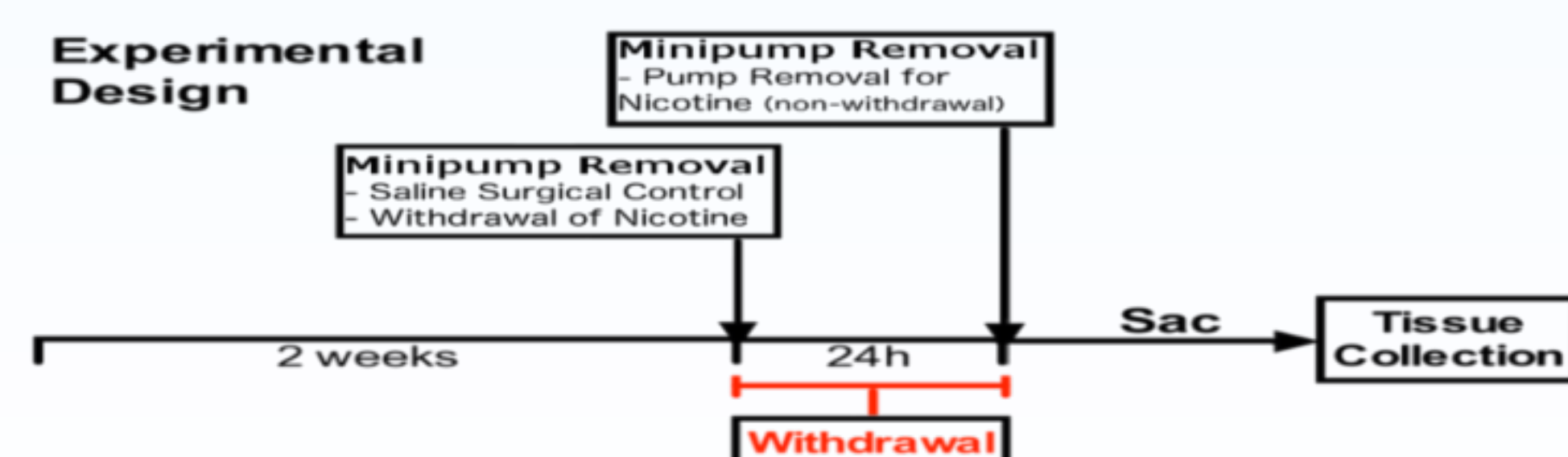
- ◆ Neuroinflammation via microglial activation may underlie the cognitive and affective withdrawal symptoms that lead to relapse.
- ◆ Suppression of microgliosis has been shown to significantly reduce opioid withdrawal and improve analgesia (Hutchinson et al. 2009)
- ◆ The striatum, and especially its ventral portion (nucleus accumbens), is a region that underpins many behavioral characteristics of both substance use disorders and withdrawal symptomology (Scofield et al. 2016).
- ◆ Microglia shift activity states from a surveilling or resting mode into an alerted or reactive state by environmental changes. Activated microglia initiate an inflammatory response via a classical M1 pro-inflammatory pathway producing TNF α , IL-6 or TLR4, or through an alternative M2 pathway producing anti-inflammatory cytokines such IL-10 and Trem2 (Cherry et al. 2014).
- ◆ **Our preliminary data indicate that significant neuroinflammation can be detected in the striatum and most acutely in the ventral striatum (nucleus accumbens) following withdrawal from chronic nicotine in mice.**
- ◆ **Furthermore, we demonstrate that minocycline, a second generation tetracycline known to have anti-inflammatory effects, modulates both the M1 and M2 cytokine mRNA levels in a brain-derived highly proliferative immortalized (HAPI) microglial cell line.**

METHODOLOGY

Microglial Cell Culture: HAPI cells were maintained in low glucose DMEM and 5% FBS for initial microglia and minocycline characterization.

Subjects: B6129F1 male mice (Taconic, age 6 weeks, 20-25g) were randomly assigned to saline, nicotine and nicotine withdrawal treatment conditions.

