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The Effect of Two Novel Anti-Inflammatory Drugs on Sensorimotor Gating and Microglial Activation in the Poly I:C Rodent Model of Schizophrenia

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Introduction

What is Schizophrenia (SCZ)?

- Chronic & debilitating neurobehavioral disorder
- Affects estimated 21 million people worldwide (WHO Statistics Sheet, 2018)
- Age at onset observed in **adolescence** or **early adulthood**
- Diagnosis based on clinical observation or self-reporting (Gejman et al., 2010)
- Costs U.S. approx. $62 billion annually for medications & other therapeutic expenses

Current Treatment

- **Antipsychotic Medications**
  - Typical – dopamine D_2 antagonists (e. g. Haloperidol)
    - Attempts to treat associated positive symptoms
  - Atypical – dopamine D_2 antagonists; act on histamine, norepinephrine, & serotonin (e. g. Clozapine, Olanzapine, & Risperidone)
    - Attempts to treat associated positive & negative symptoms
- **Psychosocial Interventions**
  - Individual & family therapy, social skills training, and vocational rehabilitation

Problems with Current Treatment

- Typical antipsychotic drugs (FGA)
  - Not designed to treat (-) symptoms
  - **Potent extrapyramidal motor side effects**

Haloperidol

- Atypical antipsychotic drugs (SGA)
  - **Dose-dependent side effects** (Solmi et al., 2017)

    Weight gain, insulin resistance/diabetes, cognitive impairment, agranulocytosis, seizures, pneumonia, myocarditis, & hypersalivation.
  - **Short time to discontinuation**

Clozapine

Neuroinflammatory Aspect

- SCZ patients shown to have **increased inflammation in CNS** (Howes & McCutcheon, 2017; Van Kesteren et al., 2017)

