

# Isflurane Preconditioning Protects Astrocytes and Neurons from Oxygen and Glucose Deprivation Independent of Innate Cell Sex

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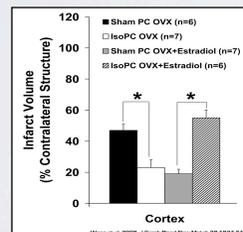
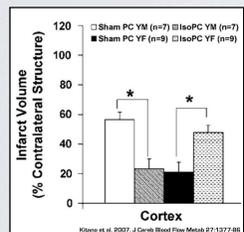
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## INTRODUCTION

Isflurane exposure can protect the mammalian brain from subsequent insults such as ischemic stroke. However, this protective preconditioning effect can be sexually dimorphic, with isflurane preconditioning (IsoPC) decreasing brain damage in young males compared to young females in a mouse model of ischemic stroke (**Background Figure 1**). Sex steroids like estradiol are partially responsible for this sexual dimorphism (**Background Figure 2**). Emerging evidence suggests that innate cell sex is an important factor in brain cell death, with male and female cells having unique sensitivities to various insults, but the role of innate cell sex in the response to IsoPC has not been investigated.

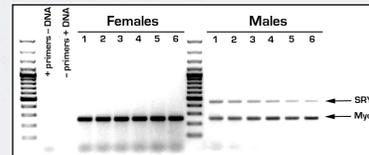
**Background Figure 1:** IsoPC protection is sexually dimorphic in young male (YM) and young female (YF) ischemic brain

**Background Figure 2:** Estradiol abolishes IsoPC protection in ovariectomized (OVX) and young female (YF) ischemic brain

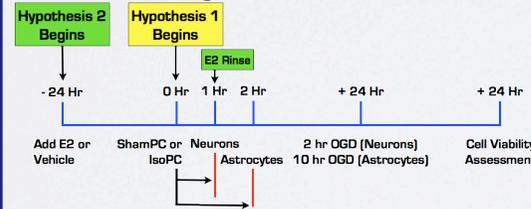


## METHODS

- Used sex-segregated cortical cultures from postnatal day 0-1 C57BL/6 mice
- Sex segregation based upon larger ano-genital distance in male pups
- IsoPC** = 3% isflurane, 92% medical air, 5% CO<sub>2</sub>
- Sham Preconditioning (ShamPC)** = 0% isflurane, 95% medical air, 5% CO<sub>2</sub>
- Preconditioning was done when astrocytes were confluent or when neurons reached day 9 in vitro
- Cell viability quantified with lactate dehydrogenase release (astrocytes) or calcein fluorescence (neurons).
- PCR confirmation of sex segregation done using the male-specific Sry gene and the autosomal Myog gene



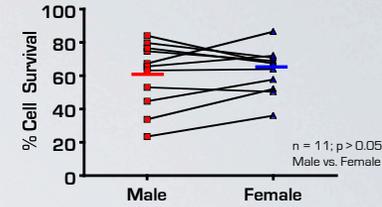
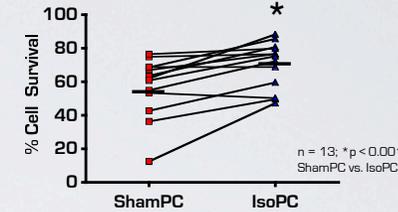
## Treatment Paradigm:



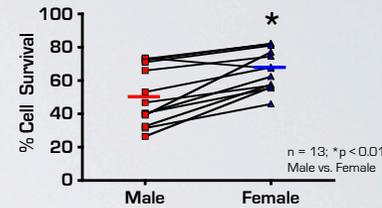
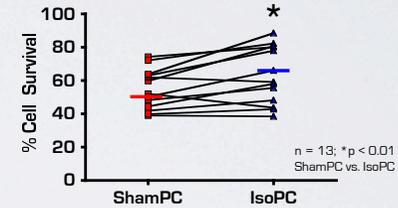
**Statistics:** Two-way ANOVA of the difference of paired means ("repeated measures") controlled for variability in absolute cell survival. Compared factors were "preconditioning treatment" vs. "sex" (hypothesis 1) or "preconditioning treatment" vs. "E2 dose" (hypothesis 2). Differences within groups were analyzed with post-hoc Student Newman-Keuls test. Each litter (i.e. culture) represents n = 1.

## RESULTS

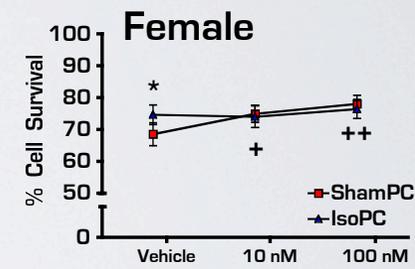
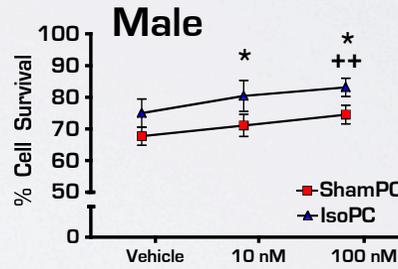
### Astrocytes



### Neurons



### Neurons



n = 9, no interaction between E2 dose and preconditioning treatment; \*p < 0.05, ShamPC vs. IsoPC; ++p < 0.05, 100 nM E2 IsoPC vs. Vehicle IsoPC

n = 9; interaction between E2 dose and preconditioning treatment, p < 0.001; \*p < 0.05, ShamPC vs. IsoPC; +p < 0.05, 10 nM E2 ShamPC vs. Vehicle ShamPC; ++p < 0.05, 100 nM E2 ShamPC vs. Vehicle ShamPC

## CONCLUSIONS

- 1) IsoPC protects astrocytes and neurons from OGD independent of innate cell sex
- 2) Innate female sex is a protective factor against OGD-induced cell death in neurons, but not in astrocytes, independent of preconditioning treatment
- 3) E2 abolishes IsoPC protection only in female neurons

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## HYPOTHESES

- 1) Isolated male and female astrocytes and neurons exhibit sex-specific responses to isflurane preconditioning (IsoPC) and subsequent oxygen and glucose deprivation (OGD), an in vitro model of ischemic stroke
- 2) Estradiol (E2) abolishes IsoPC protection in neurons regardless of innate cell sex