INVITED REVIEW

The estrous cycle and 17β-estradiol modulate the electrophysiological properties of rat nucleus accumbens core medium spiny neurons

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Abstract

The nucleus accumbens core is a key nexus within the mammalian brain for integrating the premotor and limbic systems and regulating important cognitive functions such as motivated behaviors. Nucleus accumbens core functions show sex differences and are sensitive to the presence of hormones such as 17β-estradiol (estradiol) in normal and pathological contexts. The primary neuron type of the nucleus accumbens core, the medium spiny neuron (MSN), exhibits sex differences in both intrinsic excitability and glutamatergic excitatory synapse electrophysiological properties. Here, we provide a review of recent literature showing how estradiol modulates rat nucleus accumbens core MSN electrophysiology within the context of the estrous cycle. We review the changes in MSN electrophysiological properties across the estrous cycle and how these changes can be mimicked in response to exogenous estradiol exposure. We discuss in detail recent findings regarding how acute estradiol exposure rapidly modulates excitatory synapse properties in nucleus accumbens core but not caudate-putamen MSNs, which mirror the natural changes seen across estrous cycle phases. These recent insights demonstrate the strong impact of sex-specific estradiol action upon nucleus accumbens core neuron electrophysiology.

KEYWORDS
estradiol, estrogen receptors, estrous cycle, nucleus accumbens, sex differences

1 | INTRODUCTION

A defining feature of endocrinology is the influence of gonadal hormones upon target tissues. The tissues targeted by gonadal hormones include the brain and its component cells in both males and females, with highly differential effects depending on developmental stage, sex, brain region, neuron type, and other variables, including local hormone synthesis.[1] Gonadal hormones can both temporarily and permanently modulate target tissues from seconds to days to years. Here, we review the action of one gonadal hormone, 17β-estradiol (estradiol), upon the electrophysiological properties of medium spiny neurons (MSNs). Estradiol can be a gonadal hormone, manufactured in the ovaries in females and the testes in males, and can also be produced in other organs such as the brain.[2] Both of these sources are relevant to MSNs. Estradiol is also referenced as a steroid hormone, a sex hormone, and/or a sex steroid hormone.[3] Estradiol acts across a wide timeframe,[4] from years to days, to rapid and acute modulation in minutes to seconds.[5,6]

Here, we focus upon estradiol action in the nucleus accumbens core in adult rats, in the context of biological sex and the estrous cycle. Estradiol triggers the activation of estrogen receptors (ERs). ERs can be located in various cellular compartments, including the nucleus and the plasma membrane. MSNs express nuclear ERα, ERβ, and GPER-1 during early development. In adulthood, MSNs express plasma membrane-associated ERα, ERβ, and GPER-1.[7-19] Thus, estradiol can alter MSN electrophysiological function and ultimately nucleus accumbens core output by binding to ERs on MSNs during both development and adulthood. In this review, we provide evidence supporting this theme. We first introduce the nucleus accumbens core and MSNs, and how MSN electrophysiological properties are differentiated by sex and sensitive to estradiol action. We then review the estrous cycle, and its impacts upon nucleus accumbens core MSN electrophysiological properties. We then discuss recent findings demonstrating how acute estradiol exposure rapidly and sex-specifically modulates glutamatergic excitatory synapse properties in rat MSNs, and how these alterations mirror the natural changes seen across estrous cycle phases. We discuss how rapid estradiol action on glutamatergic synapses is not detected in rat caudate-putamen. We conclude with a discussion of possible local estradiol synthesis, as well as future directions.