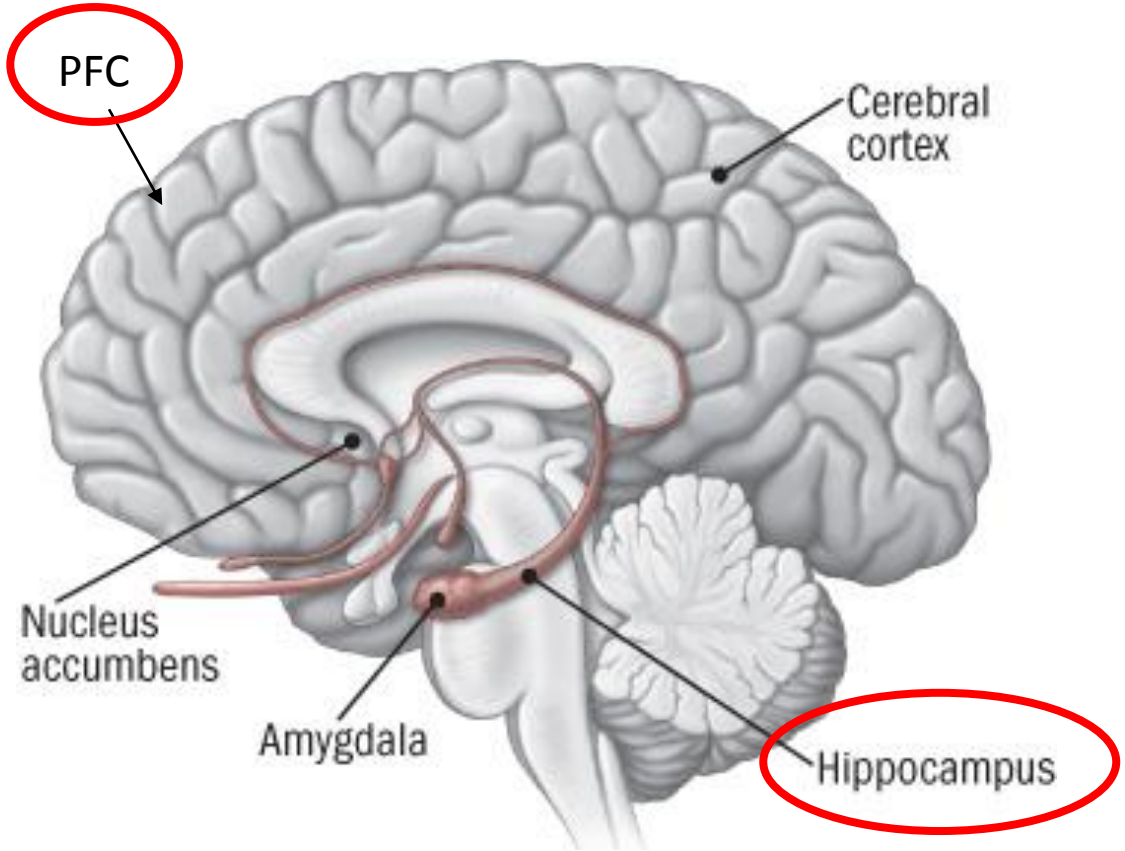

Image adapted from Harvard Health Publishing

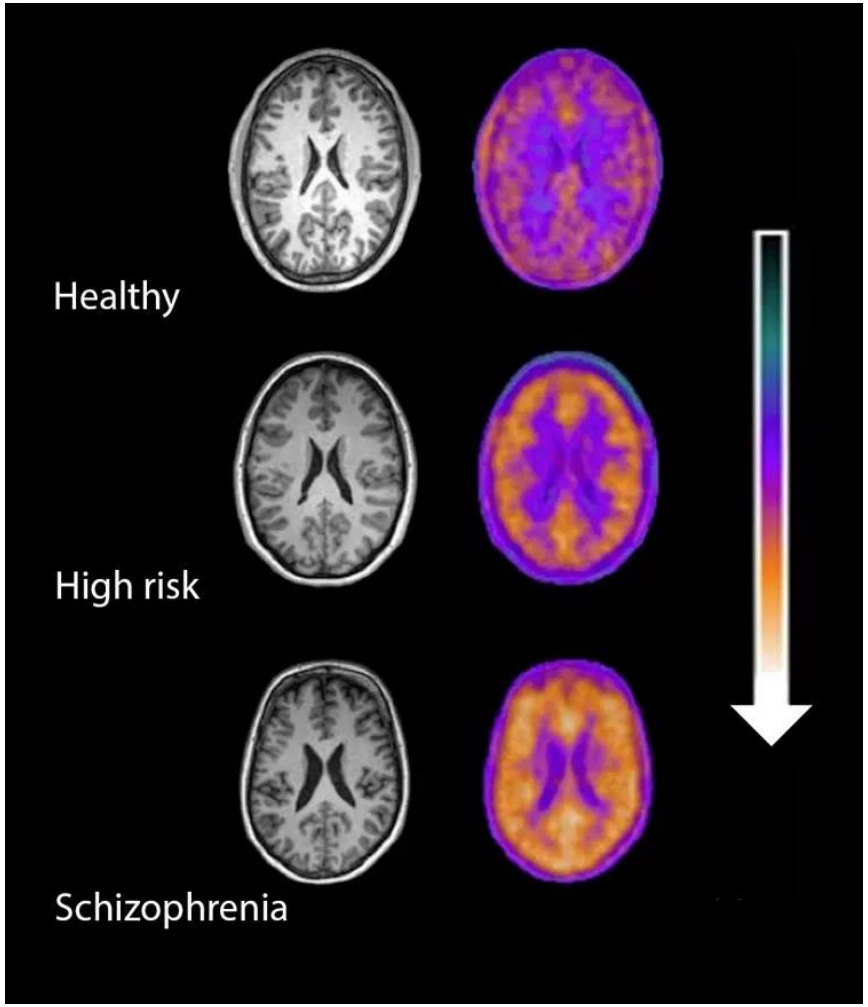

Image adapted from Bloomfield et al., 2016

Tumor Necrosis Factor-alpha (TNFα)

- Pro-inflammatory cytokine
- Implicated in some autoimmune diseases (ex. RA)
- Influence state of CNS defense cells, called **microglia**

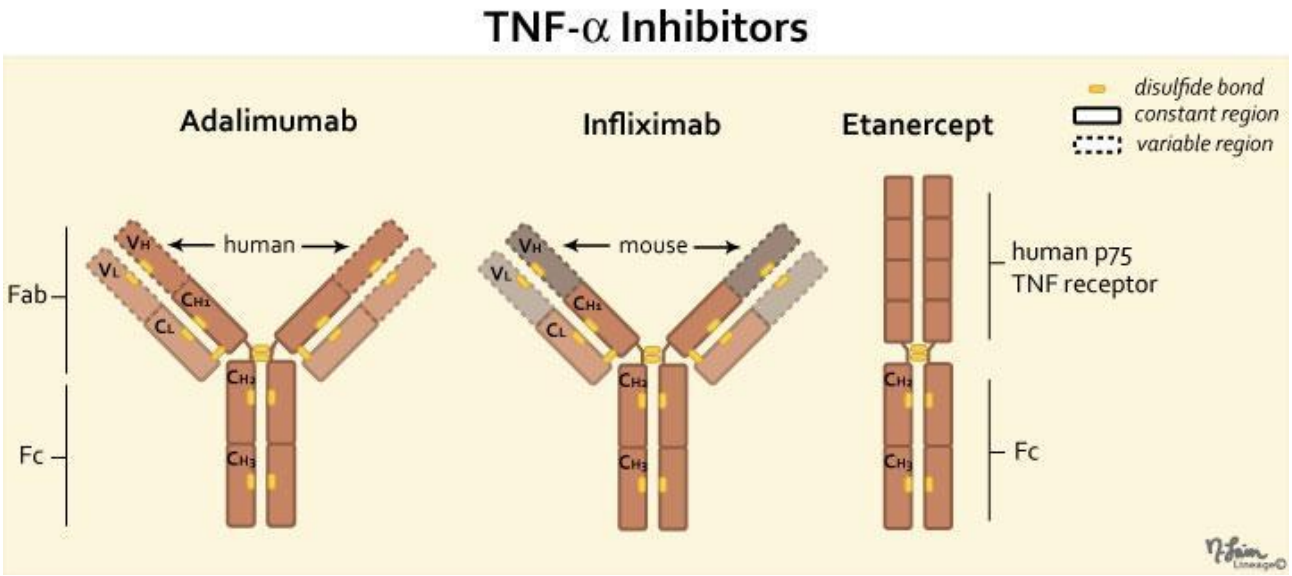

Image from MEDBULLETS:STEP 1

Microglial Cells

- Primary immune cells of the CNS & scan local environment for cellular stress (Nimmerjahn et al., 2005)
- Normally exist as anti-inflammatory, neuroprotective agents (M2 state)
- Upon activation by TNFα secretion, switch to M1 state, which is pro-inflammatory & neurotoxic
- **Activated M1 microglia** leads to overexpression of pro-inflammatory cytokines (ex. TNFα) & ROS, resulting in synaptic loss & neuronal death (Howes & McCutcheon, 2017)

