# Enhanced Expression And Activity of NAD(P)H Oxidase in Mouse Periaqueductal Gray Tissue During Morphine Antinociceptive Tolerance

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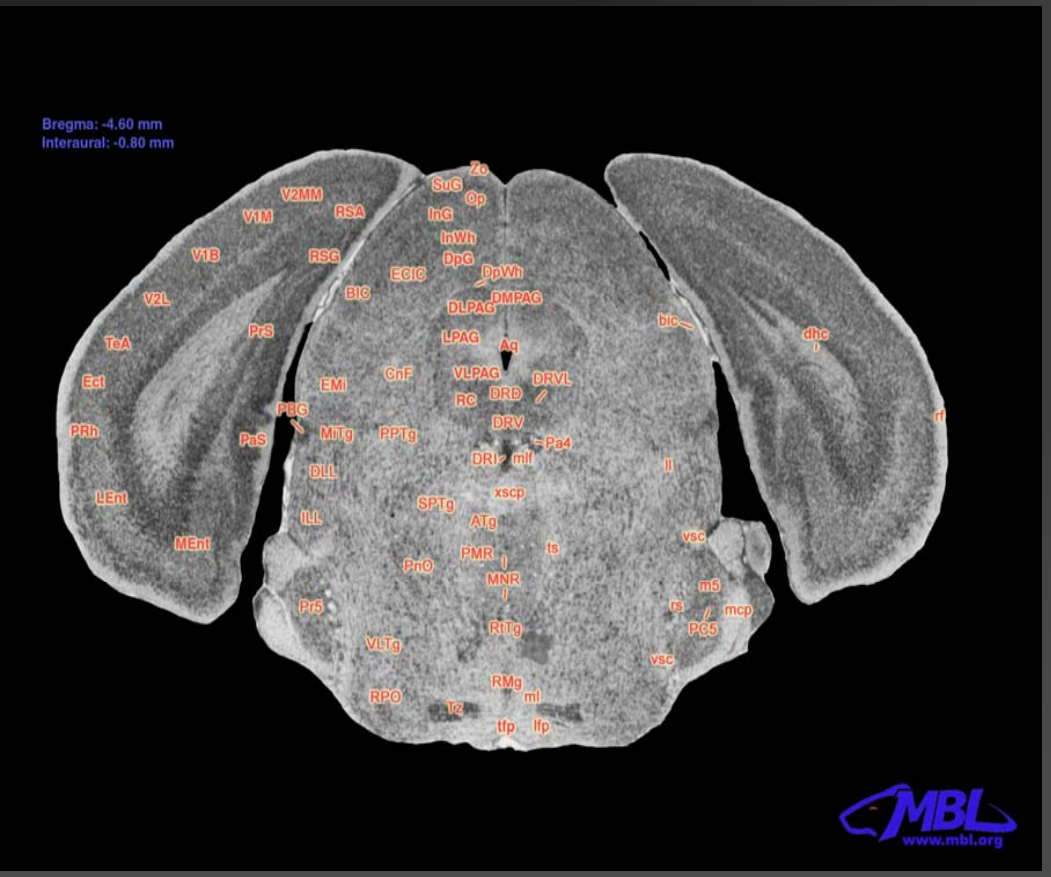
# Background: Periaqueductal Gray (PAG)

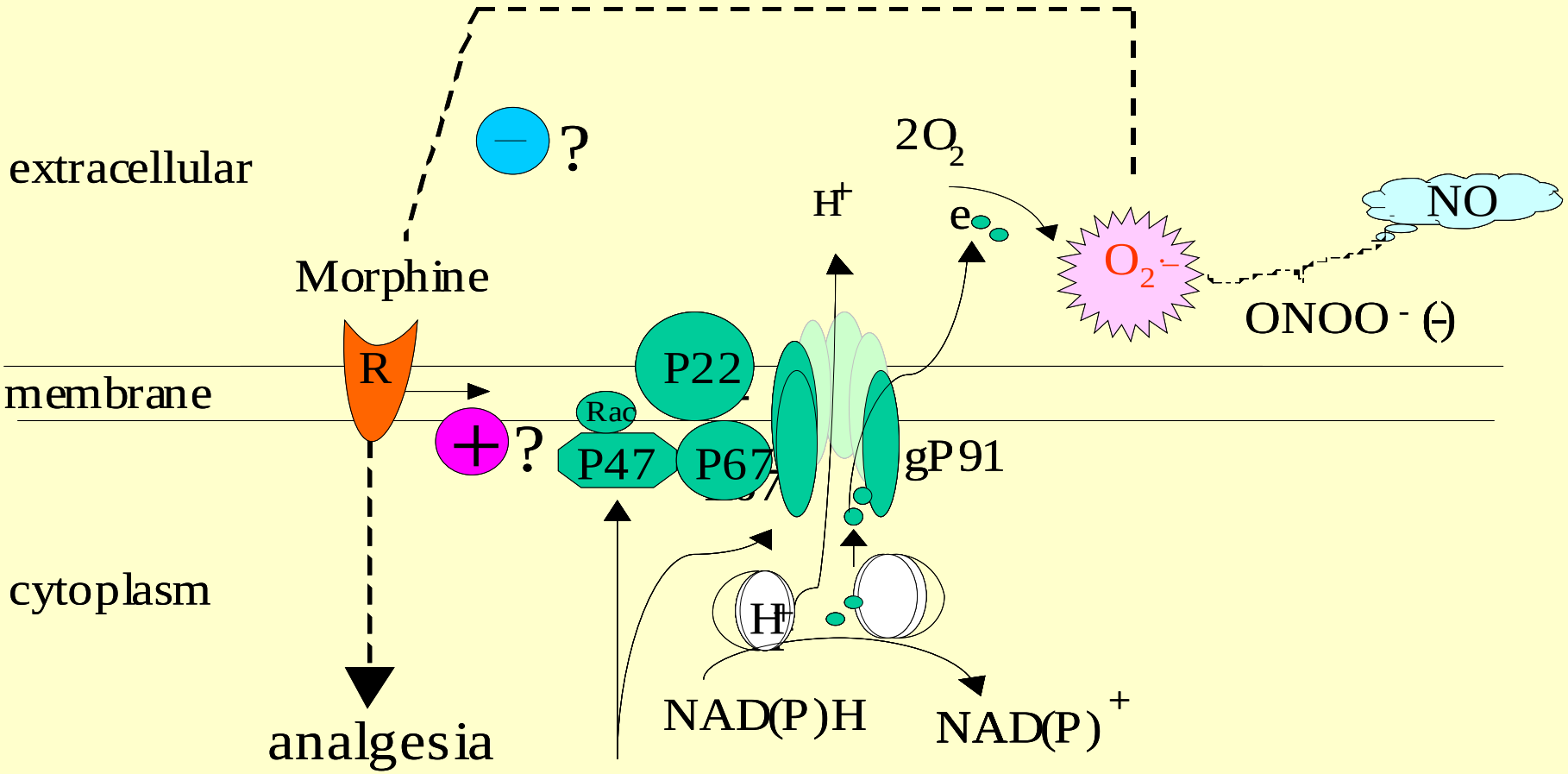



- Area surrounding cerebral aqueduct in brain stem levels 9 and 10
- Contains receptors for opiate peptides which can eliminate the perception of pain