Nicotine Delivery: Nicotine tartrate (reported as free base) was dissolved in a 0.9% sterile saline solution and infused through subcutaneous osmotic minipumps for 14 days. Osmotic minipumps were filled with either saline or nicotine at 18 mg/kg/day, which yields a mouse plasma dose of ~0.3 μ m. Withdrawal was initiated by the physical removal of the minipumps with matched saline surgical controls 24 hours before sacrifice.



RESULTS

Figure 1. Morphology in Microglia Activation States

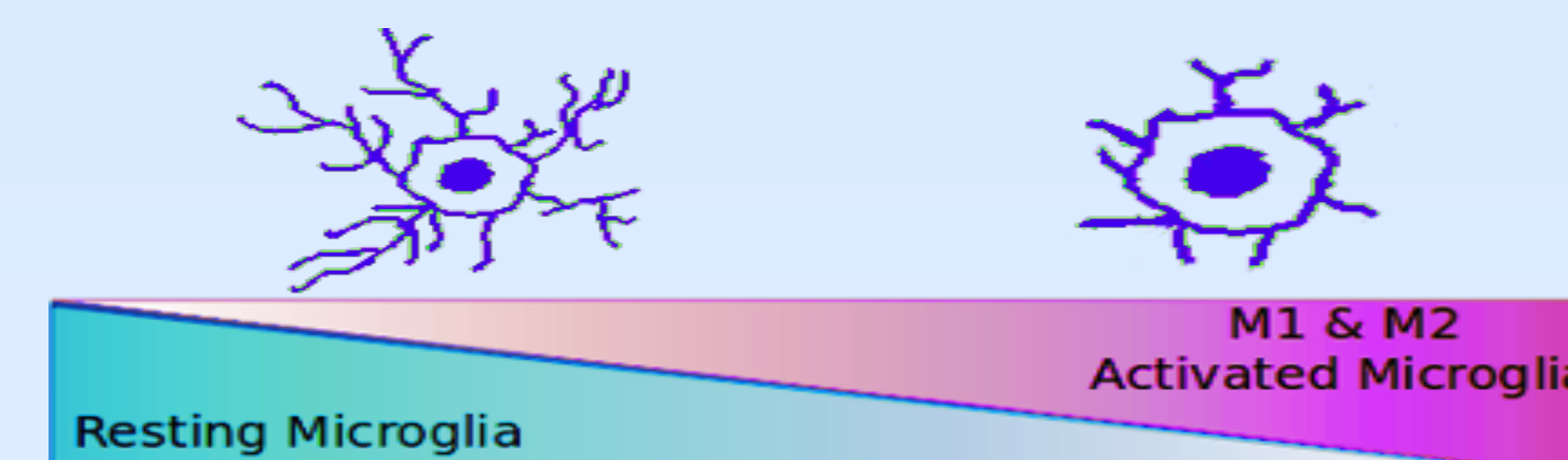
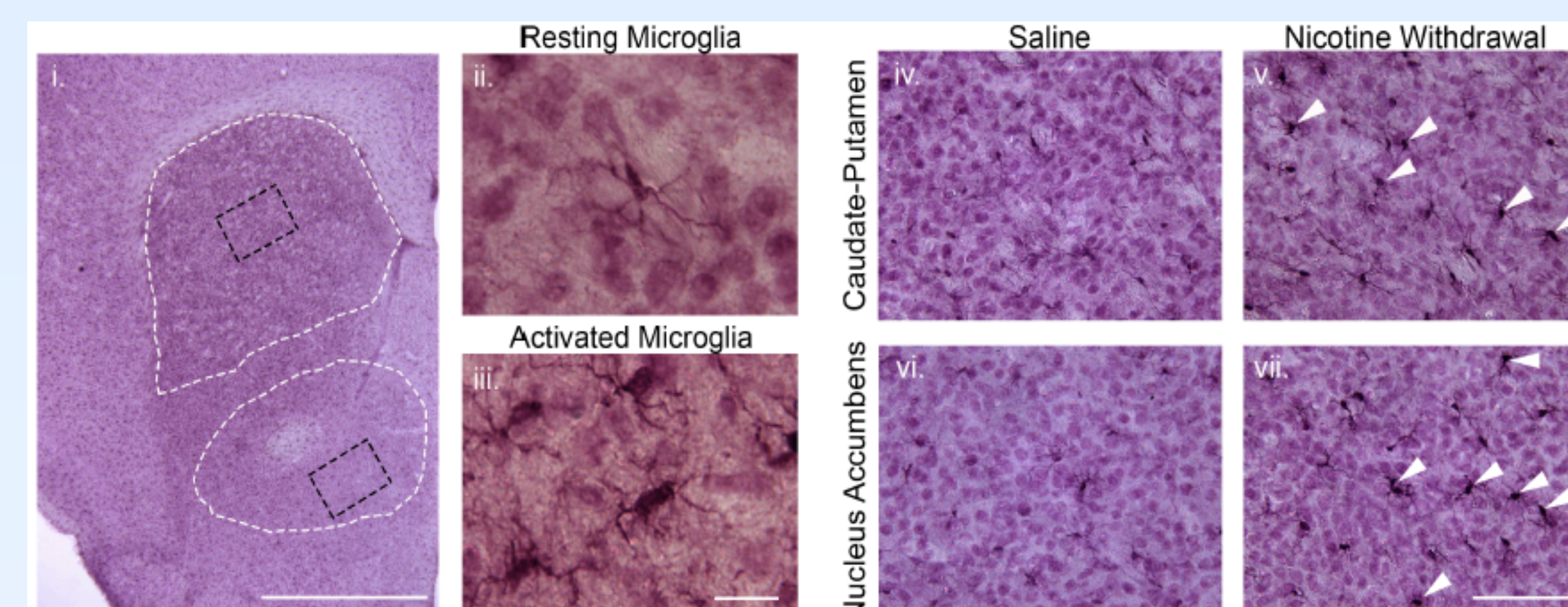


