Mechanisms underlying rapid estradiol signaling in male rat striatum

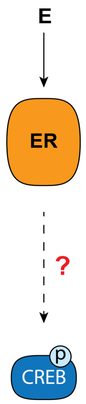
Sonal Nagpal[1], Luis A. Martinez[2], and Paul G. Mermelstein[1,2,3]

[1]Undergraduate and [3]Graduate Program in Neuroscience, University of Minnesota; [2]Department of Neuroscience, University of Minnesota

Department of Neuroscience
University of Minnesota
Driven to Discover℠

INTRODUCTION

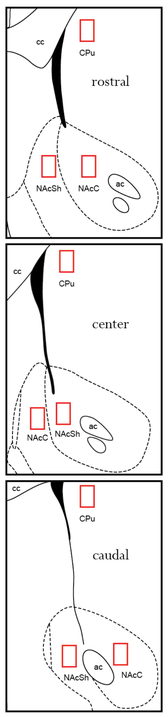
- Gonadal hormones, such as estradiol (E), can rapidly induce plasticity in brain areas implicated in addiction such as the striatum (i.e., the nucleus accumbens (NAc) and caudate putamen (CPu)).
  - In females, this effect on plasticity is associated with the induction of phosphorylated CREB (pCREB).
  - Estradiol induction of pCREB requires activation of metabotropic glutamate receptor 5 (mGluR5).
- Like females, males have E in the brain; however, it is not known if E can act through similar signaling mechanisms to influence plasticity in the striatum of males.



Hypothesis: E rapidly induces changes in pCREB in the striatum of males, and this effect is mediated via mGluR5

METHODOLOGY

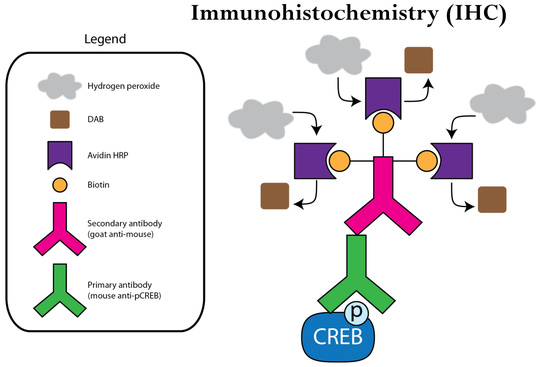
Counting domains



Timeline

- **animals arrive** (1 week)
  - gonadectomized male rats
  - Harlan Sprague Dawley
  - habituate
- **drug injection** (30 mins)
  - 1 mg/kg MPEP or Saline (Sal) vehicle
- **hormone injection** (15 mins)
  - 10 µg Estradiol (E) or Oil vehicle
- **perfuse & postfix** (1 hour)
  - 4% PFA
- **30% sucrose** (2 days)
- **section tissue & IHC** (2 days)
  - 40 µm coronal sections
  - primary (1:1000) mouse α-pCREB (Millipore)
  - biotinylated secondary goat α-mouse
- **image & analyze**
  - Leica DM4000 B light microscope
  - Image J quantification

Immunohistochemistry (IHC)

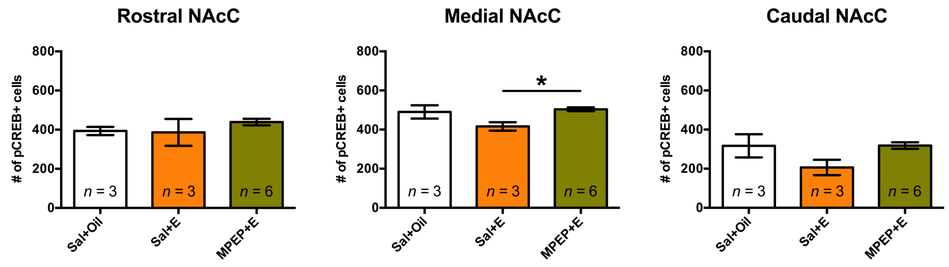


To visualize our target antigen (pCREB), first a primary antibody binds to pCREB. Next, a biotinylated secondary antibody binds to the primary to amplify the signal. Finally, a color-change reaction takes place between 3, 3' diaminobenzidine (DAB), Hydrogen peroxide, & Avidin Horseradish Peroxidase (HRP) .