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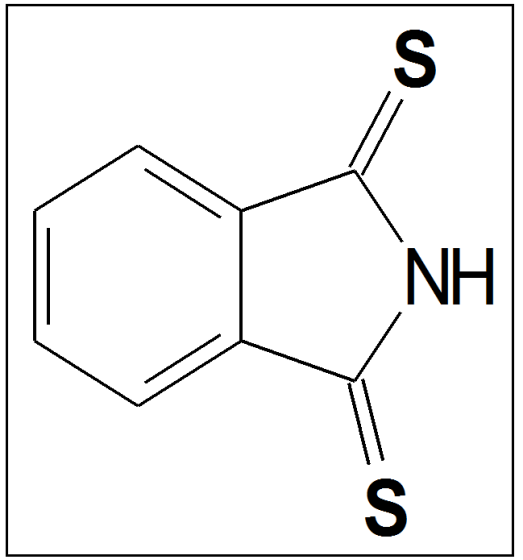
TNFα Modulator Development



PD2024
M_w = 179.0 g/mol
TNFα IC_{50} = 3 μM

- Isoindoline-derived compounds
  - PD2024 & PD340
- Small, anti-TNF molecules
  - **Destabilizes TNFα mRNA**
    - Decreases TNFα protein release & secretion
- Centrally-acting, anti-neuroinflammatory properties
  - Safe & well tolerated in small (rats) and large (dogs) animals

Polyinosinic:polycytidylic Acid (Poly I:C)

- Immunostimulant
- Interaction with TLR3
- Synthetic dsRNA (virus-like)

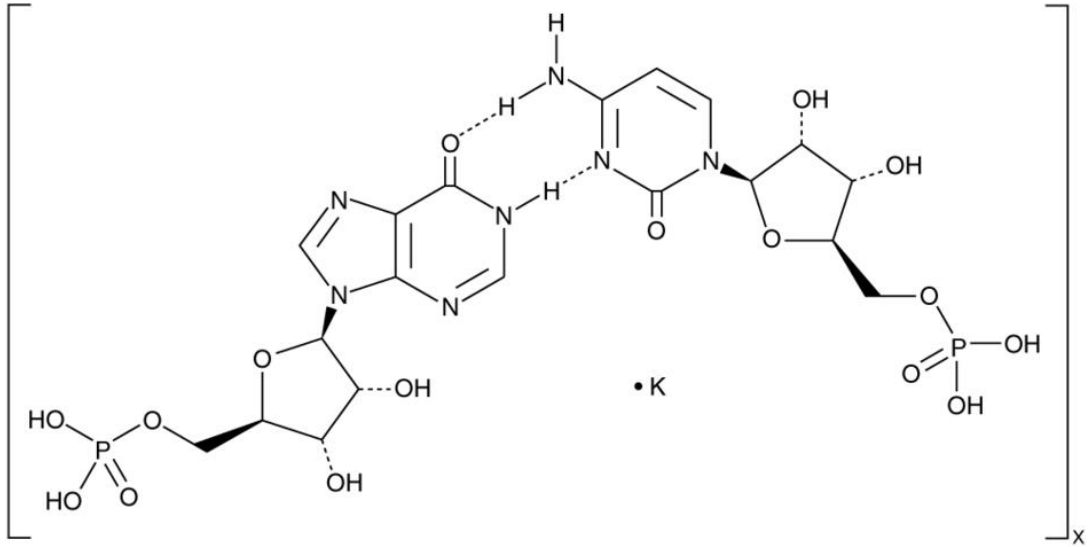


Image from: https://www.biomol.com/product_Polyinosinic-polycytidylic-Acid-potassium-salt.html?aRelated=Cay16881

- **Activates innate immune system**
- **Mimics neonatal infection in humans**

Poly I:C Rodent Model of SCZ

- **Behavioral deficits/neuropathology consistent with SCZ**
  - Sensorimotor gating
  - Cognitive
  - Dopamine hyperfunction
  - Structural abnormalities (cortical volume reduction in PFC & HPC) (Meyer, 2014)
- Symptoms emerge in offspring, reflects delayed onset as seen in humans
- Clozapine & Risperidone alleviate deficits in neonatally treated poly I:C rats

Hypotheses

1. Treatment with Poly I:C will increase TNFα protein levels similar to the neuroinflammatory response for individuals diagnosed with schizophrenia

2. Novel TNFα modulators will alleviate sensorimotor gating deficits of the rodent Poly I:C model

3. Novel TNFα modulators will reduce associated neuroinflammation via a decrease in microglial cell activation levels in the HPC and PFC, two brain areas that mediate sensorimotor gating

Experimental Design

Study Design: Experiment 1 – TNFα Protein Levels

- A total of 17 male Sprague-Dawley pups IP injected with either Poly I:C (2 mg/kg) or saline (0.9% NaCl) from P5-7
- Sacrificed at P30 (in accordance with Exp. 2 & 3 dietary manipulation)
- PFC & HPC dissected away
- Tissue subjected to **TNFα ELISA kit (Biomatik, Inc.; Wilmington, DE)**
  - Protein detection via colorimetric detection (450 nm)

Study Design: Experiment 2 – PD2024

Grouping & Conditions

- SD pups divided equally into 4 groups

(Poly I:C/Control, Poly I:C/PD2024, Saline/Control, Saline/PD2024)