# Background: Known Effect of Morphine on PAG

- Pain reduction takes place when opiates turn on inhibitory neurons in PAG
- Antinociceptive tolerance may result from perpetual action of opiates on PAG
- Morphine causes increase in intracellular [Ca+] in the PAG in chronic morphine treatment (CMT) mice

# Role of NAD(P)H Oxidase in Morphine Induced Tolerance



# Question

- Is NAD(P)H oxidase (subunits p47 and NOX-2) present in the PAG?

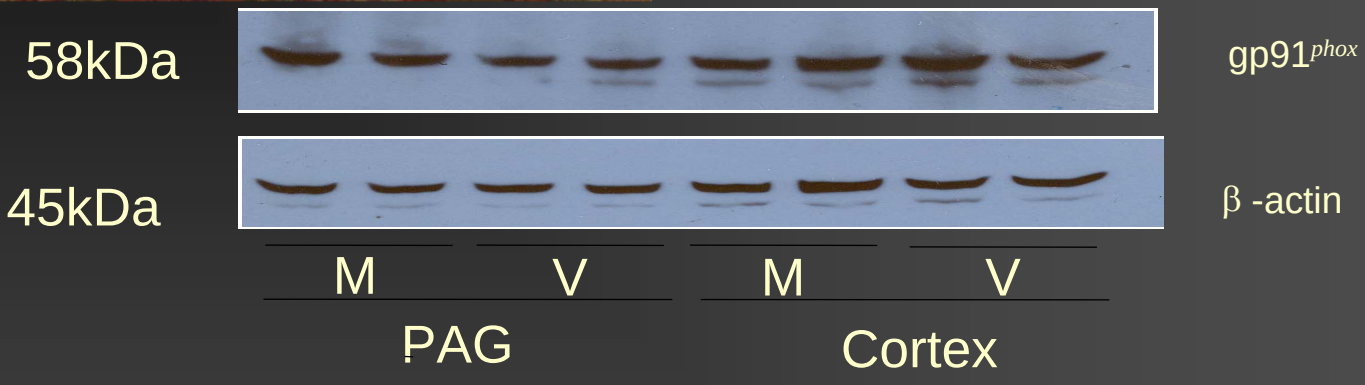
  -Approach: Immunohistochemistry

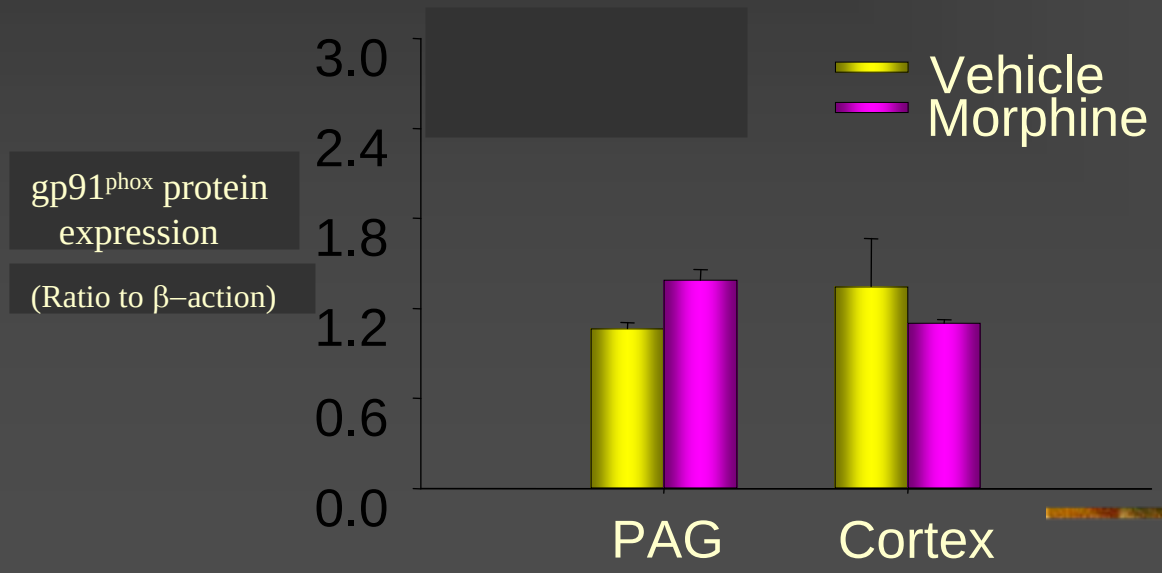
  (*process used to localize proteins in cells of tissue sections*)

# Hypothesis

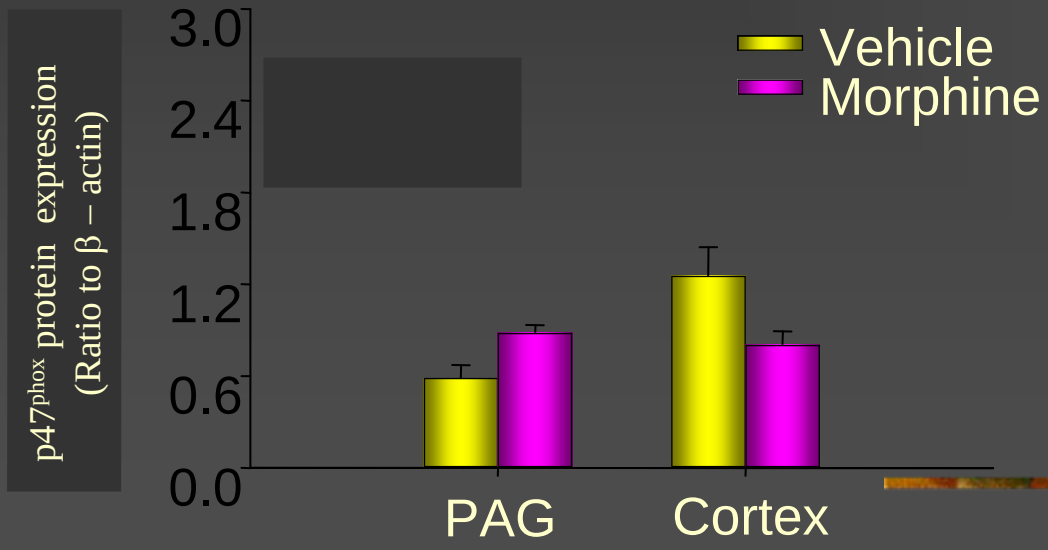
- NAD(P)H oxidase plays an important role in morphine-induced tolerance.

