Holly Korthas
UROP Project Summary

Determining Which Estradiol Receptor is Involved in Estradiol-Mediated Sensitization to Cocaine in Female Rats

A major issue present in today's society is drug addiction, having both a large economic burden and human cost. When including financial consequences such as healthcare, crime, and lost hours at work, addiction to illegal drugs costs the United States $193 billion annually (National Institute on Drug Abuse, 2015). While this is a problem that affects society as a whole, there is an increasing amount of evidence that women progress from casual drug use to drug abuse more quickly than men do (Segarra et al., 2010). It is thought that this sex difference in addiction could be due to fluctuations in hormones during the menstrual cycle, specifically estradiol (Evans et al., 2002).

Estradiol has a variety of functions in the brain. In striatal cells, a brain region that is involved in drug addiction circuitry, estradiol rapidly activates calcium signaling that increases the excitability of cells (Mermelstein et al., 1996). Interestingly, both estradiol and cocaine can rapidly induce the phosphorylation of the transcription factor CREB in the nucleus accumbens, a sub-structure of the striatum (Grove-Strawser, 2010; Nazarian et al., 2009). Furthermore, it has been found that both estradiol and cocaine induce changes in dendritic spine density in this same brain region (Peterson et al., 2014; Dumitriu et al., 2012). This seems to suggest that estradiol is capable of priming the nucleus accumbens' response to cocaine, enhancing the vulnerability to drugs of abuse in females.

Unfortunately, the mechanism underlying estradiol's potential ability to prime the response to cocaine in the nucleus accumbens is unknown. However, it is known that the nucleus accumbens contains two types of estrogen receptors (ER): ER α and ER β (Shughrue et al., 1997). While it is not yet known which ER is involved in the interaction between estradiol and cocaine, there is evidence that points specifically to ER α. In the striatum, estradiol signals through ER α and mGluR5 to induce pCREB (Grove-Strawser et al., 2010). Additionally, signaling through the receptor mGluR5 is needed for enhancement of structural plasticity and behavioral responses due to estradiol, making ER α a likely candidate (Martinez et al., 2014; Peterson et al., 2014). However, ER α's involvement in mediating these structural and behavioral effects of estradiol has not yet been tested. For my UROP, I hypothesized that estradiol signaling through ER α would be required for enhanced behavioral sensitivity and structural plasticity in response to cocaine in female rats.

In order to test my hypothesis, I used 24 ovariectomized female Sprague-Dawley rats. Eight rats were treated with the ER α agonist PPT (1 mg/ml/kg), four were treated with the ER β agonist DPN (1 mg/ml/kg) (Larson & Carroll, 2007), and 12 were treated with cottonseed oil as a control (1 mg/ml/kg). The hormones were injected subcutaneously on a two days on, two days off schedule in order to mimic the naturally occurring hormone cycle in female rats. After the first set of hormone injections, rats were injected with cocaine intraperitoneally (15 mg/ml/kg) for five consecutive days. Behavioral sensitization, or the enhanced locomotor activity that occurs with repeated exposure to cocaine, was assessed for this experiment. On the first two days

of hormone injections, rats were habituated to the locomotor chambers. Locomotor activity was then assessed on the five consecutive days of cocaine.