2 | NUCLEUS ACCUMBENS CORE MSNS DIFFER BY SEX AND ARE SENSITIVE TO ESTRADIOL

The nucleus accumbens core is a striatal mesocorticolimbic region. In humans and rodents, the nucleus accumbens core serves as a critical nexus between limbic and premotor pathways to regulate motivated behaviors, especially the cognitive processing of reward and reinforcement.[20-23] The nucleus accumbens core and other striatal regions are evolutionarily ancient and well conserved across mammals, including rats.[24,25] In both humans and animal models, non-pathological behaviors controlled by the nucleus accumbens core and other striatal regions exhibit sex differences and are modulated by hormones such as estradiol.[26-28] Likewise, the phenotype and incidence of many disorders relevant to the nucleus accumbens core and the other striatal regions show sex differences and are modulated by hormones such as estradiol.[16,29-36] These sex differences and estradiol sensitivity in non-pathological behavior and relevant disorders make understanding the fundamental mechanisms underlying estradiol action in the nucleus accumbens core an important avenue of research. Of all the striatal regions, the nucleus accumbens core stands out as particularly sensitive to estradiol, as determined in diverse non-electrophysiological contexts by multiple laboratory groups.[18,33,34,37-50] The neurobiological mechanisms of how estradiol acts on neuron function in brain regions such as the nucleus accumbens core is in most cases underexplored. This situation is largely a result of the vast majority of preclinical research employing only males, with few studies directed at females, much less during different reproductive phases and estradiol levels.[51-55] Therefore, it is important to understand whether and how estradiol can modulate nucleus accumbens core neuron electrophysiological properties. These findings not only benefit the understanding of fundamental neuroendocrinology, but also could possibly generate new personalized approaches for therapies of nucleus accumbens core-related disorders informed by sex and hormone state.

The nucleus accumbens core's predominant neuron type is the GABAergic MSN. MSNs serve as the output neurons of the nucleus accumbens core, and integrate a wide array of inputs. These inputs include dopamine, which is the most famous and well-studied input, especially in terms of estradiol action. Estradiol exposure typically augments dopaminergic action in a sex-specific manner, as demonstrated by years of research from many laboratories.[18,47,56-60] The role of estradiol and dopamine in the nucleus accumbens has been recently reviewed.[18] The MSN also receives many other inputs, including inhibitory inputs from other MSNs and interneurons, and more prominently excitatory synaptic inputs from multiple brain regions that profoundly mediate function as excitatory synaptic inputs are essential for triggering MSN action potential production.[61-68] MSNs exhibit complex developmental period and region-specific sex differences in both excitatory synapse electrophysiological properties[69-75] (Figure 1), as well as in excitatory glutamatergic synapse-related neuroanatomy. These neuroanatomical properties include synapse number, neurochemical markers associated with glutamatergic synapse, and dendritic spine density, most prominently in the nucleus accumbens core and halso in other striatal regions.[76-81] In the early 2010s, ultrastructure anatomical studies from Woolley and colleagues found increased glutamatergic synapse number in adult gonad-intact female rat caudate-putamen and nucleus accumbens when compared to males.[76,78] Supporting this finding, another study from the Woolley laboratory shows that gonad-intact adult female rats exposed to cocaine demonstrated increased miniature excitatory postsynaptic current (mEPSC) frequencies compared to males treated with cocaine and female and male controls.[77] mEPSC frequency, along with amplitude and decay, is commonly assessed excitatory synapse attributes.

These findings from Woolley and colleagues are foundational for establishing the sexually differentiated nature of glutamatergic neural circuitry in the nucleus accumbens. Soon after the publication of Wissman et al.,[77] Cao et al.[70] began testing whether prepubertal rat nucleus accumbens core MSNs showed sex differences in their electrophysiological properties, including glutamatergic synapse properties. They also tested whether perinatal exposure to masculinizing/defeminizing doses of estradiol and/or testosterone could alter glutamatergic excitatory neurotransmission in female rat nucleus accumbens, to mimic the natural masculinization process found in males. Cao et al.[70] found, in prepubertal rats, that mEPSC frequency is higher in females compared to males. Changes in mEPSC frequency are typically associated with changes at the presynaptic side of the synapse, such as synapse number of differences in internal calcium sensitivity related to vesicle release probability. No sex differences in mEPSC amplitude or decay are detected prepuberty. mEPSC amplitude and decay is typically related to the properties of the postsynaptic side of the synapse, such as synaptic strength based upon AMPA receptor number and biophysical properties. The sex difference in mEPSC frequency is eliminated in females exposed to