# Western Blot Analysis of the NOX-2 subunit of NAD(P)H Oxidase in PAG

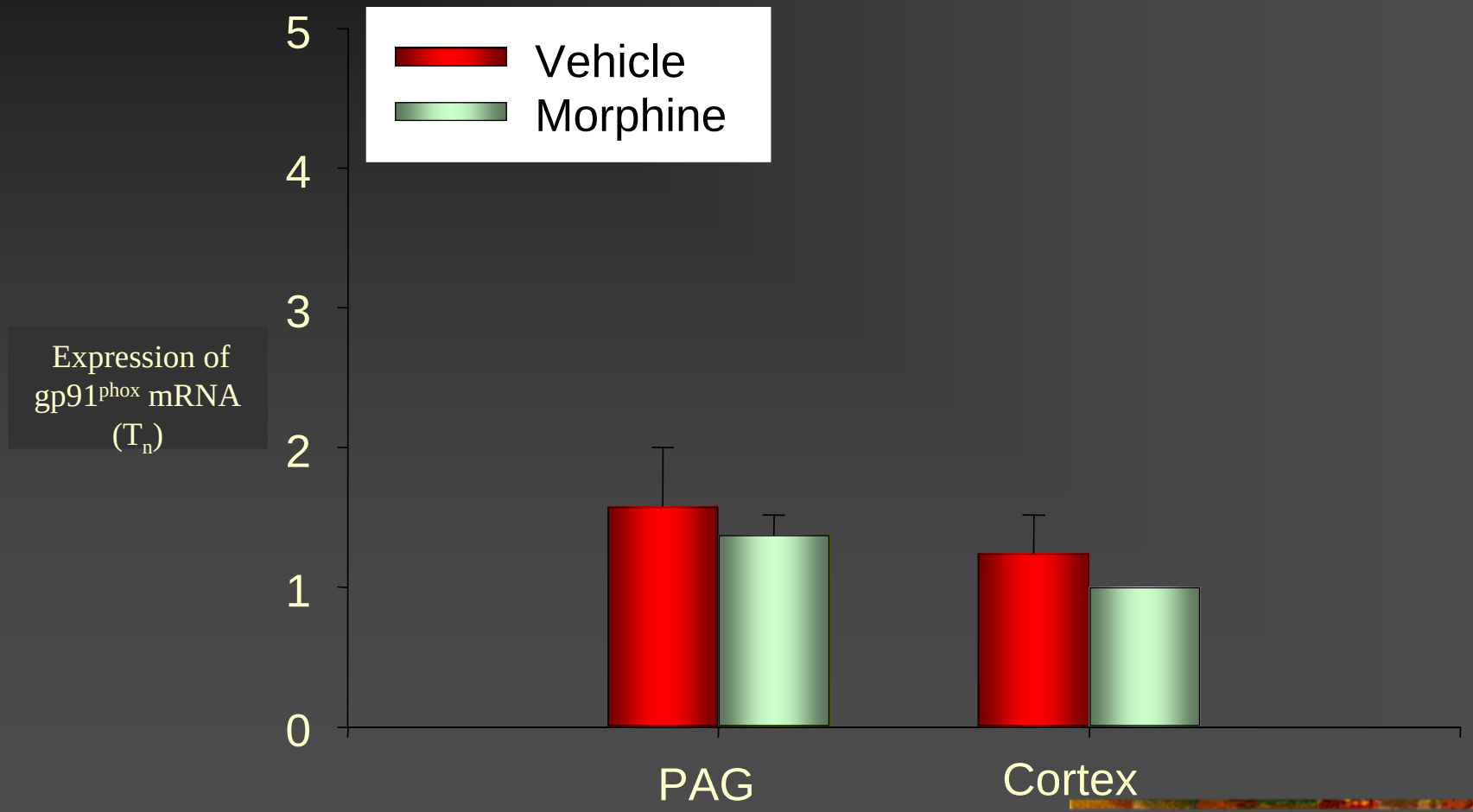


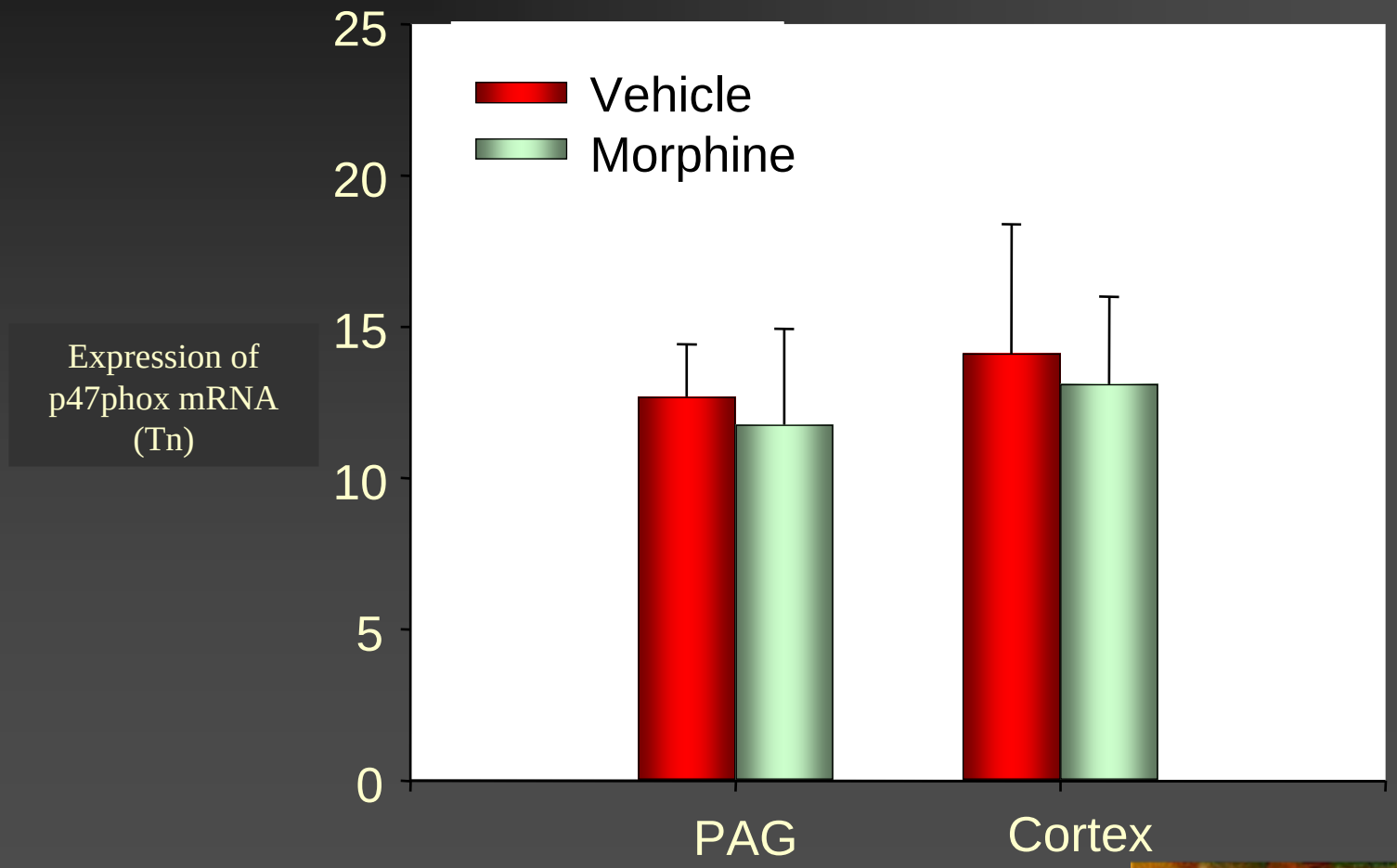
# Western Blot Analysis of the p47 subunit of NAD(P)H Oxidase in PAG