In order to analyze statistical significance, separate two-way ANOVAs were conducted on the locomotor activity between the first and fifth day of cocaine treatment for the PPT and DPN conditions. For the PPT treated group, I found that there was not a significant effect of locomotor activity on the first day versus the fifth day of cocaine ($p = 0.1913$), nor was there an effect of drug (either PPT or cottonseed oil) ($p = 0.2397$) (Figure 1). For the DPN treated group, I found that there was not a significant effect of locomotor activity on the first day versus the fifth day of cocaine ($p = 0.5558$), nor was there an effect of drug (either DPN or cottonseed oil) ($p = 0.7439$) (Figure 2). These results do not support my hypothesis that ER α is the ER involved in estradiol's mediation of cocaine response in females. Had my hypothesis been supported, I would have seen an increase in locomotor sensitization in rats treated with the ER α agonist PPT when compared to animals treated with an oil control and animals treated with the ER β agonist DPN. There are a few possibilities as to why I did not get the results I expected. First, the group sizes were relatively small (PPT n = 8, DPN n = 4, oil n = 12). A larger group size, especially for the DPN condition, would serve to increase the statistical power of the treatments. Additionally, it could be possible that ER α does not mediate the enhanced response to cocaine due to estradiol on its own. Perhaps ER α works in concert with ER β or another receptor to mediate this interaction.

I think that I effectively accomplished the objectives of my UROP project. My goal at the outset was to gain experience as an independent scientific investigator by testing to see if ER α was responsible for the effects of estradiol on sensitization to cocaine. Although time did not allow me to complete the molecular analysis that I intended to do, I valued my experience in the UROP program because I learned a lot about experimental design and gained valuable experience in a prominent behavioral assay, as well as to work around unexpected directions in my data and to the original plan that I had proposed. Most importantly, I learned how to work independently in a laboratory setting on an independent project that I was able to take ownership for.

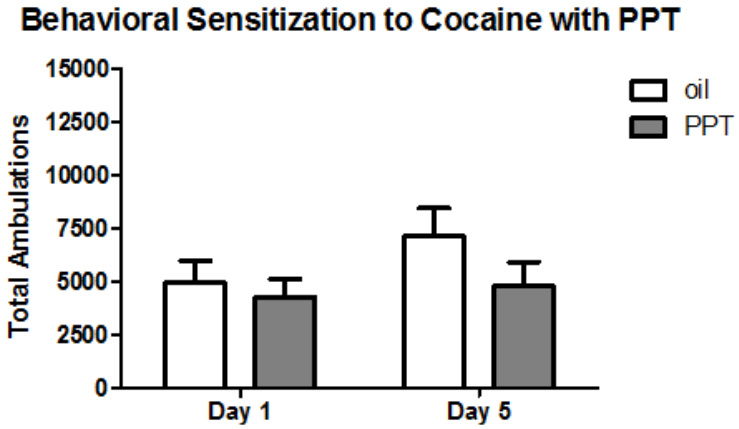

Figure 1. This figure shows the difference in locomotor activity on Day 1 of cocaine treatment versus Day 5 for PPT treated or oil treated animals. Error bars indicate the standard error of the mean. There was not a significant effect of time or of drug in this condition.

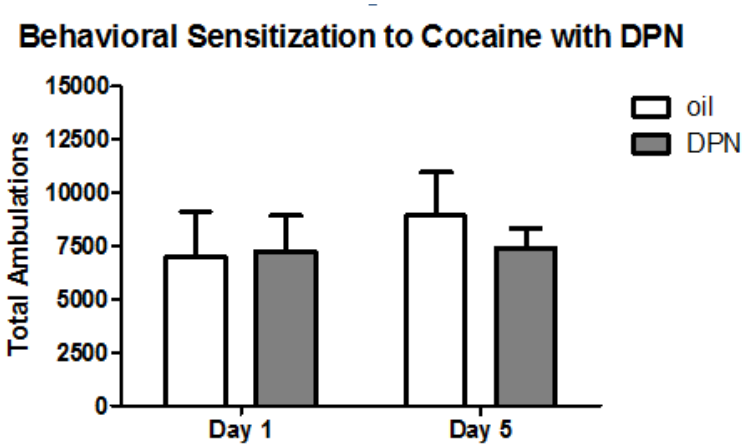

Figure 2. This figure shows the difference in locomotor activity on Day 1 of cocaine treatment versus Day 5 for DPN treated or oil treated animals. Error bars indicate the standard error of the mean. There was not a significant effect of time or of drug in this condition.

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