FIGURE 1 Developmental of rat medium spiny neuron electrophysiological properties in the context of striatal region, sex, and estrous cycle. ↺, indicates estrous cycle-dependent sex differences are detected

Sex differences in medium spiny neuron electrophysiology across striatal region and development				
Electrophysiological category	Developmental stage	Caudate putamen	Nucleus accumbens core	Nucleus accumbens shell
Intrinsic excitability	Prepuberty	F>M _Dorris et al., 2015 [69]_	F=M _Cao et al., 2016 [70]_	F=M _Willett et al., 2016 [72]_
	Postpuberty	F ↺ M→ _Willett et al., 2020 [73]_	F ↺ M→ _Proaño et al., 2018; 2020 [74,75]_	?
Excitatory synaptic input	Prepuberty	F=M _Dorris et al., 2015 [69]_	F>M _Cao et al., 2016 [70]_	F=M _Willett et al., 2016 [72]_
	Postpuberty	F=M _Willett et al., 2020 [73]_	F ↺ M→ _Proaño et al., 2018; 2020 [74,75]_	?

masculinizing/defeminizing doses of estradiol or testosterone as neonates.[70] These findings indicate that sex differences in excitatory synaptic signaling onto MSNs in the nucleus accumbens core are present long before adulthood and are induced by hormone action during either the perinatal or prenatal periods. This organizational action by estradiol during early development then prepares the MSN to receive activational estradiol action upon mEPSC frequency and amplitude during the adult estrous cycle.

3 | THE ESTROUS CYCLE

Reproductively active rodents, such as rats, and primates, such as humans, show cyclical fluctuations of hormones, including estradiol and progesterone, amongst others. In humans, cyclical fluctuations occur in ovarian production of estradiol and progesterone over the approximately 28-day menstrual cycle, with peak circulating plasma levels of estradiol during the late follicular phase.[82] Female rats exhibit an analogous approximately 4–5-day estrous cycle, with similar but not identical fluctuations in ovarian hormones.[83–85] Both cycles feature distinctive phases. Each phase, including metestrus, diestrus, proestrus, and estrus, exhibits distinct hormone concentration profiles, in addition to concomitant changes in reproductive organs, the brain, and associated behaviors.[83–89] For example, the diestrus phase begins with relatively low levels of estradiol and progesterone that then gradually increase. The length of the diestrus phase can vary. Circulating levels of estradiol peak during the morning of proestrus phase (early proestrus, also called proestrus a.m.) and circulating levels of progesterone peak during the afternoon of proestrus phase (late proestrus, also called proestrus p.m.), which can exert differential actions on neuron function,[90] including MSNs.[74] Estrus phase is when select effects of estradiol and progesterone linger, even though the circulating concentrations of these hormones are low. Estradiol and progesterone action trigger distinct processes, and the combination of both is necessary for the expression of the full variety of mating behaviors. We also note that the estrous cycle is one natural cycle of many that modulates neural functions, and, although an important feature of

neuroendocrinology and neurobiology, the ovarian cycle does not make females inherently more variable than males.[32,91,92] Our focus in this review on the estrous cycle and 17β-estradiol is meant to highlight one of many potent hormones that can have differential actions depending on the systemic states of sex and/or gender. Although most nucleus accumbens core hormone research has concentrated on estradiol,[17,93] including our own, there is evidence that circulating progesterone levels correlate with several nucleus accumbens core MSN excitatory synapse properties.[74] In general, the estrous cycle and its associated hormones such as progesterone and estradiol are relevant for MSN and nucleus accumbens core function.