# Gene Expression Level of the NOX-2 subunit of NAD(P)H Oxidase in PAG



# Gene Expression Level of the p47 subunit of NAD(P)H Oxidase in PAG



# Protocol

- 3 groups of mice: naïve, placebo pellet, and morphine pellet (morphine tolerant)
- Performed a two-day immunohisto-chemistry protocol that included over-night incubation with the primary antibody
- Qualitatively analyzed results by taking pictures of images obtained by microscope

# Results

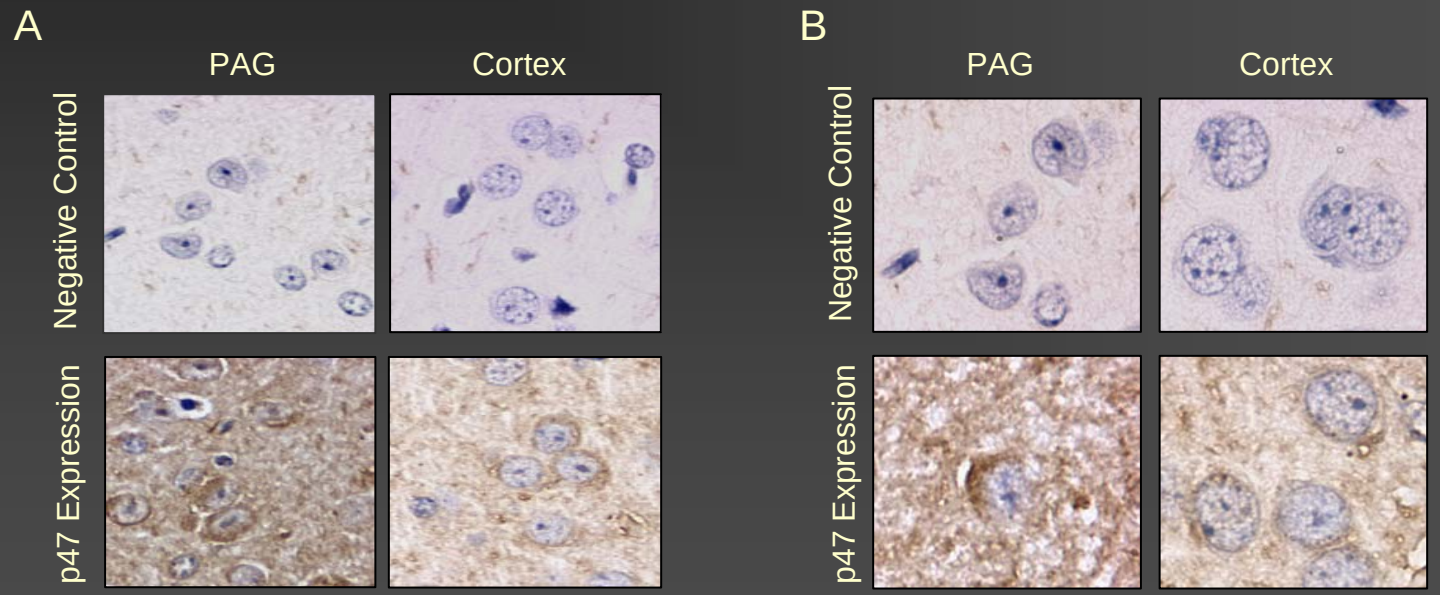



Figure 1: Expression of the p47 antigen in the periaqueductal gray and cortex of placebo pellet mouse brain tissue. A) 400X magnification. B) 1000X magnification.