Figure 2. Nicotine Withdrawal Induces Microglia Pro-inflammatory Activation in the Striatum



Treatment	Level of Microgliosis	
	Caudate Putamen	Nucleus Accumbens
Saline	+ - ++	+ - ++
Withdrawal	++	++ - +++

Figure 3. Baseline Activation of M1 Pro-inflammatory Phenotypes

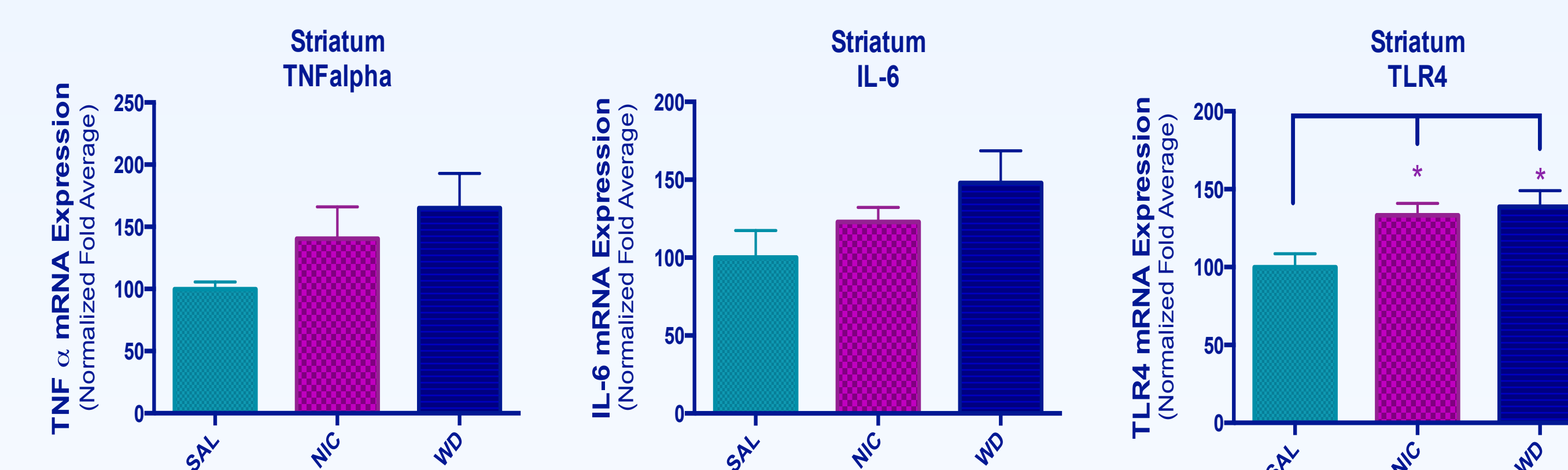
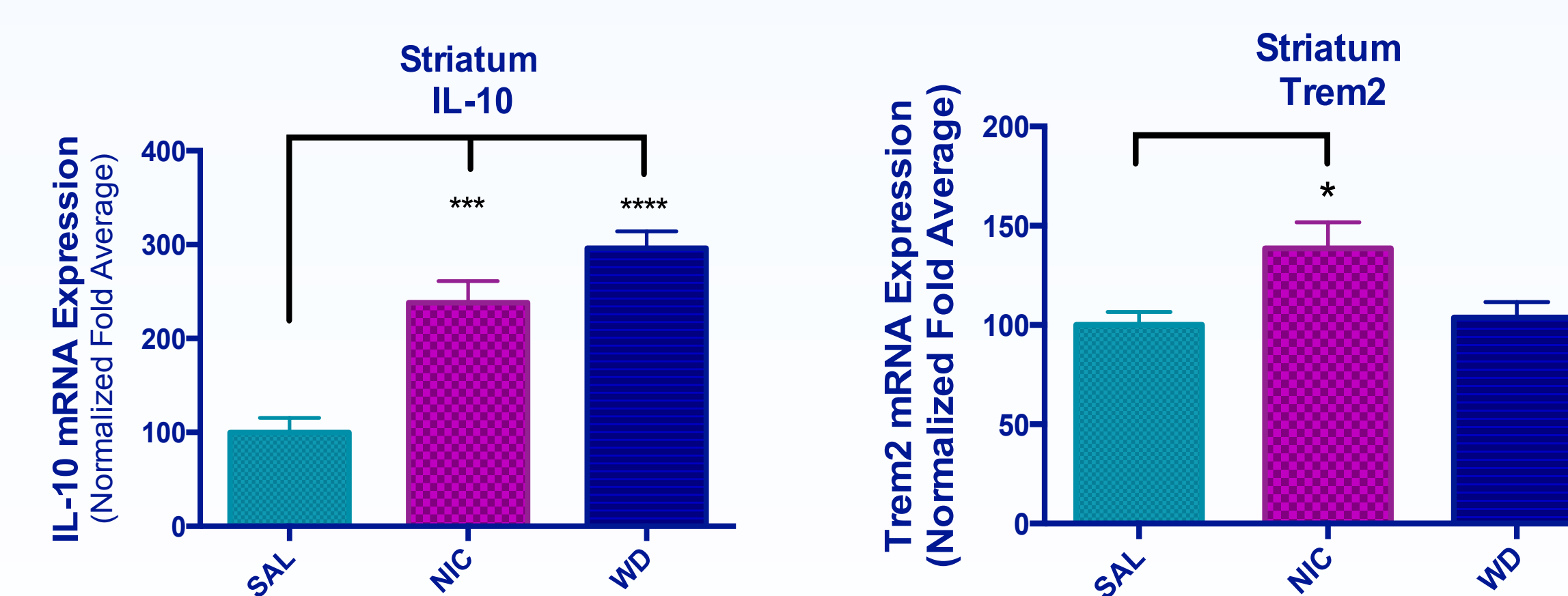
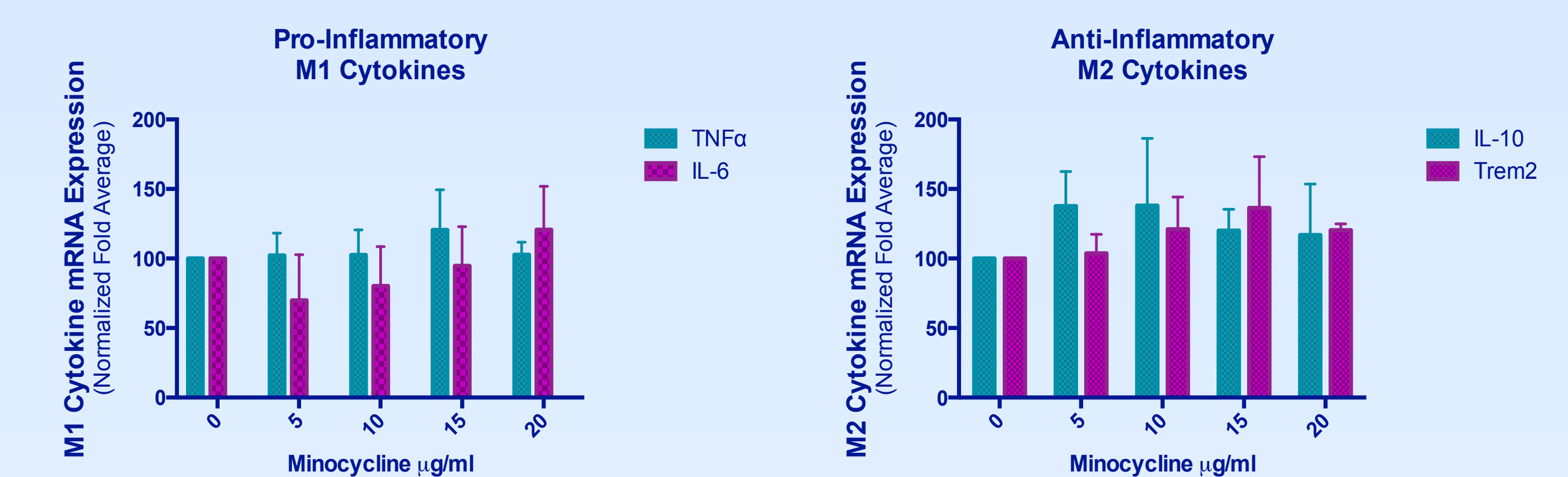


