# Estrous Cycle-Dependent Sex Differences in Rat Dorsal Striatal MSN Excitability

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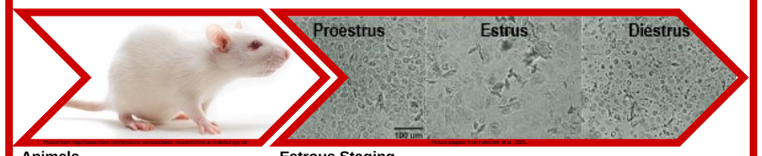
## Abstract

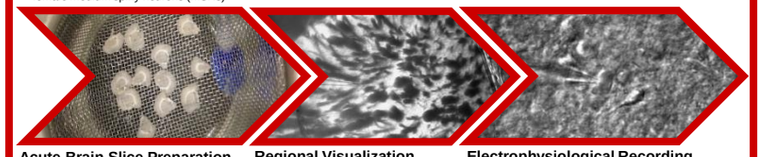
The neuroendocrine environment in which the brain operates is both dynamic and differs by sex. How this unstable neuroendocrine state affects neuron properties has been significantly neglected in neuroscience research. Behavioral data across humans and rodents indicate that natural changes in steroid sex hormone exposure affect sensorimotor and cognitive function in both normal and pathological contexts. These behaviors are critically mediated by the dorsal striatum: a well-conserved constituent of the basal ganglia that is instrumental for forebrain function, various forms of learning, and sensorimotor performance. In the dorsal striatum, medium spiny neurons (MSNs) are the predominant and primary output neurons. As such, MSNs are fundamental components of the circuits which underlie striatal-mediated behaviors. Importantly, MSNs express membrane-associated estrogen receptors and demonstrate estrogen sensitivity. However, the effects of cyclical hormone changes across the estrous cycle on the basic electrophysiological properties of MSNs have not been investigated. **Here, I test the hypothesis that dorsal striatal MSN intrinsic excitability is a dynamic property that is modulated in adult females across the estrous cycle via the associated changes in steroid sex hormone levels**. I performed whole-cell patch clamp recordings on male, diestrus female, proestrus female, and estrus female MSNs in acute brain slices obtained from adult rat dorsal striatum. Assessment and analysis of the electrophysiological properties is ongoing, with a particular emphasis on intrinsic excitability and miniature excitatory synaptic currents (mEPSC). Preliminary results indicate that the properties that govern cellular excitability differ over the course of the estrous cycle for female MSNs. Additional analysis is needed to further inform these results. Overall, given the estrous-dependent sex differences in the normal and pathological behavioral output of circuits involving the dorsal striatum, understanding the nature of neuroendocrine modulation of MSN function is an important research goal.

## Methods




**Animals**
- 8 male, 9 diestrus, 8 proestrus, and 9 estrus female Sprague-Dawley CD IGS Rats
- Age: postnatal day 70 to 90
- Intrinsic excitability (current stimuli): 31 male, 30 diestrus, 27 proestrus, and 31 estrus female medium spiny neurons (MSNs)

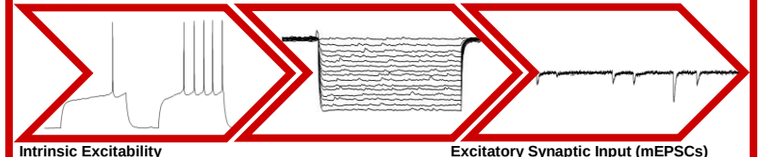
**Estrous Staging**
- Estrous status assessed via vaginal cytology
- Levels of testosterone, estradiol, and progesterone measured from trunk blood collected at sacrifice



**Acute Brain Slice Preparation**
- Rats were deeply anesthetized with isoflurane gas and killed by decapitation. The brain was dissected rapidly into ice-cold, oxygenated sucrose artificial cerebrospinal fluid (ACSF). Serial 300 µm coronal brain slices containing the dorsal striatum were prepared using a vibratome and incubated in regular ACSF for 30 minutes at 35ºC, and at least 30 minutes at room temperature (21-23 ºC). Slices were stored submerged in room temperature, oxygenated ACSF for up to 5 hours after sectioning in a large volume bath holder.

**Regional Visualization**
- After resting for ≥1 hour after sectioning, slices were placed in a Zeiss Axioscope equipped with IR-DIC optics, a Dage IR-1000 video camera, and 10X and 40X lenses with optical zoom. Slices were superfused with oxygenated ACSF heated to 30ºC.

**Electrophysiological Recording**
- Whole-cell patch-clamp recordings were made from medium spiny neurons (MSNs) in the dorsal striatum using glass electrodes (4-8 MΩ) containing 0.1-0.2% Biocytin Tracer (Vector Laboratories) internal solution. Signals were amplified, filtered (2 kHz), and digitized (10 kHz) with a MultiClamp 700B amplifier attached to a Digidata 1550A system.



**Intrinsic Excitability**
- Recordings were made in current clamp to assess intrinsic neuronal electrophysiological properties. Neurons underwent at least three series of depolarizing and hyperpolarizing current injections to elicit basic neurophysiological properties (Meitzen et al. 2009).