- Poly I:C groups IP injected with Poly I:C (2 mg/kg) from P5-7
- Saline groups IP injected with saline (0.9% NaCl) from P5-7
- All animals weaned at P21, dietary manipulation began at P30
- PD2024 groups received PD2024 until P67
- Control groups received a normal diet until P67

Study Design: Experiment 3 – PD340

Grouping & Conditions

- SD pups divided equally into 6 groups

(Poly I:C/Control, Poly I:C/PD340 – 10 mg/kg, Poly I:C/PD340 – 30 mg/kg, Saline/Control, Saline/PD340 – 10 mg/kg, Saline/PD340 – 30 mg/kg)

- Poly I:C groups IP injected with Poly I:C (2 mg/kg) from P5-7
- Saline groups IP injected with saline (0.9% NaCl) from P5-7
- All animals weaned at P21, dietary manipulation began at P30
- PD340 groups received PD340 (10 mg/kg or 30 mg/kg) until P67
- Control groups received a normal diet until P67

Study Design: Experiments 2 & 3

Prepulse Inhibition (PPI)

- Behaviorally tested on PPI
  - During adolescence (P44-46) and adulthood (P60-66)

Sacrifice & Immunohistochemistry (IHC)

- Sacrificed at P67, PFC & HPC dissected away, subjected to IHC
- IHC examined microglial cell activation using the Iba1-GFP conjugated antibody system

Prepulse Inhibition (PPI)

Used to measure auditory sensorimotor gating

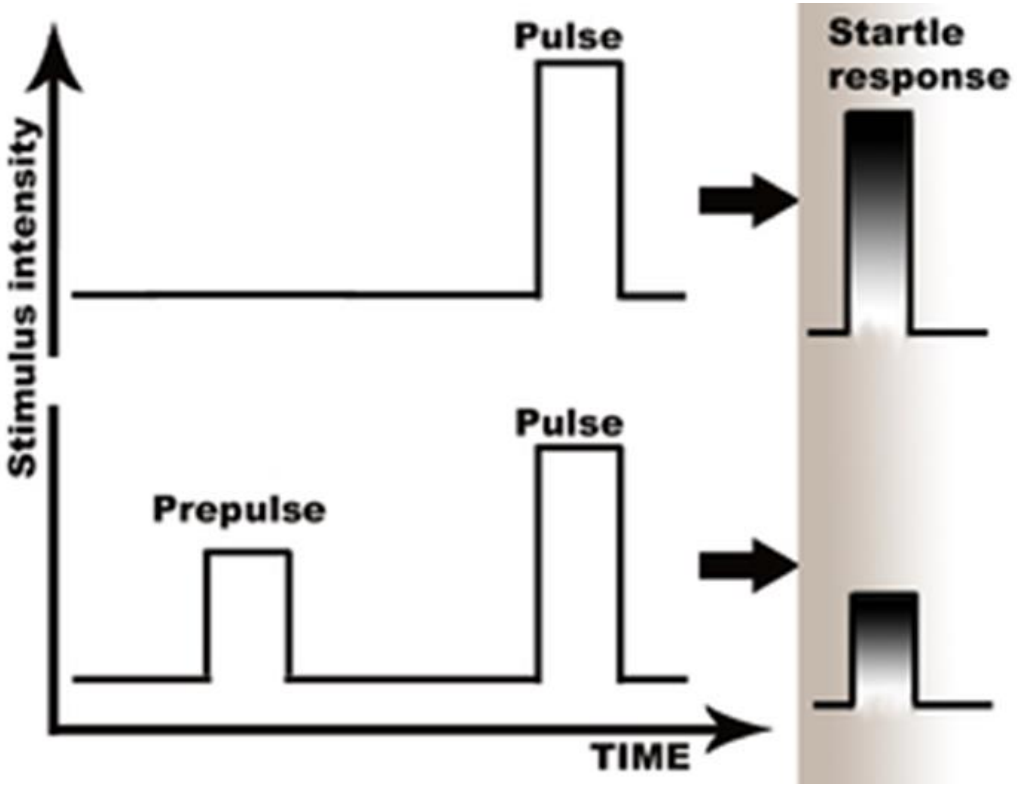


Image from: https://en.wikipedia.org/wiki/Prepulse_inhibition#/media/File:Prepulse_Inhibition_schematically.png

Immunohistochemistry (IHC)