Figure 4. Baseline Activation of M2 Anti-inflammatory Phenotypes



RESULTS

Figure 5. Minocycline Dose Curve in HAPI Rat Microglial Cell Line



CONCLUSION

- ◆ Withdrawal from nicotine induces activation and remodeling in microglia as seen in the phenotypic characterization visualized by ionized calcium binding adapter molecule 1 (IBA1) staining.
- ◆ Within the striatum, the nucleus accumbens demonstrates the greatest inflammatory response during nicotine withdrawal.
- ◆ Baseline microglial activation of both the M1 and M2 pathways occurs during nicotine treatment and withdrawal.
- ◆ Early experiments with minocycline, a known M1 activation inhibitor, result in a trending change in both M1 and M2 cytokine levels in an immortalized microglial cell line.

FUTURE DIRECTONS

- ◆ Minocycline will be tested in vivo with the chronic nicotine/withdrawal paradigm to observe the microglial inflammatory response in both the M1 and M2 activation states.
- ◆ Behavioral testing will be carried out to monitor withdrawal-induced anxiety-like behavior using Open Field and Marble Burying tests.
- ◆ As a part of a quad-partite synapse, microglia communicate with both astrocytes and neurons. Cell type specific sequencing will be used to interrogate the molecular changes in the transcriptome for both activated microglia and astrocytes.

ACKNOWLEDGMENTS

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