Overview of Talk

• Sex differences in the brain – how do they arise?
• The Reward System in the Brain: Estradiol modulation of striatal dopamine release.
• Sex differences in addiction and the neural mechanisms mediating these differences in rats.
• Stress, Social Support and sex differences in addiction.
Chromosomal sex determines gonadal sex which determines most sex differences in the brain and behavior – there may also be effects of XX or XY chromosomes.

- In humans, testosterone masculinizes the brain.
- In rodents, estradiol aromatized from testosterone masculinizes the brain.

Development of Sex Differences in the Brain

This is a critical period!

Thanks to Jaclyn Schwartz for the slide.
ALL SEX DIFFERENCES ARE NOT THE SAME

A. Qualitative Differences

B. Quantitative Differences

C. Population Differences

D. Underlying Mechanisms Differ

A. Developmental Origins

Organizational: genetic and/or hormone initiated trait

Contingent: internal or external factors (including epigenetic factors) acting on a sexually dimorphic individual

B. Statistical Characteristics

i. Bimodal Distribution

ii. Average / Mean Difference

iii. Frequency Distribution

C. Functional Expression of Trait

Sex Difference in Behavioral Expression of Trait

i. Underlying Mechanisms Differ

No Sex Difference in Behavioral Expression of Trait

i. Underlying Mechanisms the Same
Motivation depends on the Reward System

- Estradiol Enhances Dopamine Release in Dorsal Striatum
  - Amphetamine (AMPH)-induced striatal dopamine (DA) release varies with estrous cycle in vitro and in vivo
  - Ovariectomy attenuates and Estradiol treatment reinstates K⁺, AMPH and cocaine-induced increases striatal DA in females but not males.
  - Estradiol acts directly on the striatum to rapidly enhance DA release in vitro and in vivo: there are membrane receptors for E - membrane ER (mER)
  - 17β-estradiol most potent endogenous hormone produced of the class of hormones known as “estrogens”

Estradiol delivered to dorsolateral striatum enhances Amphetamine-induced DA in dialysate of OVX females

Estradiol rapidly down-regulates D2-dopamine receptor binding in females but not in males

Bazzett & Becker, Brain Research, 637 (1994) 163-172
Estradiol enhances cocaine-induced increase in DA in striatum of females but not males

No effect of estradiol in NAc

Males > Females in NAc DA increase induced by cocaine

Cummings et al (Alcohol and Drug Dependence, 2014)

How does estradiol act in the brain?

**Nuclear Estradiol Receptors (ER-α and ER-β) vs. Membrane Estradiol Receptors (mER)**

- mER for both ER-α and ER-β plus GPER
- mER-α and mER-β couple with mGluR in brain to mediate rapid effects of E
- mER found in the brain, heart, bone (osteoclasts), fibroblasts, fat, breast – throughout the body!
FSCV in anesthetized males and females

Gelatin → Vehicle
Estradiol Benzoate (EB) → ER

Treatment (SC)

OVX/CAST
2 weeks

Stimulated DA release
30 min

Cocaine (IP)

Peak [DAc] → Release
Tau → Reuptake

Effect of estradiol on stimulated DA in males and females: response after cocaine as % pre-cocaine

20Hz 24p

[DAc] (% pre-cocaine)

Vehicle EB Vehicle EB

[DAc] (% pre-cocaine)

Vehicle EB Vehicle EB

Females Males

100 200 300

8 7

8 7

* p<0.05 compared by treatment
# p<0.05 compared by sex

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Multiple estradiol receptor subtypes expressed in ventral striatum

FSCV with selective estradiol receptor agonists

- Propyl pyrazole triol (PPT) → ERα
- Diarylpropionitrile (DPN) → ERβ

Katie Yoest
Mechanisms Mediating Sex Differences in Addiction

- Estradiol enhances dopamine release in dorsolateral striatum
- Estradiol down regulates D2 dopamine receptors in dorsal striatum
- **Sex differences in nucleus accumbens in ER receptors mediating effect**
- **Sex difference in the enhancement by estradiol on dorsal striatal dopamine release in females**
E2 acting at mER decreases GABA and D2DR resulting in greater DA release and activation of the direct pathway with less inhibition of the indirect pathway.

**Dorsal striatum**

- **DLS**
- **GABA Neuron in Indirect Pathway** – less GABA release after E2

**Incentive Salience**

- **Incentive Valence**
- **Acquisition of rewarded behaviors**

**DEVELOPMENT OF AUTOMATIC BEHAVIORS**

**INITIATION OF MOTIVATED BEHAVIORS**
What are the implications of sex differences and hormonal influences on motivation?

**Sex differences in drug-taking**

Why are there Sex Differences in Motivation?