Detection technique, selectively identifies protein(s) in cells

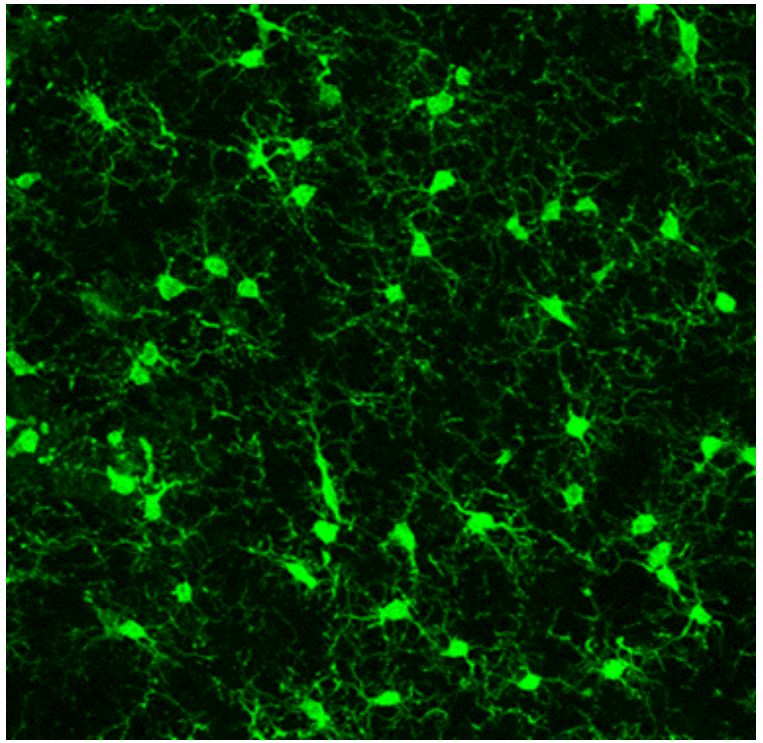


Image from Exp. 3

Results

Figure 1. TNFα Protein Levels Following Saline or Poly I:C Administration Between P33-35.

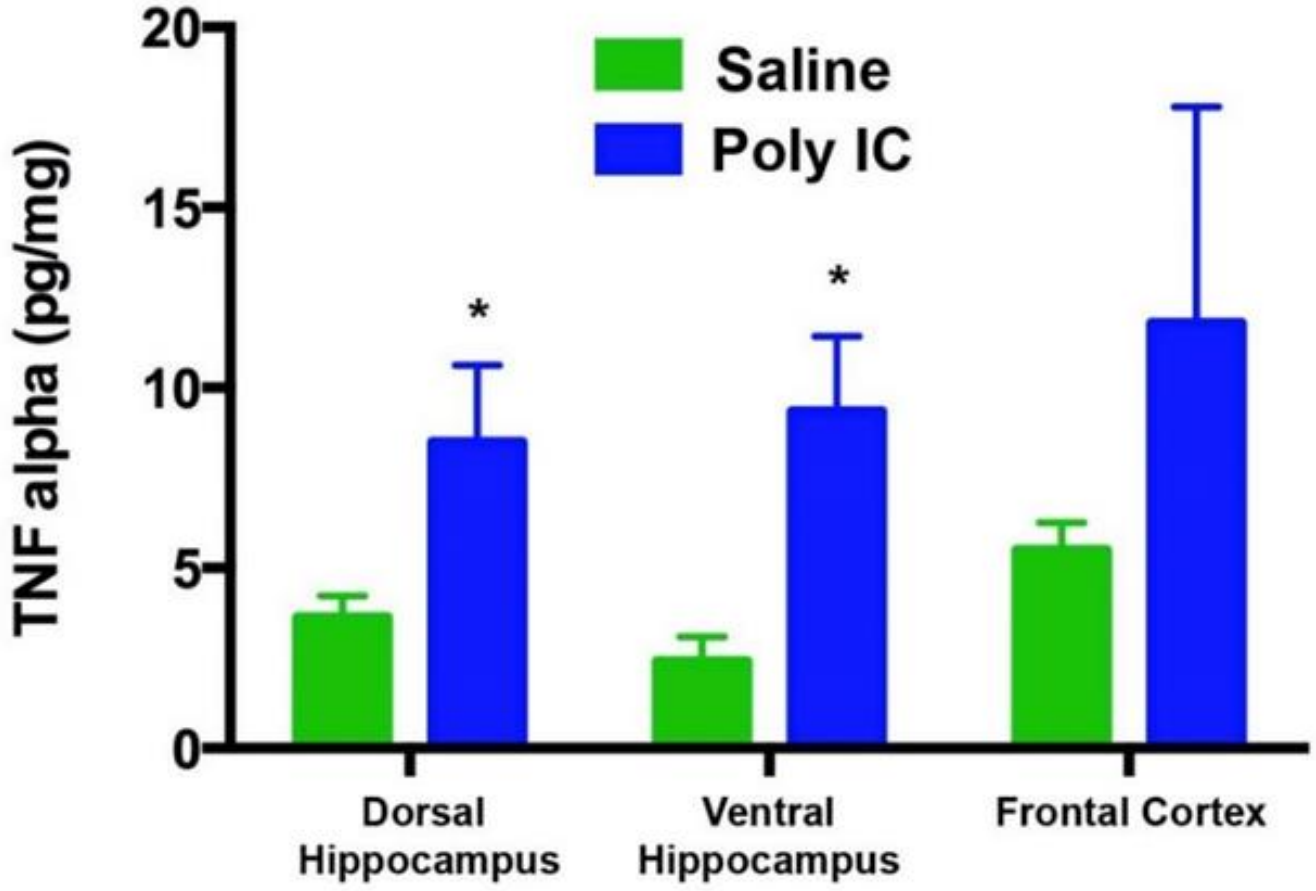


Figure 2. Experiment 2: PPI Performance in Adolescents and Adults.