4 | THE ESTROUS CYCLE INFLUENCES NUCLEUS ACCUMBENS CORE MSN ELECTROPHYSIOLOGY: ROLE OF ESTRADIOL

The estrous cycle has been shown to influence neuron physiology across multiple brain regions.[73,75,90,94–97] Aside from the select regions already studied, we suspect that even more regions are sensitive to the estrous cycle than is now established as a result of the general lack of investigation into the neurobiology relevant to women's health. In naturally cycling female rats, MSNs show changes in both intrinsic excitability and excitatory synapse properties across estrous cycle phases, which generates sex differences compared to male MSNs[74,75] (Figure 2). Intrinsic excitability encompasses a variety of biophysical parameters that collectively embody the responsiveness of a neuron to electrical input. These parameters include such properties as resting membrane potential, and also properties such as action potential generation, rheobase, and input resistance, which are typically assessed by injecting neurons with electrical current and measuring changes in the output voltage.[98] For example, a decreased input resistance (a measurement of the change in voltage to injected current), as well as an increased rheobase (the amount of current necessary to elicit an action potential), typically indicates decreased excitability. Changes in MSN excitability alter how a MSN responds to synaptic input and directly relate to changes in circuit output and behavior.[99–105]

Excitatory synapse
↑ mEPSC frequency
↓ mEPSC amplitude

Intrinsic excitability
↓ Resting membrane potential
↓ Input resistance
↑ Rheobase
↓ Tau
↑ Delay to first AP
↓ FI slope

Excitatory synapse
↓ mEPSC frequency
↓ mEPSC amplitude

Intrinsic excitability
↑ Resting membrane potential
↑ Input resistance
↓ Rheobase
↑ Tau
↓ Delay to first AP
↑ FI slope

Excitatory synapse
↓↓ mEPSC frequency
↑ mEPSC amplitude

Intrinsic excitability
↓ Resting membrane potential
↓ Input resistance
↑ Rheobase
↓ Tau
↑ Delay to first AP
↓ FI slope

Excitatory synapse
↑ mEPSC frequency
↓ mEPSC amplitude

Intrinsic excitability
↓ Resting membrane potential
— Input resistance
↑ Rheobase
— Tau
↑↑ Delay to first AP
↑ FI slope