- Sex differences in the neural systems important for maternal motivation result in sex differences in motivated behaviors in general (Becker & Taylor, 2007).
- Sex differences in neural systems mediating motivation to engage in reproductive behaviors.
- Sex differences in the role of hormones in modulation of energy intake also play a role in sex differences in motivation
  * females estradiol decreases feeding behavior
  * males eat more than females due to sexual differentiation of hypothalamus
Sex Differences in Drug Abuse and Addiction: More than Demographics

- Time from first exposure to drug to chronic drug use is shorter for women than for men – true for alcohol and cocaine and heroin.
- Females present for treatment consuming MORE cocaine than men, even though there time of use is shorter.
- Reasons for initiation of drug use: women report self-medication for depression, stress and anxiety most often; men and boys report they engage in risky behaviors that include taking drugs to be part of the group.
**Cocaine Self-Administration: Estradiol enhances cocaine self-administration and being female also increases intake**

Hu, Crombag, Robinson, & Becker 2004 Neuropsychopharmacology

Utah Addictions Update 6-8-18

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**Estradiol does NOT enhance cocaine self-administration in Male Rats**

Jackson, Robinson, and Becker
Neuropsychopharmacology (2006)

Lisa Jackson
Estradiol Enhances Motivation to take Cocaine

Sex Differences in Motivation: Females work harder for cocaine than males in bHR rats
The choice paradigm: cocaine vs. pellets

One nose poke delivers a banana-flavored pellet the other cocaine
When rats can choose tasty pellets or cocaine at the beginning most choose pellets

Many weeks later, some rats choose cocaine over pellets. More females than males choose cocaine. Perry et al PLOSOne, 2014.

More females choose cocaine and they tend to choose cocaine more rapidly than do male rats

Utah Addictions Update & Perry et al PLOSOne 2014.
Cocaine-induced DA Response in NAc is Attenuated in Cocaine Preferring Male and Female Rats

A. Early SA (Week 2) — Late SA (Week 7)
   - ABST (n=11)
   - PP (n=5)
   - CP (n=9)

B. Early SA (Week 2) — Late SA (Week 7)
   - CP Females (n=5)
   - CP Males (n=4)

Perry et al., Neuropsychopharmacology 2015
Utah Addictions Update 6-8-18
Stressful life events and addiction


Results
Having 2 or more stressful life events in the past year increased the odds of having a new AUD, TUD, CUD, and OUD (OR=3.14, 2.15, 5.52, and 3.06, respectively) or ongoing AUD, TUD, and CUD (OR=2.39, 2.62, and 2.95, respectively) compared to 0 or 1 stressful life event. A stress by gender interaction for new vs. absent AUD demonstrated that having 2 or more stressful life events was associated with increased odds of new AUD in men (OR=2.51) and even greater odds of new AUD in women (OR=3.94).

Effect of Prenatal Stress
(maternal restraint stress days 15-21 gestation)
on cocaine-taking behavior

Utah Addictions Update 6-8-18
Prenatal stress advances day of acquisition of cocaine self-administration in male offspring, but not females.

Thomas, Hu, Bhatnagar, Lee, and Becker (2009)

Prenatal stress enhances cocaine intake in male offspring, but not females.

Thomas et al. (2009)
Prenatal Stress increases the proportion of females exhibiting the highest breaking points

Females met more addiction-like criteria than males and PS females more addiction-like criteria than No PS females

Thomas & Becker (2018)
Oxytocin & Social Support

Effects of oxytocin on cortisol reactivity and conflict resolution behaviors among couples with substance misuse

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\textasteriskcentered{ARTICLE INFO}

Keywords: Oxytocin, Couples, Substance use, Cortisol, Gender, Sex

\textasteriskcentered{ABSTRACT}

Social stress, particularly in the form of dyadic conflict, is a well-established correlate of substance use disorders (SUD). The neuropeptide oxytocin can enhance prosocial behavior and mitigate addictive behaviors. These effects may be, in part, a result of oxytocin's ability to attenuate hypothalamic pituitary adrenal (HPA) dysregulation. However, only one study to date has examined the effects of oxytocin on neuroendocrine activity or conflict resolution behavior among couples. Participants (N = 33 couples or 66 total participants) were heterosexual couples in which one or both partners endorsed substance misuse. Using a double-blind, placebo-controlled, repeated-measures design and an evidence-based behavioral coding system, we compared the impact of oxytocin (40 IU) vs. placebo on cortisol reactivity and conflict resolution behaviors.

Women, oxytocin attenuated cortisol response following the task. Oxytocin was also associated with increased Distress Maintaining Attributions and decreased Relationship Enhancing Attributions. Among men, oxytocin associated with decreased Distress Maintaining Attributions, and both oxytocin and placebo yielded declines in Relationship Enhancing Attributions. The findings support emerging hypotheses that oxytocin may have differential effects in men and women, and indicate the need for future efforts to translate oxytocin's positive neurobiological effects into therapeutic behavioral changes.