**Excitatory Synaptic Input (mEPSCs)**
- In a subset of recordings, oxygenated ACSF containing $GABA_A$ receptor antagonist picrotoxin (PTX, 150 µM, Fisher) and voltage-gated sodium channel blocker tetrodotoxin (TTX, 1 µM, Abcam Biochemicals) was bath applied to abolish action potentials and inhibitory post-synaptic current events. Once depolarizing current injection no longer elicited an action potential, MSNs were voltage-clamped at -70 mV and miniature excitatory post-synaptic current events (mEPSCs) were recorded for at least 5 minutes. Input resistance was reassessed after recording and cells were discarded if input resistance changed more than 20%.

## Cyclical changes in neuroendocrine state, behavior, and the dorsal striatum

The neuroendocrine environment in which the brain operates is both dynamic and differs by sex. Importantly, females exhibit a cyclical fluctuation in steroid sex hormones, while males do not (Figure 1).

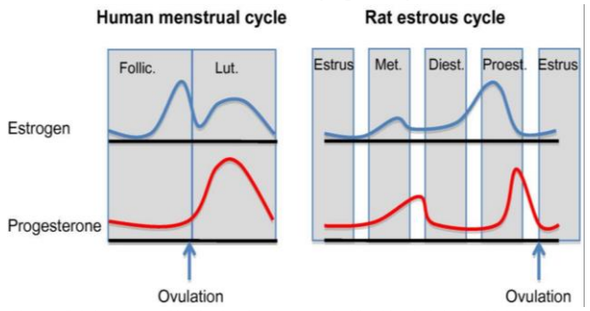



Figure 1. Relative estrogen (blue lines) and progesterone (red lines) levels during the 28 day human menstrual cycle and the 4 day rat estrous cycle. Time of ovulation is indicated by blue arrows. From Lebron-Milad et al., 2012.

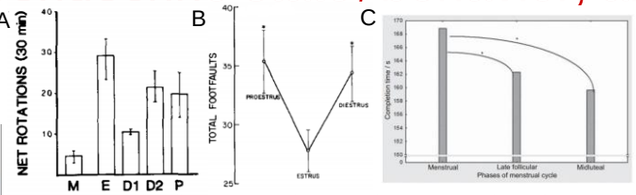
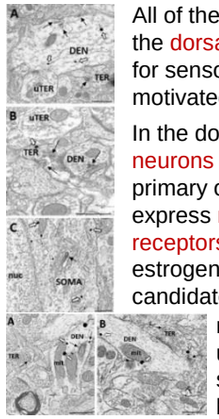



Figure 2. Cycle-dependent differences in dorsal striatal-mediated behaviors. (A) The influence of estrous cycle on rotational behavior from Becker et al.,1982 and (B) sensorimotor performance on a balance beam task from Becker et al.,1987 in the female rat and (C) performance in the O'Connor Finger Dexterity Test through menstrual cycle phases by human females from Simic et al., 2010.

These cyclical hormone changes correlate with changes in behavior. In both humans and non-humans, the mid-cycle increase in circulating estradiol is associated with:
- Increased locomotor activity (Figure 2A)
- Improved limb coordination (Figure 2B & 2C)
- Increased sensory perception for numerous modalities
- Enhanced place learning behavior
- Increased drug-seeking and intake