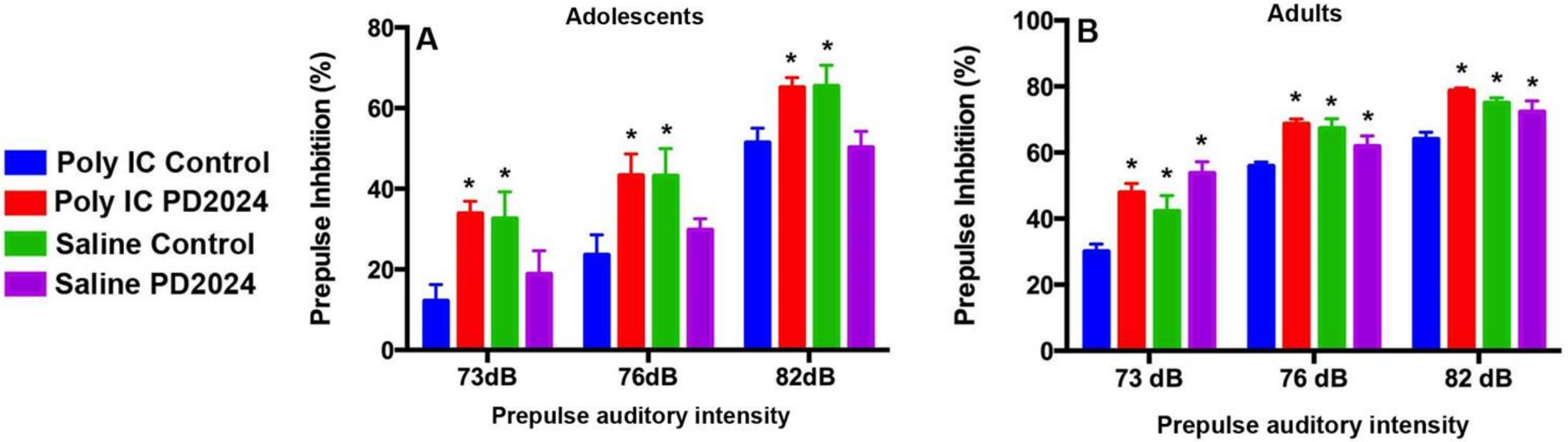


Figure 3. Experiment 2: Microglial Cell Activation in the PFC and HPC.

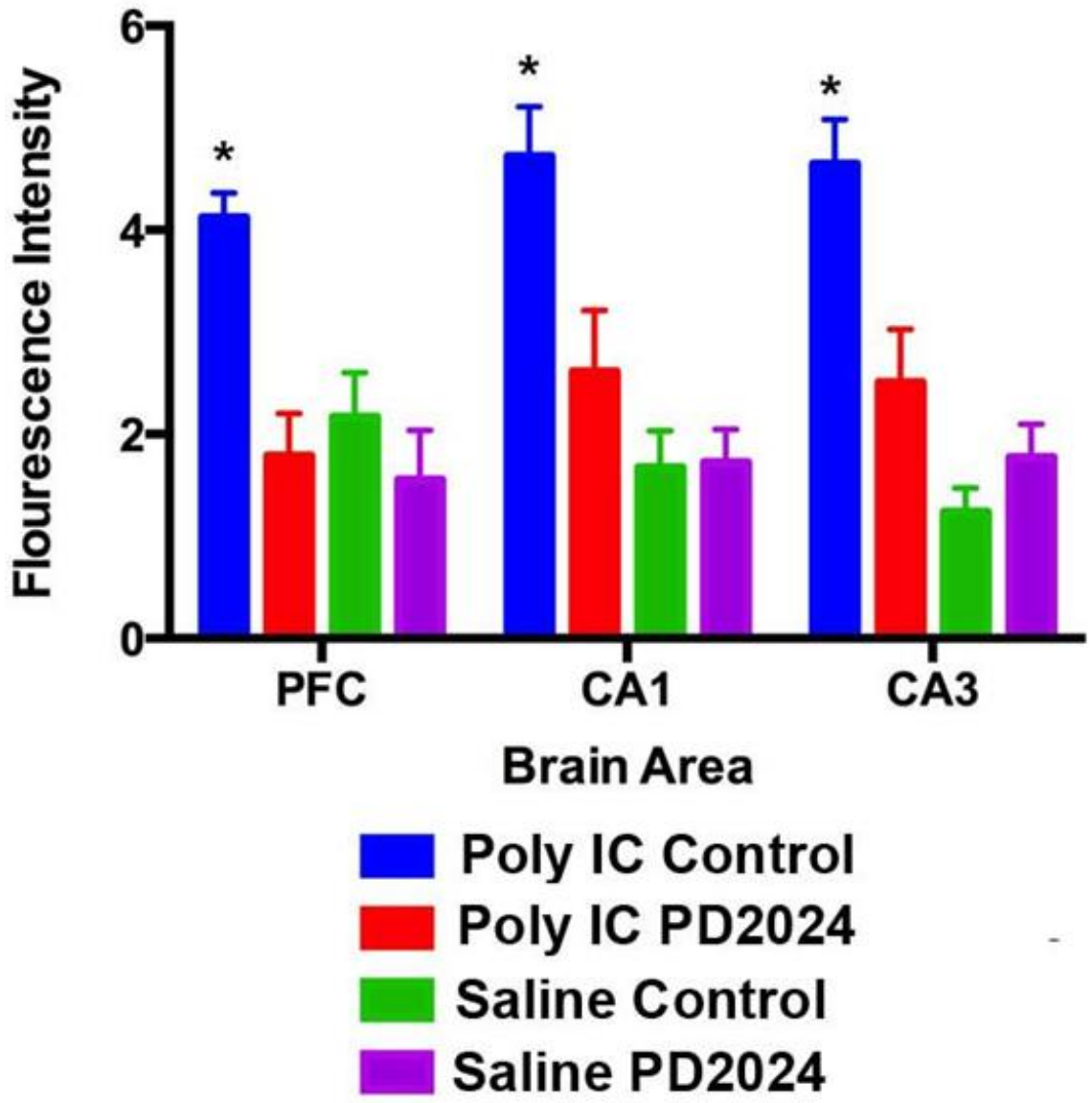


Figure 4. Experiment 2: Representative Images of Iba1-GFP Labeled Microglia Cells in the PFC.