1. Introduction

Social stress is a well-established correlate of substance use disorders (SUD). Clinical studies indicate that social stress exacerbates craving, substance use, and relapse among individuals with SUD (Back et al., 2010; Higley et al., 2011). One particular type of social stress which is especially salient in the development, maintenance, and treatment of SUD is dyadic conflict among couples. Existing research indicates that SUD has detrimental effects on dyadic functioning acutely and over time (Boden et al., 2013). Maladaptive dyadic functioning can precipitate excessive substance use and impede effective treatment outcomes (Cranford et al., 2011; Keyes et al., 2011; Quigley et al., 2013; Rodriguez et al., 2013; Testa et al., 2014). For example, research has established a strong temporal association between dyadic treatment and maintenance of abstinence (Grosso et al., 2013). One recent study demonstrated that higher relationship satisfaction among women in SUD treatment was associated with experiencing fewer cravings (Owens et al., 2013). Findings from clinical trials also consistently demonstrate that dyadic interventions for result in significantly higher rates of abstinence compared to individual treatments for SUD (Powers et al., 2008; Stanton and Shadish, 1997). While the efficacy of addressing dyadic functioning within behavioral SUD interventions is well-established, much remains unknown about the efficacy of medications to address dyadic functioning as well concurrent substance use problems.

The neuropeptide oxytocin (OT) is associated most saliently parturition and lactation (Ludwig and Leng, 2006). OT is commonly administered as an intranasal spray and may enhance dyadic functioning. Research translating OT’s neurobiological effects into behavioral changes is needed.

Effects of Social Housing and Oxytocin in Female Rats on Self-Administration Behavior

Utah Addictions Update 6-8-18
Social Housing attenuates drug taking in female but not male rats
Westenbroek, Perry, & Becker (BBR, 2013)

Isolated females take more cocaine than pair housed females or males

Isolated females work harder for cocaine than pair housed females or males

Social Housing attenuates motivation for METH in females:
Oxytocin (0.3mg/kg i.p.) decreases motivation for METH (0.3mg/kg i.v.)

self-administration (5 days/week)  
week 1  FR1 (3 days) | week 2-5  PR (2 days) | week 6-7  PR (VEH or OT)

Westenbroek et al., 2018
Individual Variability in Breaking Point was altered by housing conditions

A. Individual Variability

B. Oxytocin decreased BP for Intermediate and High BP females independent of housing
TYPES OF SEX DIFFERENCES

A. Qualitative Differences
- Male Behavior
- Female Behavior

B. Quantitative Differences
- Female Behavior
- Male Behavior

C. Population Differences
- Male Distribution of Behaviors
- Female Distribution of Behaviors

D. Underlying Mechanisms Differ
- Male Neural Mechanisms
- Female Neural Mechanisms

Effect of Estradiol in striatum

Initial DA response to cocaine and AMPH
# Types of Sex Differences

## A. Qualitative Differences

- **Male Behavior**
- **Female Behavior**

## B. Quantitative Differences

- **Male Behavior**
- **Female Behavior**

## C. Population Differences

- **Male Distribution of Behaviors**
- **Female Distribution of Behaviors**

## D. Underlying Mechanisms Differ

- **Male Neural Mechanisms**
- **Female Neural Mechanisms**

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### Vulnerability for Addiction

Becker and Koob (2015)  Utah Addictions Update 6-8-18

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## Sex Differences in Addiction to Different Classes of Drugs in Rodents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Stage of Addiction Cycle</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Binge/Intox/escalation</strong></td>
<td><strong>Withdrawal/Negative Affect</strong></td>
<td><strong>Preoccupation/Anticipation</strong></td>
</tr>
<tr>
<td>Alcohol</td>
<td>Amount of intake F&gt;M**</td>
<td>Withdrawal symptoms M&gt;F**</td>
<td>Stress-induced reinstatement F&gt;M**</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Escalation of use F&gt;M*</td>
<td>Withdrawal symptoms F&gt;M**</td>
<td>Stress-induced reinstatement F&gt;M*</td>
</tr>
<tr>
<td></td>
<td>Amount of intake and motivation F=M**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Incidence F&gt;M***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opiates</td>
<td>Reward/conditioned place preference F&gt;M**</td>
<td>Symptoms/duration of symptoms M&gt;F**</td>
<td>Stress-induced reinstatement F&gt;M**</td>
</tr>
<tr>
<td></td>
<td>Acquisition of self-administration F faster than M F-M**</td>
<td>Acoustic startle as withdrawal index M=F**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Motivation F&gt;M**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>Stress promotes initiation in females and not males*</td>
<td>Stress and anxiety F&gt;M**</td>
<td>No sex difference in stress-induced reinstatement</td>
</tr>
<tr>
<td></td>
<td>Greater physical signs of withdrawal F&gt;M**</td>
<td></td>
<td>Stress promotes relapse in females*</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>Anxiogenic symptoms F&gt;M*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anxiolytic symptoms M&gt;F*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No sex differences in precipitated withdrawal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cue-induced reinstatement F&gt;M*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = qualitative    ** = quantitative    *** = population