# Results

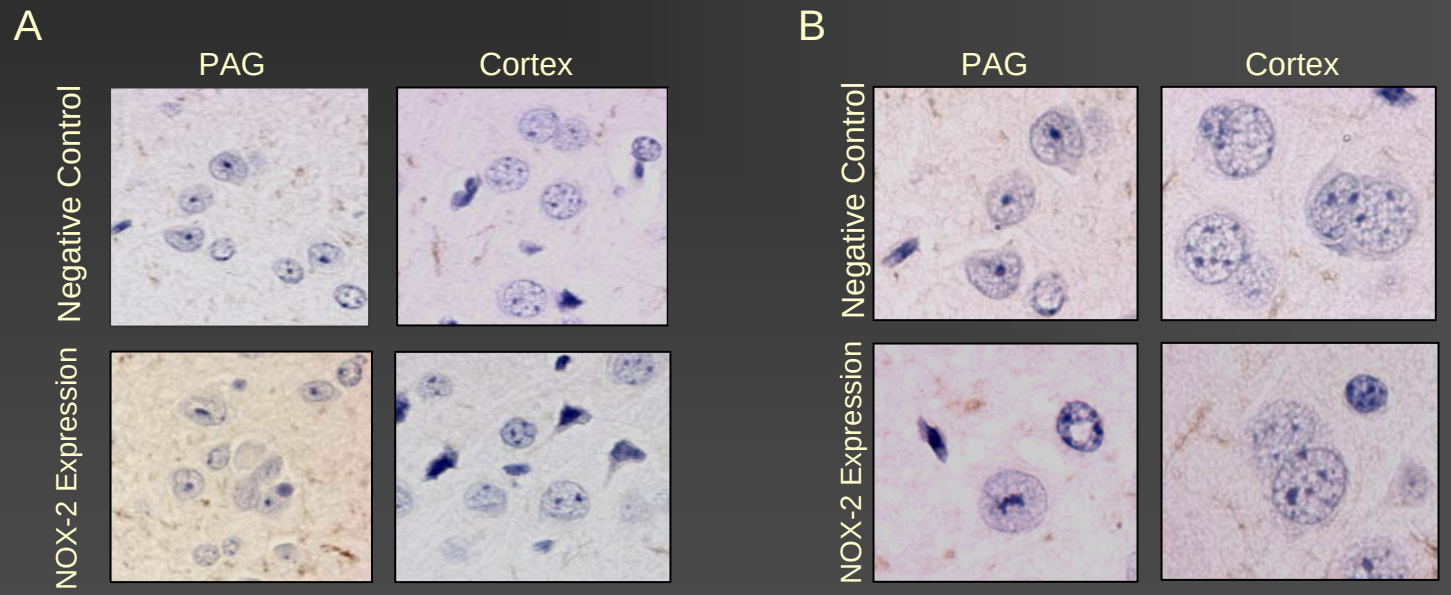



Figure 2: Expression of the NOX-2 antigen in the periaqueductal gray and cortex of placebo pellet mouse brain tissue. A) 400X magnification. B) 1000X magnification.

# Conclusion

- NAD(P)H oxidase is present in the PAG of mice brain tissue

# Future Direction

- Perform ESR to detect the levels of superoxide in the PAG
- Perform HPLC to assess the functioning of NAD(P)H Oxidase in the PAG

# Results

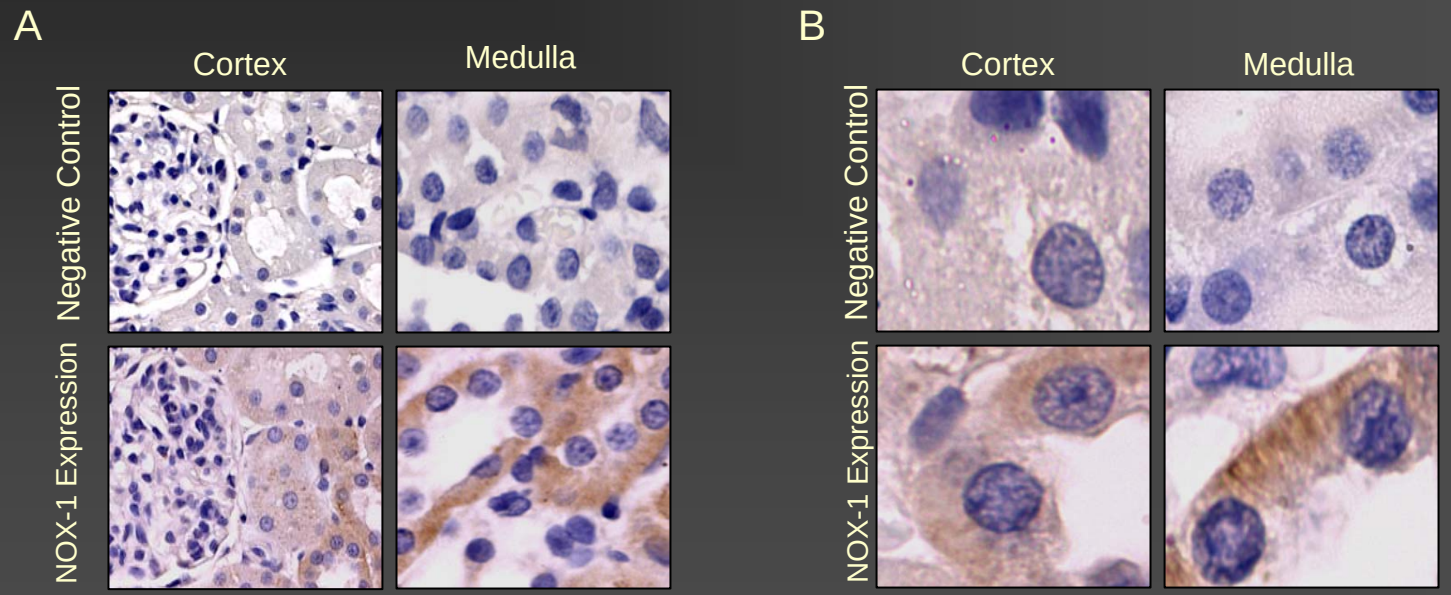



Figure 3: Expression of the NOX-1 antigen in the cortex and medulla of rat kidney tissue. A) 400X magnification. B) 1000X magnification