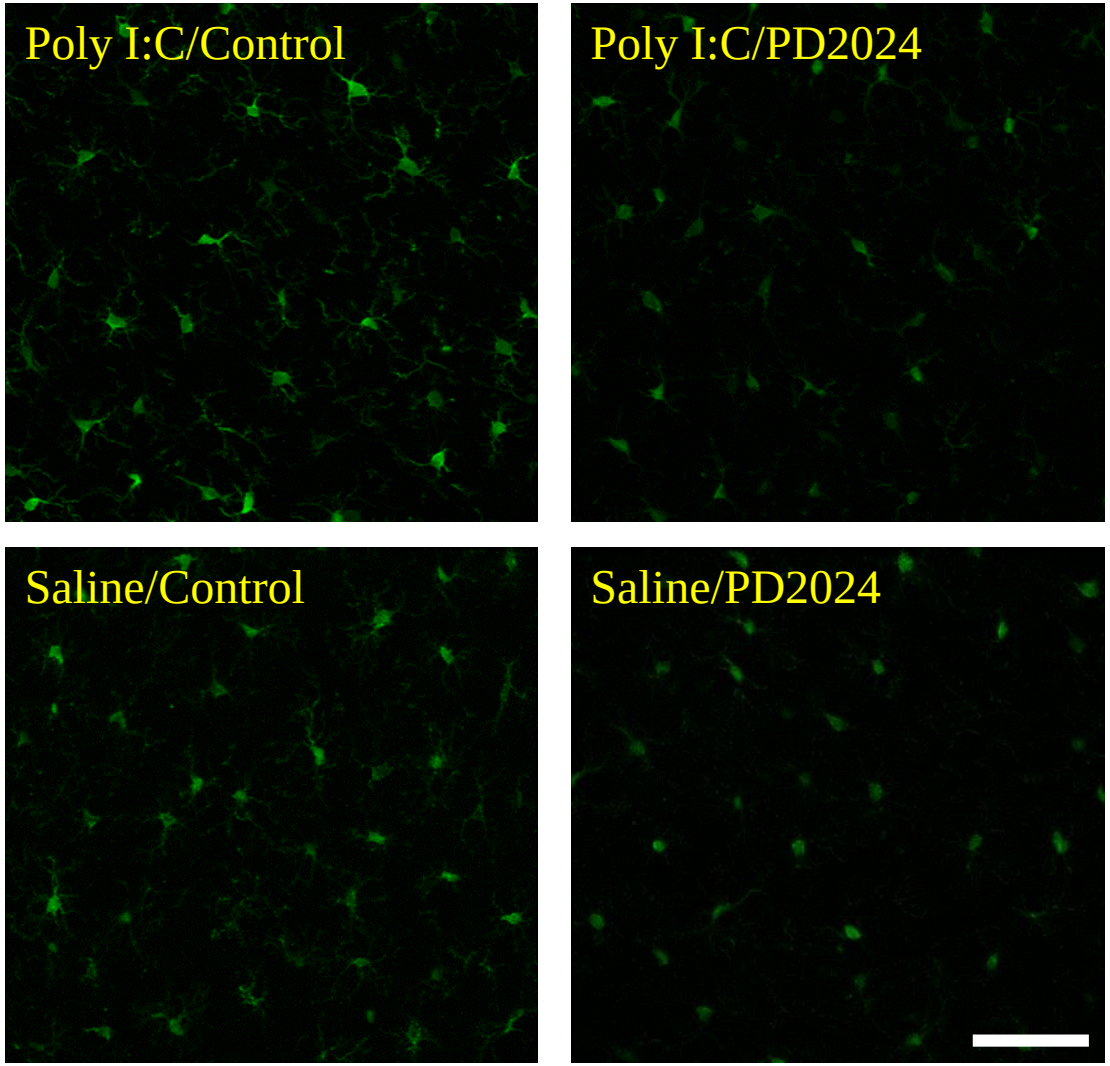


Figure 5. Experiment 3: PPI Performance for Adolescents and Adults.