# Sex Differences in Addiction to Different Classes of Drugs in Humans

![Stage of Addiction Cycle Table]

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<tr>
<th>Drug</th>
<th>Binge/Intoxication</th>
<th>Withdrawal/Negative Affect</th>
<th>Preoccupation/Anticipation</th>
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</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Escalation of use F&gt;M*</td>
<td>Negative affect F&gt;M*</td>
<td>Stress or anxiety-induced relapse F&gt;M*</td>
</tr>
<tr>
<td></td>
<td>Amount of intake M&gt;F**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Incidence M&gt;F***</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Amount of Intake F&gt;M**</td>
<td></td>
<td>Cue-induced relapse F&gt;M*</td>
</tr>
<tr>
<td></td>
<td>Incidence M&gt;F***</td>
<td></td>
<td>Cue-induced craving F&gt;M*</td>
</tr>
<tr>
<td>Opiates</td>
<td>Incidence M&gt;F***</td>
<td>Negative affect F&gt;M*</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>Stress promotes initiation in women*</td>
<td></td>
<td>Higher cortisol predicts relapse in women*</td>
</tr>
<tr>
<td></td>
<td>Females acquire self-administration at lower doses than males**</td>
<td></td>
<td>Lower cortisol and craving predict relapse in men*</td>
</tr>
<tr>
<td></td>
<td>Amount of intake F&gt;M**</td>
<td></td>
<td>Stress promotes relapse in women*</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>Women report enhanced subjective ratings in response to smoked cannabis and progress from use to disordered use more rapidly than men*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = qualitative  
** = quantitative  
*** = population

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## Sex Differences in Vulnerability for Addiction

- Estradiol enhances acquisition and escalation of drug taking in females.
- Prenatal stress increases drug taking in males and increases the proportion of females that meet addiction-like criteria.
- Social Housing attenuates motivation for cocaine and methamphetamine in female rats.
- Oxytocin ameliorates breaking point for methamphetamine in females after treatment for 2 weeks. This may be mediated by changes in Nac DA - stay tuned.
- Stress may differentially affect development of addiction vulnerability in males and females via different mechanisms.

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By studying females you may discover important insights that are relevant for addiction in both females and males.

Sex differences in the brain are *fundamental*. Understanding sex differences will improve understanding of neural function of both sexes.

Thank You!!
# Estrous cycle vs. Menstrual cycle

Table 2. Equivalent phases of the reproductive cycle in women and rats/mice.

<table>
<thead>
<tr>
<th></th>
<th><strong>WOMEN</strong></th>
<th><strong>RATS/MICE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Follicular</strong></td>
<td>Estradiol secretion from ovaries as follicle develops</td>
<td>Estradiol secretion from ovaries as follicle develops</td>
</tr>
<tr>
<td><strong>Peri-ovulatory</strong></td>
<td>Rapid increase in estradiol that triggers luteinizing hormone surge that induces ovulation</td>
<td>Rapid increase in estradiol that triggers luteinizing hormone surge that induces progesterone release and ovulation</td>
</tr>
<tr>
<td><strong>Luteal</strong></td>
<td>Release of relatively high concentrations of both estradiol and progesterone from the corpus luteum</td>
<td>No equivalent phase</td>
</tr>
</tbody>
</table>

There are many resources available to get up to speed on what is known about sex differences in the brain and body.

Considering sex as a biological variable in preclinical research.


Female rats are NOT more variable than male rats!

(Fem StDev/mean)/(Fem StDev/mean + Male StDev/mean)

Histogram of CV ratios
No Sex Difference – even when there is an effect of estrous cycle on the measure

Becker, Liang & Prendergast (2016)

If there is a sex difference in the data there is still no sex difference in variance

Becker, Liang & Prendergast (2016)
Sex as a Biological Variable

Are female rats more variable than male rats? No!!

<table>
<thead>
<tr>
<th>Measure</th>
<th>Male</th>
<th>Female</th>
<th>STDEV / MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavior</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Neurochemistry</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Electrophysiology</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Non-Brain Measures</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

- Male and Female STDEV / MEAN values are nearly identical across all measures.
- No significant differences in variability between male and female rats.

Becker, Liang & Prendergast (2016)