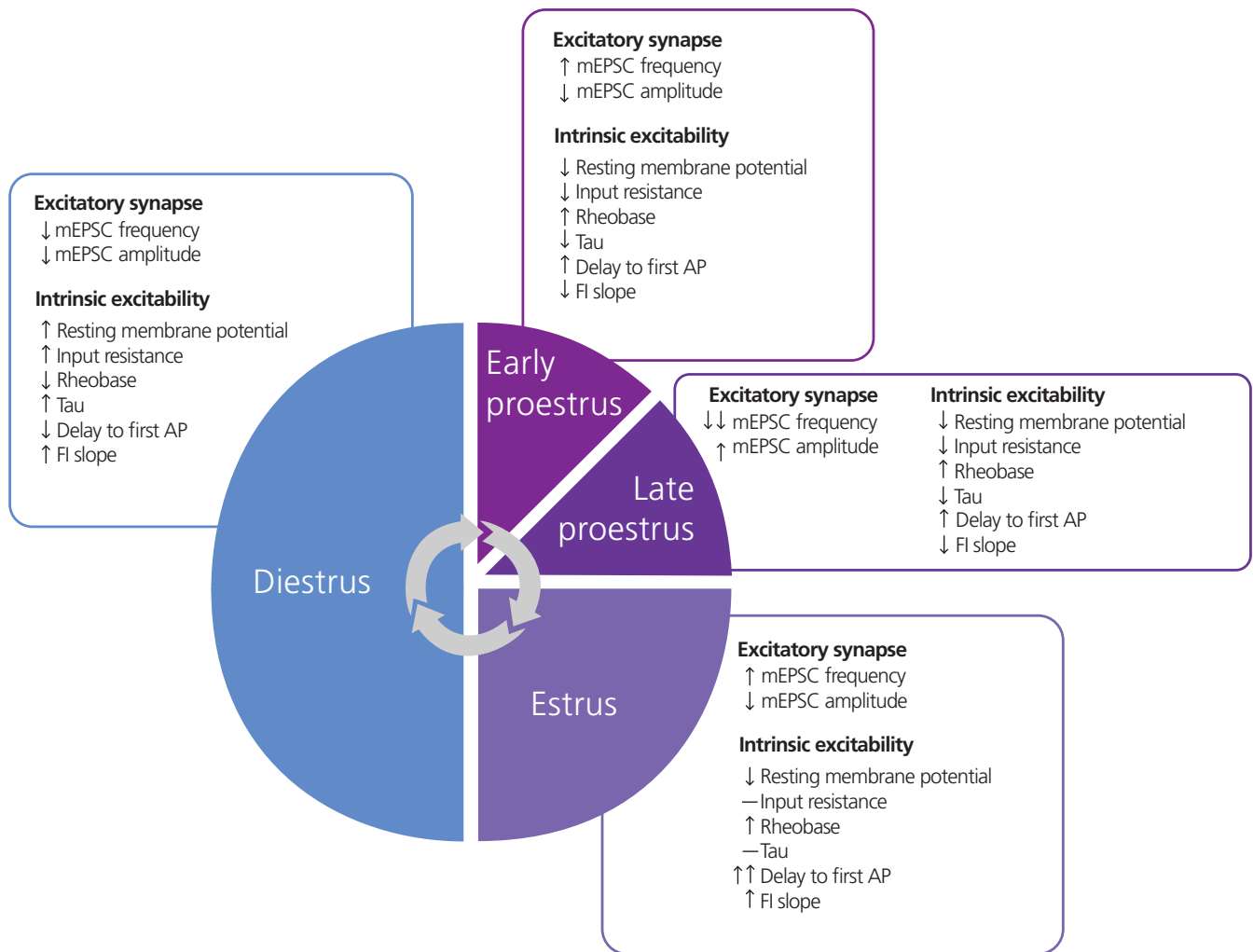Early proestrus

Late proestrus

Diestrus

Estrus

FIGURE 2 Schematic indicating changes in female rat nucleus accumbens core medium spiny neuron electrical properties across the estrous cycle. Changes in each attribute are depicted in comparison to an overall value over all cycle phases. Acronyms: ↑, increased value; ↓, decreased value; —, intermediate; AP, action potential; AP, action potential; FI, evoked firing rate-to-positive current curve; mEPSC, miniature excitatory postsynaptic current; Tau, time constant of the membrane; Input resistance, input resistance in the linear range. Input resistance in the rectified range follows a similar pattern. Not pictured on this schematic are the other following properties that change across the estrous cycle: inward rectification, input resistance in the rectified range, mEPSC decay, action potential width, and action potential afterhyperpolarization peak amplitude also differ across the estrous cycle. Data integrated from Proaño et al.[74,75]

MSNs recorded in early proestrus, late proestrus, and estrus phases showed hyperpolarized resting membrane potentials accompanied by decreases in input resistance and increases in rheobase compared to diestrus phase, among other select changes. These electrophysiological differences in intrinsic excitability properties are abolished when females are ovariectomized.[75] They are restored upon exposure to an exogenous estradiol replacement paradigm,[106] designed to mimic the changing temporal circulating levels of estradiol during the diestrus and proestrus phases of the rat estrous cycle.[107] In our study, acute brain slices of nucleus accumbens core were made 2 h after the last injection of estradiol into the rat. Thus, MSNs were recorded during the equivalent of proestrus phase.[106] In this preparation, MSNs exhibited decreased membrane excitability that was consistent with that demonstrated by MSNs in females in the early and late proestrus and estrus phases. MSNs did not exhibit membrane

excitability consistent with that found in the diestrus phase. Consistent with MSNs recorded from the proestrus phase in naturally cycling females, MSNs recorded from the gonadectomized females receiving exogenous estradiol showed decreased input resistance in both the linear and rectified range, hyperpolarized resting membrane potential, and increased rheobase.[106] These changes in intrinsic excitability induced by estradiol are hypothesized to be mediated by activation of membrane ERs expressed by MSNs, which in turn can act through intermediaries such as metabotropic glutamate receptors (MGluRs) to trigger intracellular signal cascades that ultimately modulate ion channel function and expression.[17,108] This longer, more genomic-like action of membrane ERs could explain why the changes in intrinsic excitability persist for several hours after the last exposure to estradiol, as well as naturally during proestrus and estrus phases. What changes are induced in MSN ion channel expression and function by