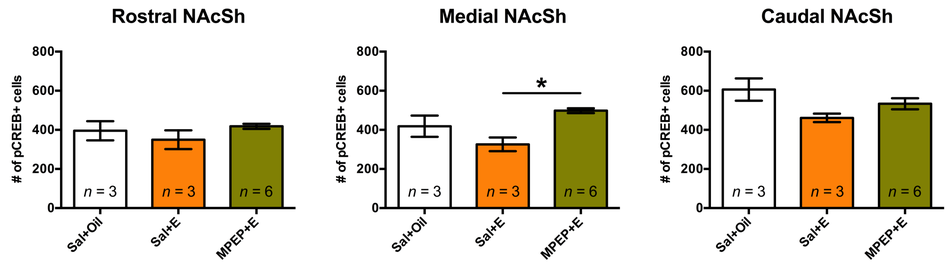
RESULTS

Nucleus Accumbens (NAc)

a. NAc core (NAcC)

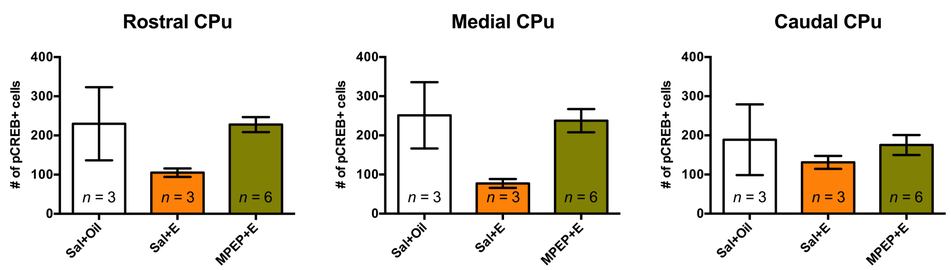


b. NAc shell (NAcSh)



Caudate Putamen (CPu)

c.

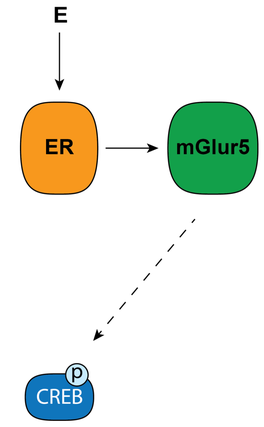


a & b. Number of cells expressing pCREB protein in the two subdivisions of the nucleus accumbens (NAc), the NAc core (NAcC) and NAc shell (NAcSh). Graphs left to right are organized coronally from rostral to caudal. Estradiol (E) tended to decrease pCREB expression at medial and caudal levels of the NAc compared to oil (Sal+E vs. Sal+Oil), and pretreatment with the mGluR5 antagonist MPEP tended to block this effect of E (MPEP+E vs. Sal+E). However, statistical significance was only seen for the medial NAcC ($F(2,9) = 5.69$, $p < .05$) and medial NAcSH ($F(2,9) = 9.14$, $p < .05$). Tukey's post-hoc comparisons revealed that in both areas, MPEP+E males had more pCREB+ cells compared to Sal+E treated males.

c. Number of cells expressing pCREB protein in the caudate putamen (CPu). Although E treatment tended to decrease pCREB expression compared to oil treatment (Sal+E vs. Sal+Oil), and this effect tended to be blocked by pretreatment with MPEP prior to E, these effects were not statistically significant at any level of the CPu.

CONCLUSIONS

- E tended to decrease pCREB expression in the NAc and in the CPu of male rats, and this decrease was prevented in animals that received the mGluR5 antagonist MPEP.
  - Effects were not statistically significant for most areas analyzed, likely due to low statistical power
  - However, the pattern of results are in agreement. with our hypothesis that E can rapidly influence pCREB expression in the striatum via mGluR5 activation.



- The effects of E on pCREB expression in the striatum of males are opposite of what is observed in females
  - E may inhibit plasticity in addiction pathways in males, in contrast to its enhancing effects in females

FUTURE DIRECTIONS

- Is E acting directly in the striatum to influence pCREB expression in this area?
  - Inject E directly into this brain area
- Does E influence structural plasticity in the striatum of males?
  - Measure effects of E on dendritic structure within this brain area

ACKNOWLEDGMENTS

The authors would like to thank Holly Korthias and Brittni Peterson for their technical assistance. This project was funded by the Undergraduate Research Opportunities Program(UROP) to Sonal Nagpal and NIH grant DA035008 to P.G.M.