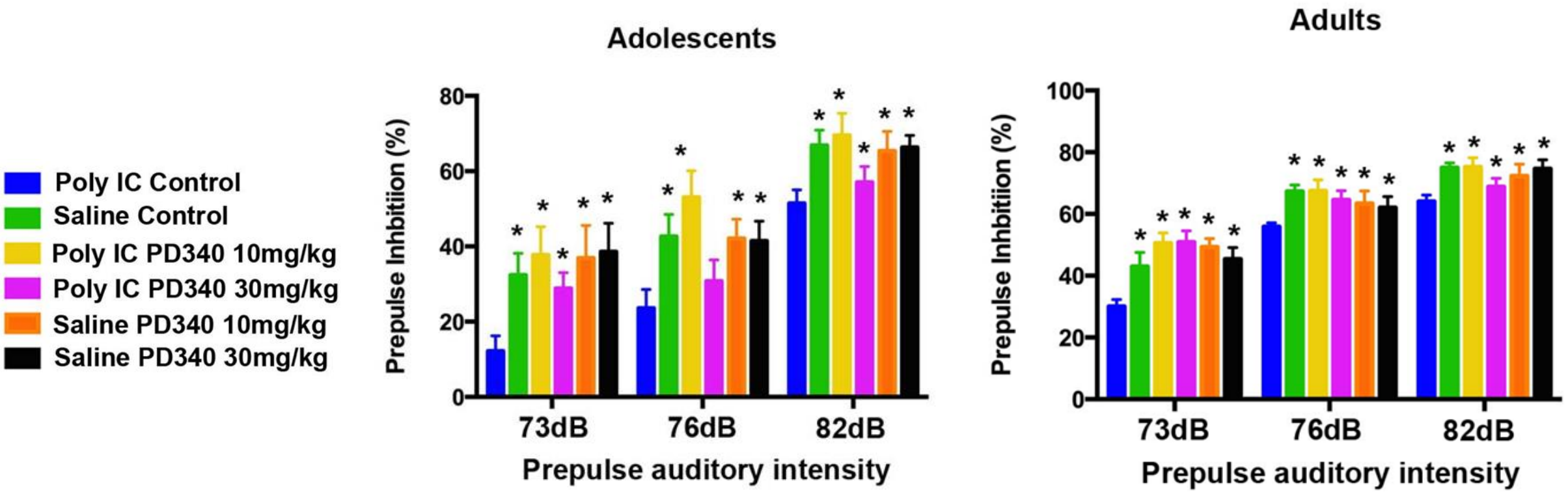
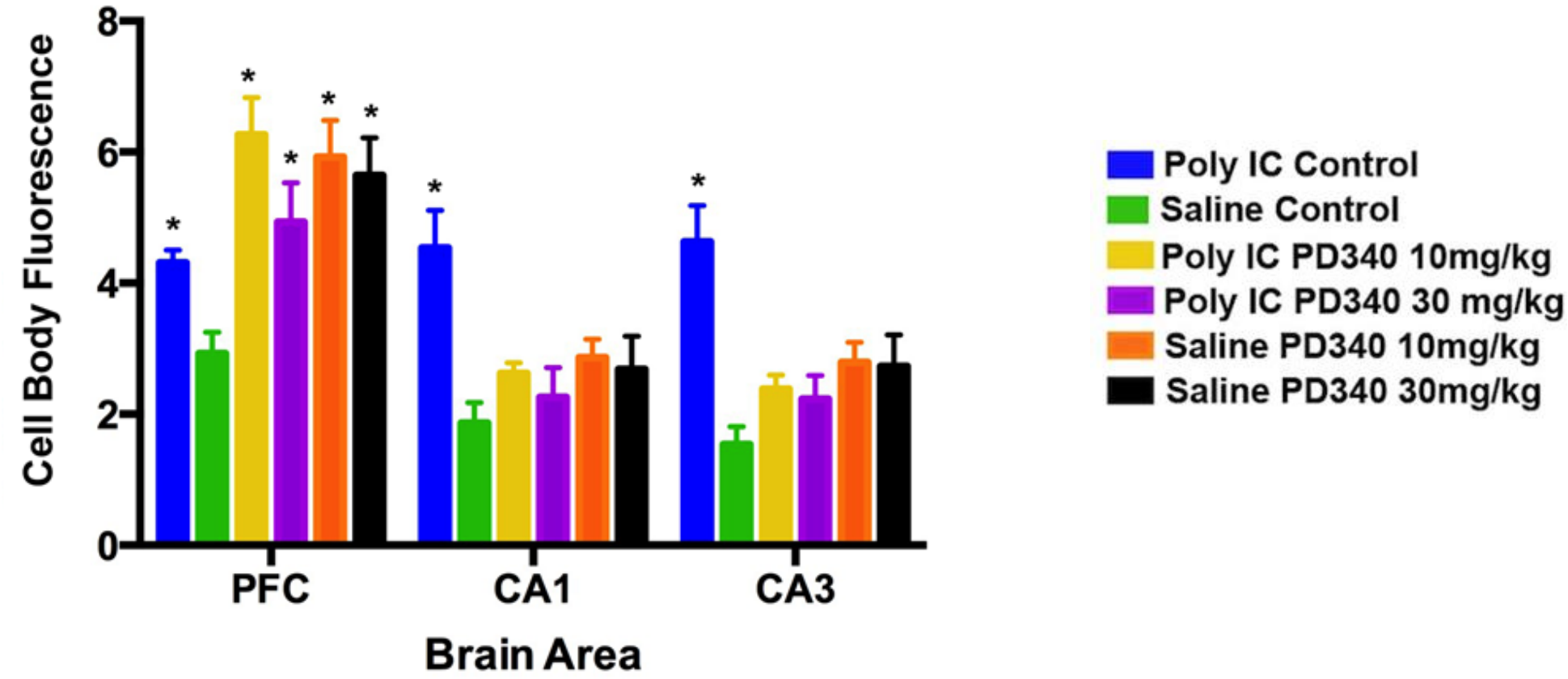


Figure 6. Experiment 3: Microglial Cell Activation in the PFC and HPC.



Conclusions & Future Directions

Conclusions

- Neonatal Poly I:C **resulted in** behavioral deficits in adolescence & adulthood, consistent with clinical observation & diagnosis of SCZ.

- Two novel TNF-alpha modulators (PD2024 & PD340) **alleviated** sensorimotor gating deficits in adolescence and adulthood.
  - Decreased microglial cell activation known to mediate sensorimotor gating

Future Work & Directions

- PD2024 and PD340 adjunctively used with antipsychotic drugs in the Poly I:C and other rodent models of SCZ.

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Journal

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Questions?