estradiol is not yet known, beyond L-type calcium channel function.[12] Given that estradiol induces a hyperpolarized resting membrane potential, accompanied by decreased input resistance, as well as increased rheobase, multiple biophysical mechanisms could underlie these changes. For example, estradiol could induce alterations in leak or inwardly rectifying (IRK) potassium channel expression, perhaps accompanied by changes in voltage-gated and/or leak sodium channel expression. One working hypothesis is that IRK potassium channel and voltage gated sodium channel expression both change in response to estradiol exposure, but in opposite directions. Potassium channels have been shown to be modified by estradiol in other neuron types.[109–111]

Regarding excitatory synapse properties, mEPSC frequency, amplitude, and decay robustly differed across the estrous cycle. Specifically, mEPSC frequency increased during early proestrus and estrus phases compared to diestrus phase, and significantly decreased in late proestrus phase compared to all other phases.[74,75] This drop in mEPSC frequency in late proestrus phase is unrivaled in magnitude, and we consider this represents the largest natural decrease in mEPSC frequency ever reported in the literature. Interestingly, although mEPSC frequency falls, MSNs in late proestrus phase maintain intrinsic excitability comparable to that measured in early proestrus and estrus. This curtailment of excitatory synapse activity is mediated by a rapid onset and acute activational effect of estradiol,[112] and also by a potential synergistic action with progesterone.[74] A common anatomical correlate of changes in mEPSC frequency are changes in dendritic spine density. It is not yet known whether MSN hdendritic spine properties naturally change across the estrous cycle, although pilot data from our laboratory supports this conclusion. In adult gonadectomized female rats and hamsters, a series of estradiol injections decreases nucleus accumbens core dendritic spine density by activating metabotropic glutamate receptor 5 (mGluR5) and endocannabinoid signaling.[11,12,80,81,113] No sex differences or estradiol effects have been detected on other anatomical attributes such as MSN soma size, density or striatal region volume.[114,115]

By contrast to mEPSC frequency, mEPSC amplitude is significantly increased in late proestrus phase compared to the diestrus, early proestrus, and estrus phases of the cycle. Regarding mEPSC decay, there are also significant differences across the estrous cycle that are quantitatively established to be the product of changes in mEPSC amplitude. These electrophysiological differences in excitatory synapse properties are abolished when females are ovariectomized,[75] and are consistent with a previous study comparing proestrus phase female and male nucleus accumbens core MSNs.[77] These changes in mEPSC properties are not restored using the exogenous estradiol replacement paradigm.[106] There are several possible explanations for this lack of restoration, with the most salient being that estradiol action on nucleus accumbens core MSN excitatory synapse properties is regulated by the rapid action of estradiol that acutely modulates and maintains the suppression of excitatory synapse properties such as mEPSC frequency.[112] Such a rapid action of estradiol may not be detected by the technique employed in the exogenous estradiol replacement study,[106] given that animals are sacrificed 2 h after the

last estradiol injection. A temporal difference in the impact of estradiol on electrophysiological properties, especially between the seconds to minutes to hours timescale, has been demonstrated in other systems.[109] There may also be differences in MSN electrophysiology in response to repeated doses of estradiol because several nucleus accumbens core related behaviors are sensitive to estrogen priming.[116–118] It is unknown whether inhibitory synaptic inputs and synaptic plasticity change across the estrous cycle.