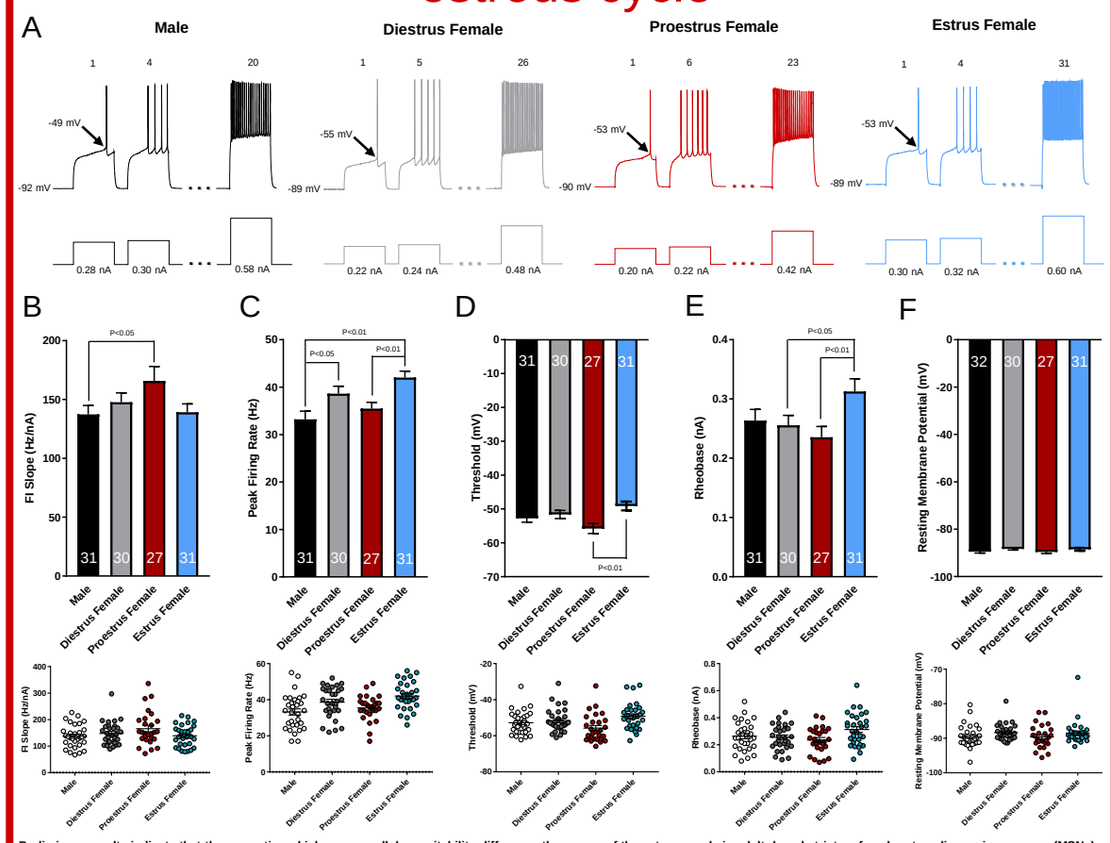



All of these behaviors are mediated by the dorsal striatum, a critical brain region for sensorimotor, learning, and motivated behavior.

In the dorsal striatum, medium spiny neurons are the predominant and primary output neuron. These neurons express membrane-associated estrogen receptors (Figure 3) and demonstrate estrogen sensitivity, making them prime candidates to play a role in the neurobiological mechanisms underlying cycle-dependent sex differences observed in behavior.

Figure 3. Electron micrographs indicating estrogen receptor α (lower panels A & B) and G-protein couple estrogen receptor 1 (upper panels A, B, & C) are localized to GABAergic neurons in adult female rat dorsal striatum from Almey et al., 2016. Black arrows indicate respective estrogen receptor and white arrows indicate GABA labeling.

## Female rat MSN excitability differs over the estrous cycle




**Preliminary results indicate that the properties which govern cellular excitability differ over the course of the estrous cycle in adult dorsal striatum female rat medium spiny neurons (MSNs).** (**A**) Representative recordings of male (black), diestrus female (grey), proestrus female (red), and estrus female (blue) MSN responses to positive current stimuli. The number of action potentials elicited for each stimulation is listed above each trace. Threshold measurements are indicated with arrows. Resting membrane potential measurements are listed to the left of each trace. The positive current stimulus application used to evoke each response is described below each trace. (**B**) The rate of increase in the number of action potentials elicited in response to increasing positive current stimuli (FI Slope) is elevated in proestrus female MSNs relative to male MSNs (p<0.05). (**C**) The maximum frequency of action potentials evoked by positive current stimulation (peak firing rate) is higher for estrus female MSNs relative to proestrus female MSNs (p<0.01) and male MSNs (p<0.01) as well as for diestrus female MSNs relative to male MSNs (p<0.05). (**D**) Threshold, or the potential at which the action potential initiates, is hyperpolarized for proestrus female MSNs relative to estrus female MSNs (p<0.01). (**E**) Rheobase, or the current input required to elicit an action potential, is elevated for estrus female MSNs relative to proestrus (p<0.01) and diestrus female MSNs (p<0.05). (**F**) Resting membrane potential does not differ by sex or by estrous cycle stage. Individual MSN data for each property analyzed are below each respective bar graph. Mean value is indicated by a horizontal line. Error bars reflect the standard error of the mean. The number of MSNs sampled per group for each property are within each bar in white. T-tests were used to assess differences between experimental groups. Significant differences are indicated with connecting lines and related p values.

## Conclusions

- Cyclical hormone changes modulate the electrical properties of dorsal striatal MSNs in female rats.
- Female MSNs exhibit differences in action potential production, peak firing rate, threshold, and rheobase over the course of the estrous cycle.
- Thus, an estrous cycle-dependent sex difference exists in rat dorsal striatal MSN excitability.

**Developmental Effects on Regional Sex Differences in MSN Electrophysiology**

| Electrophysiological Property | Developmental Stage | Dorsal Striatum | Nucleus Accumbens Core | Nucleus Accumbens Shell |
|---|---|---|---|---|
| Intrinsic Excitability | Pre-puberty | F>M | F=M | F=M |
| | Post-puberty | F◯ M⟶ | ? | ? |
| Excitatory Synaptic Input | Pre-puberty | F=M | F>M | F=M |
| | Post-puberty | ? | F>M (Wissman et al., 2011) | F=M (Wissman et al., 2011) |

## Future Directions

- Further determine which intrinsic electrophysiological properties are altered by the estrous cycle
- Analyze excitatory synaptic input for estrous cycle-dependent sex differences
- Determine the most appropriate statistical analyses to perform for this data set
- Determine which hormone(s) are driving these effects
- Elucidate the cellular mechanisms underlying these effects

## Acknowledgements

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