# Results

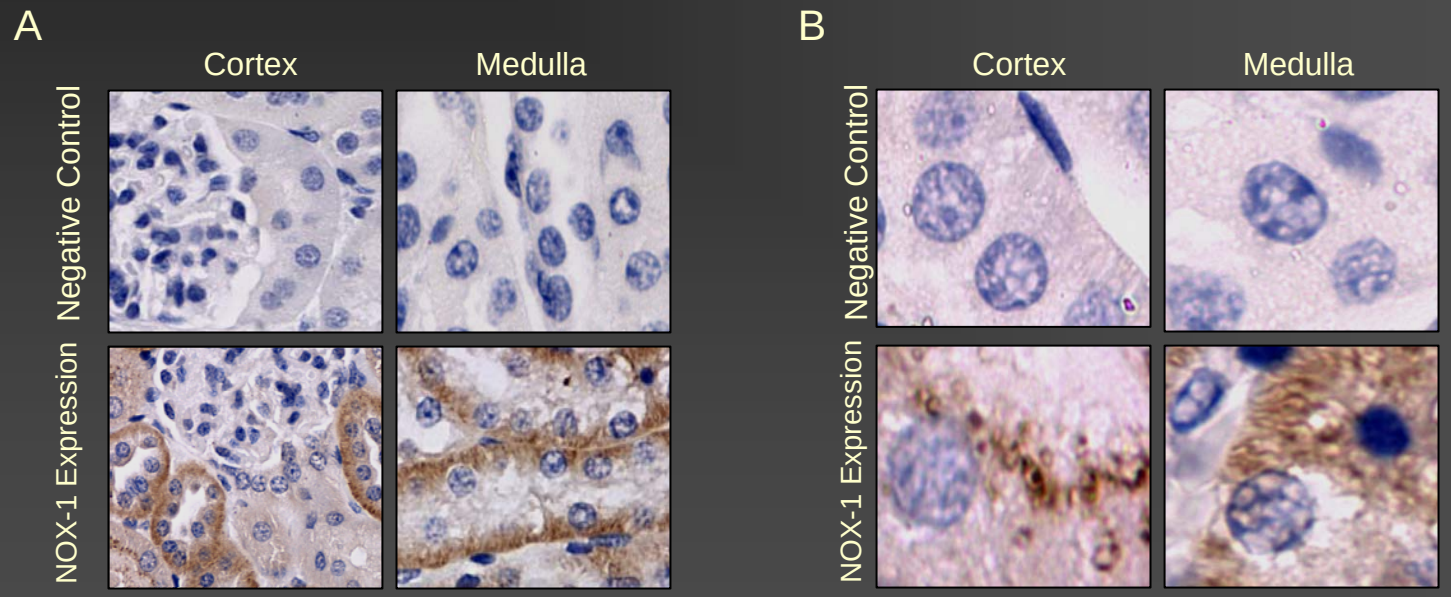



Figure 4: Expression of the NOX-1 antigen in the cortex and medulla of mouse kidney tissue. A) 400X magnification. B) 1000X magnification

# Results

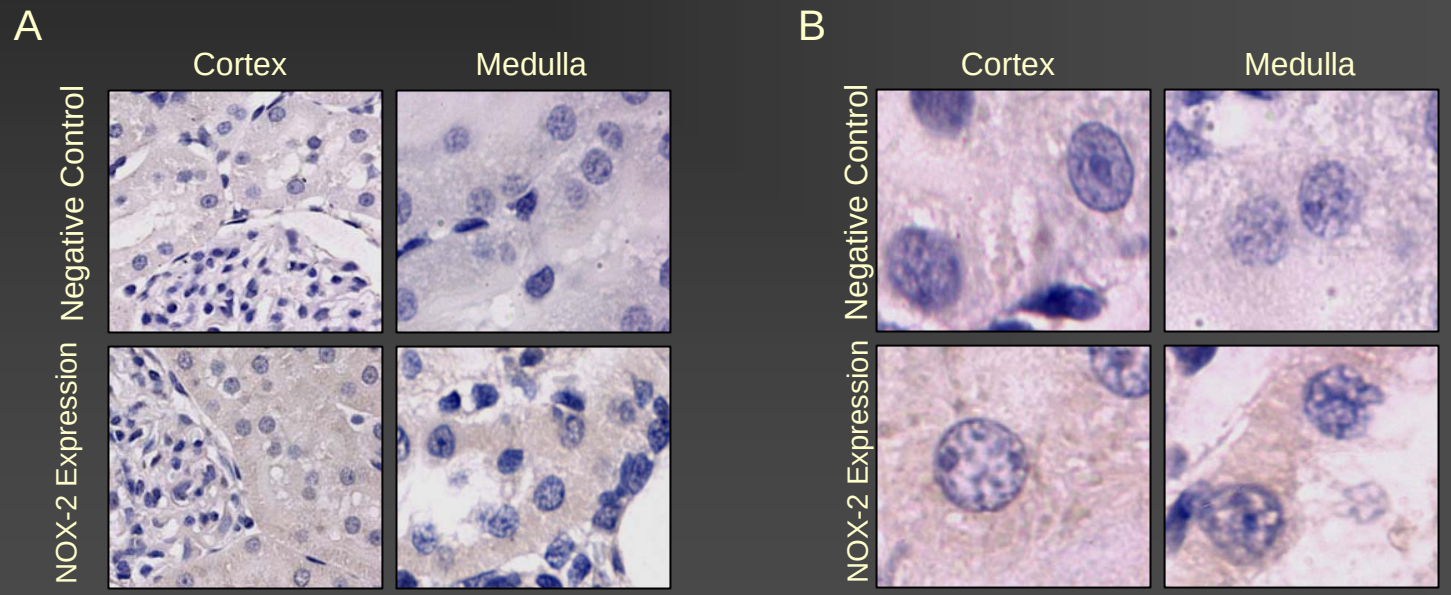



Figure 5: Expression of the NOX-2 antigen in the cortex and medulla of rat kidney tissue. A) 400X magnification. B) 1000X magnification

# Results

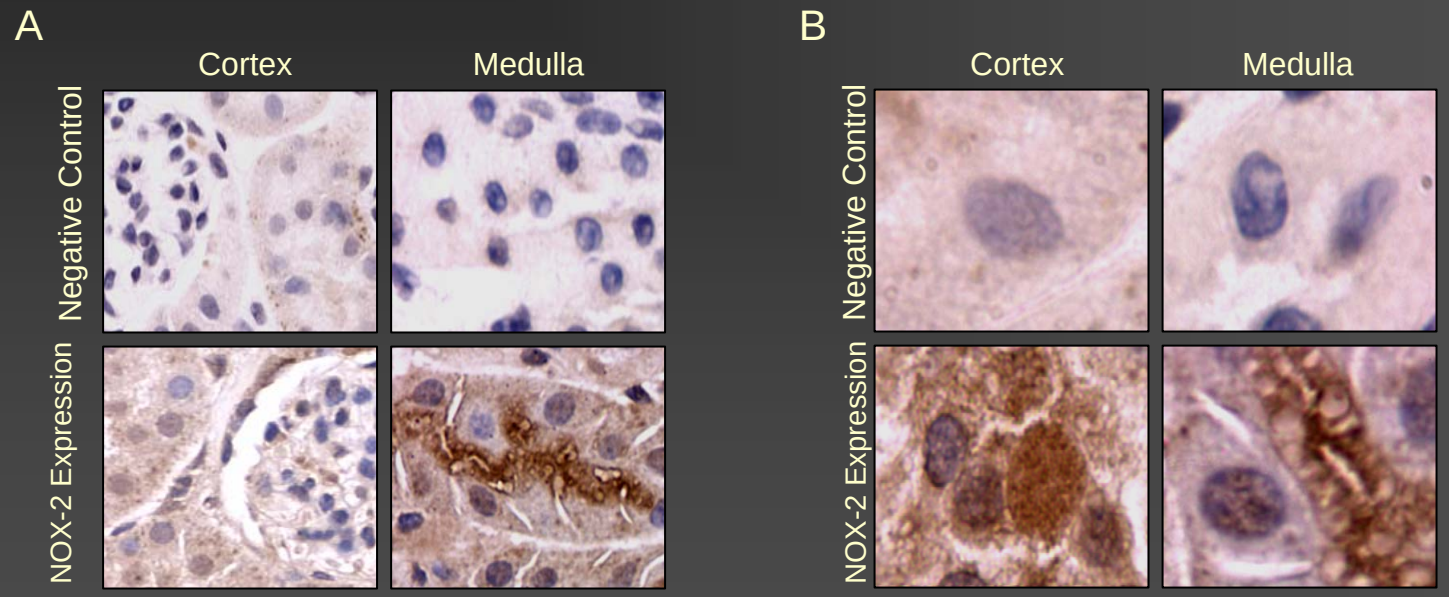



Figure 6: Expression of the NOX-2 antigen in the cortex and medulla of mouse kidney tissue. A) 400X magnification. B) 1000X magnification