There are at least two competing interpretations for the changes in MSN excitatory synapse and intrinsic excitability properties across the estrous cycle. First, estrous cycle sensitive properties directly facilitate related changes in nucleus accumbens core function resulting in altered behaviors. A second interpretation posits that changes in excitatory synaptic input and intrinsic excitability compensate for each other to mitigate differential circuit output, perhaps via an estrous cycle associated homeostatic plasticity mechanism, as detailed in other circumstances.[119,120] This second interpretation is based upon potentially compensatory changes in excitatory synapse properties and intrinsic excitability between diestrus phase MSNs and early proestrus and estrus phases MSNs. Arguing against this possibility, the late proestrus phase of the estrous cycle demonstrates different patterns of nucleus accumbens core MSN electrophysiological properties compared to other phases of the cycle, corresponding with this phase's differential hormonal, behavioral, and reproductive functions. The potentially compensatory changes in excitatory synapse properties and intrinsic excitability exhibited between diestrus phase compared to early proestrus and estrus phases dissipated during late proestrus phase. During late proestrus phase, changes in excitatory synapse properties are disengaged from changes in intrinsic excitability, and vice versa. Another line of evidence arguing against homeostatic plasticity is that different estradiol exposures result in differential effects on MSN electrophysiological properties, as well as the differential relationship of MSN electrophysiological properties to circulating levels of either estradiol, progesterone, or the combination of both, as may occur during the estrus phase.[74,106,112] There are also varying timeframes by which electrophysiological properties remain altered in response to estrous cycle phase. For example, several electrophysiological properties during the estrus phase display a more diestrus phase-like phenotype, others assume a more proestrus phase-like (both early and late) phenotype, while others exhibit more intermediate values (Figure 2). Overall, the specific alterations in MSN electrophysiological properties across the estrous cycle are dissociable and inducible by differential estradiol (and likely progesterone) exposures. We favor the first interpretation: changing electrophysiological properties across the estrous cycle directly facilitate changes in nucleus accumbens core function, mostly regarding its role in motivated reproductive behaviors.[121] These behaviors could potentially include the changes in sexual receptivity detected during the estrous cycle,[18,89] paced mating behavior,[122] sexual reward,[123] sensorimotor locomotor, and exploratory/anxiety-related behaviors,[36,117,118,124] and, at least in some species, mate choice and pair bonding.[125,126] We do note that correlation is not causation, and an important future direction will be to
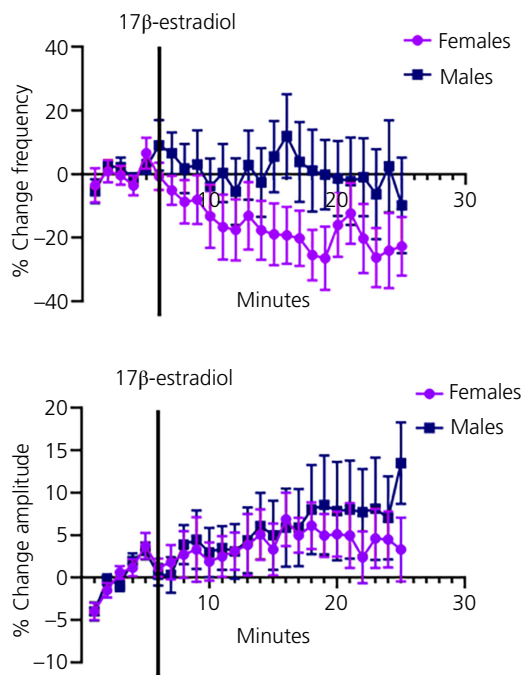
FIGURE 3 Estradiol rapidly modulates excitatory synapse properties in nucleus accumbens core medium spiny neurons within minutes of exposure. Top: Plotting the normalized change in miniature excitatory postsynaptic current (mEPSC) frequency versus time indicates that adult female rat nucleus accumbens core medium spiny neurons (MSNs) ($n = 16$) are robustly sensitive to rapid estradiol action upon mEPSC frequency while adult male nucleus accumbens core MSNs are not ($n = 14$). Bottom: Plotting the normalized change in mEPSC amplitude versus time indicates that adult female ($n = 16$) and male ($n = 14$) rat nucleus accumbens core MSNs are weakly sensitive to rapid estradiol action upon mEPSC amplitude. Vertical line indicates application of 100 nm 17β-estradiol. Vehicle alone controls showed no effect on either mEPSC frequency or amplitude (data not shown). Estradiol application exerts no effect on mEPSC frequency or mEPSC amplitude in either female or male MSNs recorded from the adult rat caudate-putamen. Data replotted from Krentzel et al.[112]

establish causal roles of estrous cycle action and ovarian hormone effects in the nucleus accumbens core to specific behavioral phenotypes.