# Results

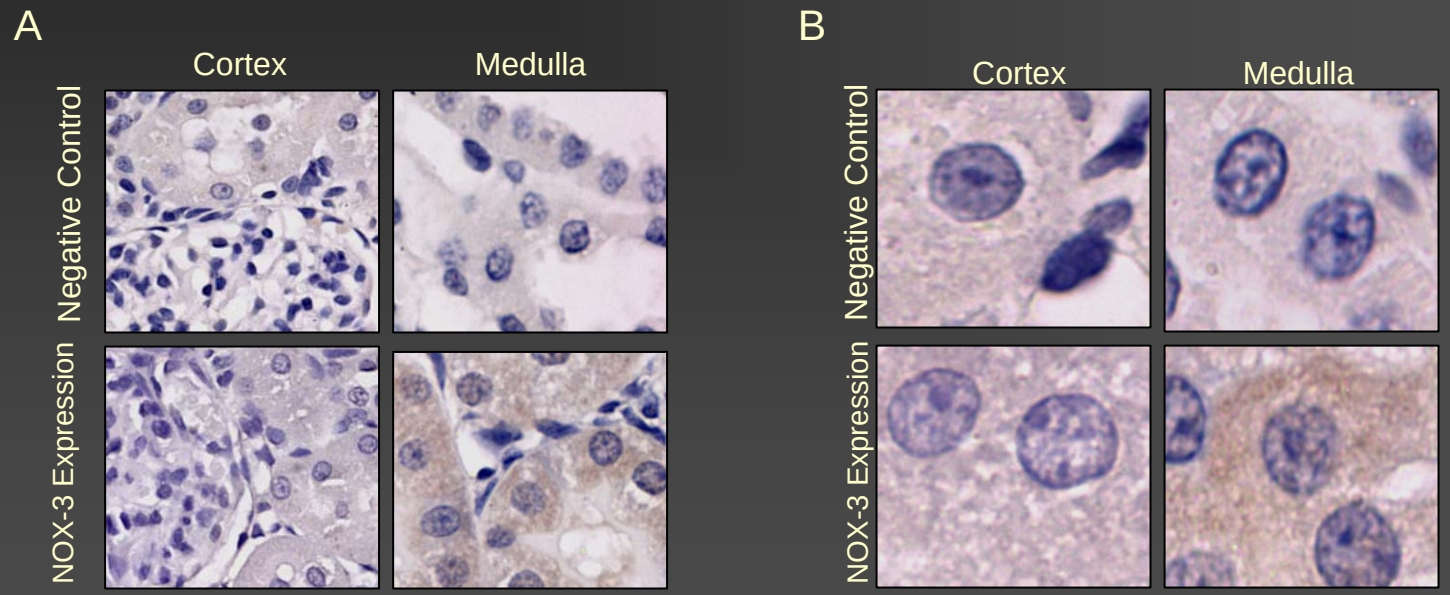



Figure 7: Expression of the NOX-3 antigen in the cortex and medulla of rat kidney tissue. A) 400X magnification. B) 1000X magnification

# Results

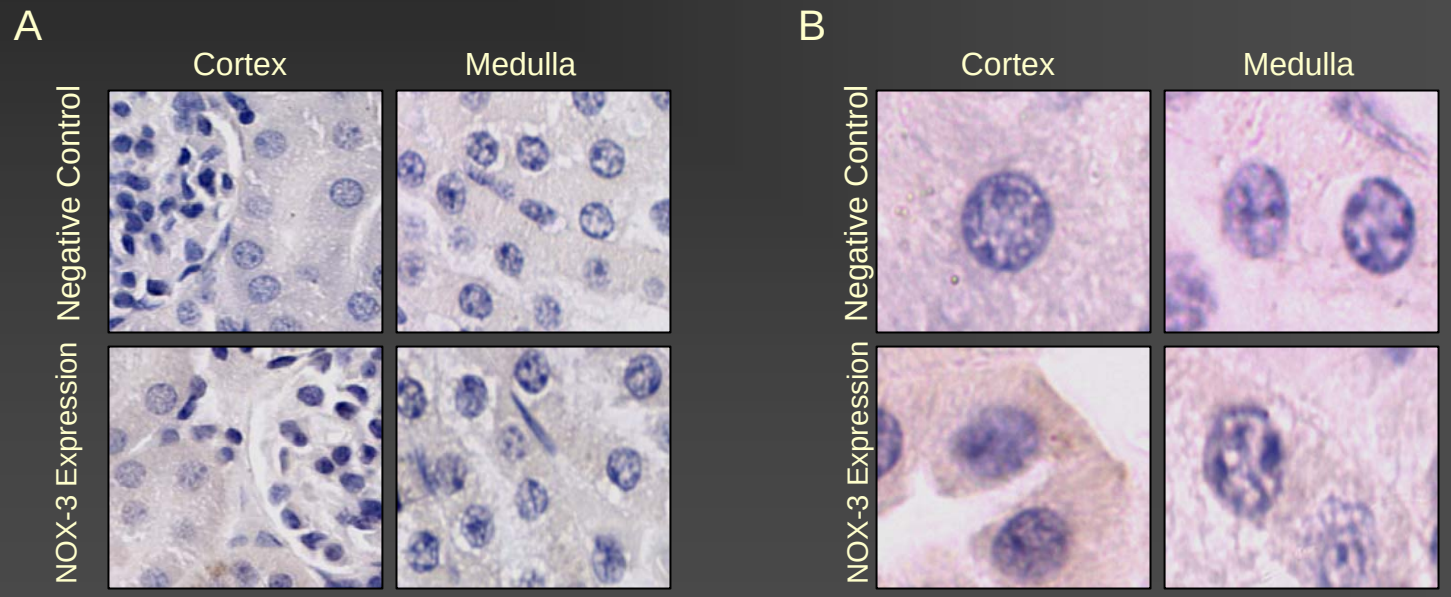



Figure 8: Expression of the NOX-3 antigen in the cortex and medulla of mouse kidney tissue. A) 400X magnification. B) 1000X magnification

# Results

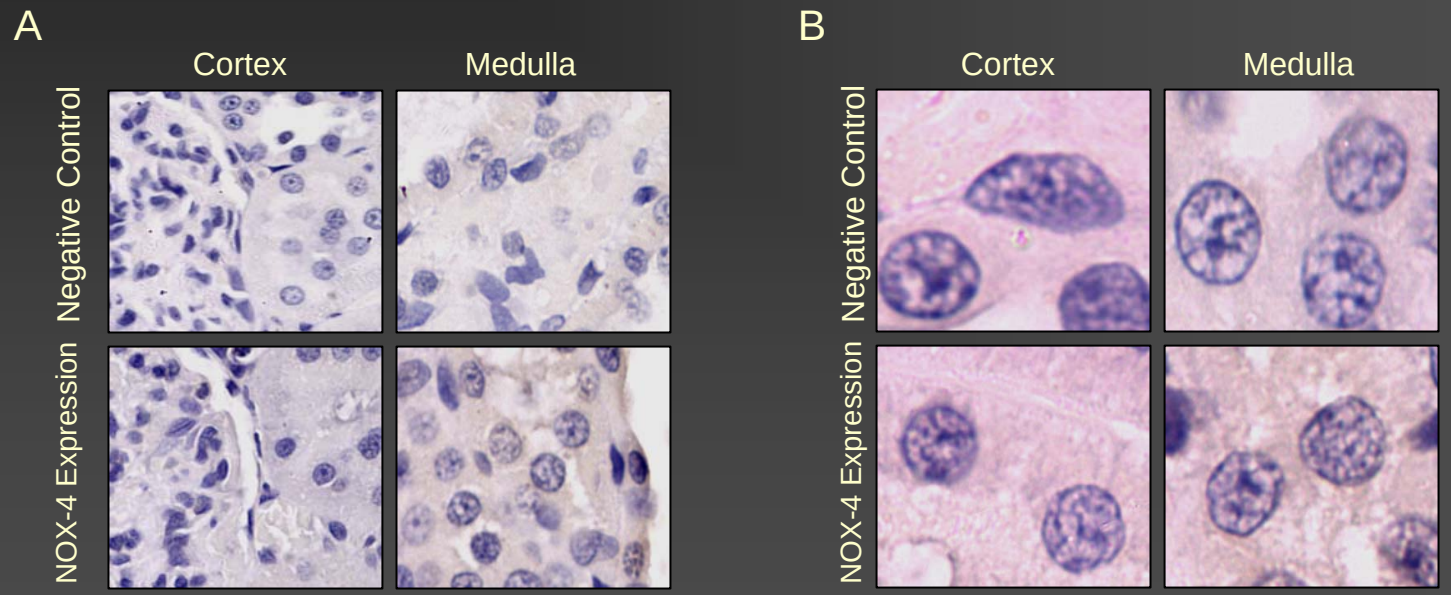



Figure 9: Expression of the NOX-4 antigen in the cortex and medulla of rat kidney tissue. A) 400X magnification. B) 1000X magnification

# Results

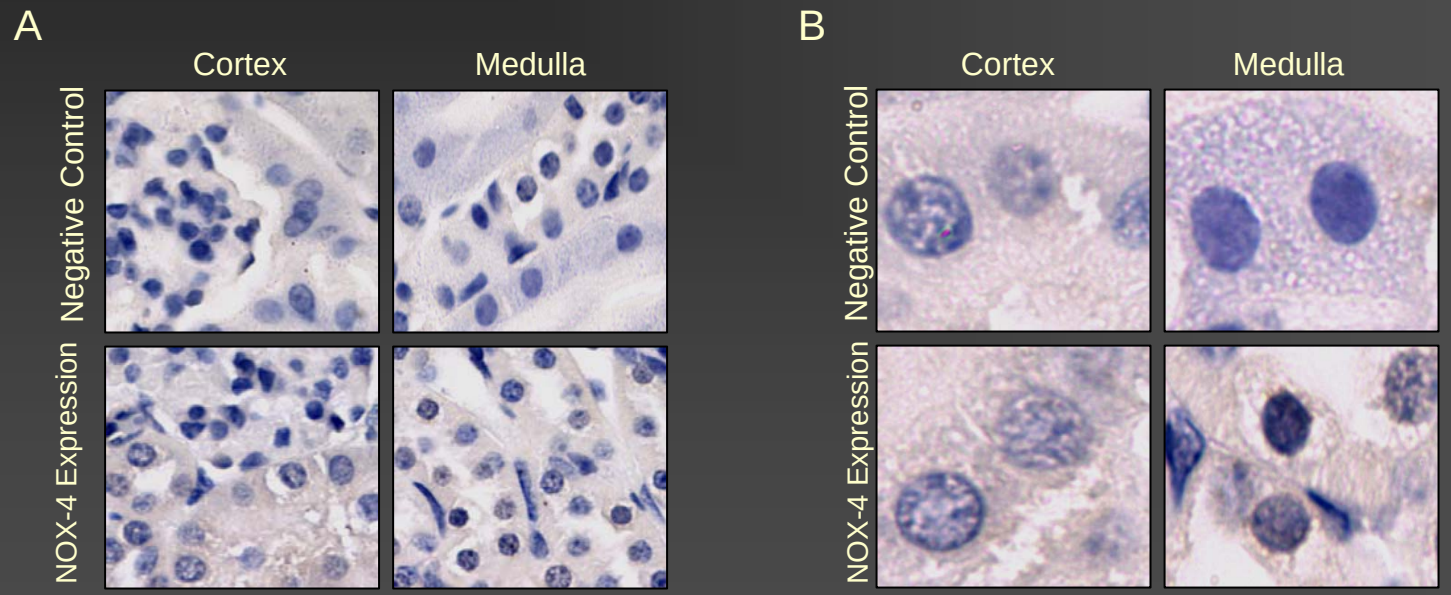



Figure 10: Expression of the NOX-4 antigen in the cortex and medulla of mouse kidney tissue. A) 400X magnification. B) 1000X magnification

# Conclusion

- There are some differences between rat and mouse kidney tissue in their expression of the NOX isoforms

# Future Direction

- Positive controls for NOX-3 and NOX-4 antigens in mice and rat kidney tissue

# Acknowledgements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- Dr. William Dewey, Ph.D.
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- Program for Summer Research Experience of Undergraduates in Pharmacology & Toxicology

# Bibliography


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