5 | ESTRADIOL RAPIDLY MODULATES EXCITATORY SYNAPSE PROPERTIES IN A SEX-SPECIFIC MANNER IN RAT NUCLEUS ACCUMBENS CORE BUT NOT CAUDATE-PUTAMEN

Consistent with the hypothesis that estradiol acutely regulates a suppression of excitatory synapse properties, estradiol rapidly modulates nucleus accumbens core MSN excitatory synapse properties within minutes of exposure in adult female rats[112] (Figure 3). This action of estradiol is present in MSNs recorded in the nucleus accumbens core but not the caudate-putamen, and shows sex-specificity in its actions upon mEPSC frequency but not mEPSC amplitude. mEPSC frequency

robustly decreases in response to estradiol in female MSNs recorded across estrous phases but not male MSNs. To our knowledge, this is the first demonstration of an acute action of estradiol on excitatory synapse properties upon MSNs in any striatal region. The decrease in mEPSC frequency in response to estradiol does not correlate with any MSN intrinsic electrical property. Considering that mEPSC frequency is primarily associated with changes in presynaptic properties (i.e., number of synapses and/or neurotransmitter release), it is logical that MSN intrinsic properties do not relate to mEPSC frequency.

mEPSC amplitude moderately increases in response to estradiol in both male and female MSNs[112] (Figure 3). This change in mEPSC amplitude is considerably smaller in magnitude than that observed in mEPSC frequency. Thus, overall, acute estradiol exposure engenders a decrease in glutamatergic excitatory neurotransmission onto female MSNs, whereas, in males, if anything, it subtly augments excitatory neurotransmission. The protocol used in our laboratory to record mEPSCs specifically targets those generated by activation of glutamatergic AMPA channels.[75] The increase in mEPSC amplitude generated by acute estradiol exposure is a result of amplified AMPA receptor signaling. This augmentation could occur via AMPA receptor trafficking to the membrane, phosphorylation or some other mechanism. MSN dendritic-expressed estrogen receptor α may directly facilitate this effect. Pilot experiments from our laboratory support this conclusion, although these data remains to be published. MSNs exhibiting concurrent lower rheobase values and more hyperpolarized action potential thresholds show the greatest positive percent change in mEPSC amplitude in response to estradiol. Collectively, these data indicate that increased MSN excitability, as measured by a reduced rheobase and action potential threshold, associates with increased likelihood of estradiol-sensitivity in mEPSC amplitude. Rheobase is a strong predictor of whether a MSN is a D1 or D2 MSN subtype (D1 > D2; as reviewed previously[127,128]), however, these strict definitions of MSN subtypes tend to break down in the nucleus accumbens core.[129] Sex differences and estradiol sensitivity between MSN subtypes remains an active avenue of research.[127,130] These data predict that D2 subtype MSNs exhibit enhanced sensitivity to estradiol action upon mEPSC amplitude. Further testing with D1 or D2 labeled neurons in a relevant animal model must be conducted to confirm this prediction. Because rheobase or any other property fails to correlate with the impact of estradiol upon mEPSC frequency, it is likely that both MSN subtypes are sensitive to estradiol action upon mEPSC frequency.

The detected decrease in mEPSC frequency and amplitude likely indicates a locus of action that modulates the presynaptic terminal and postsynaptic dendrite respectively. Regarding changes in mEPSC frequency, it is possible that estradiol could be acting either directly on the presynaptic glutamatergic synaptic terminals or acting on the MSN (or perhaps even some other neuron type local to that MSN in the nucleus accumbens core) to trigger a retrograde messenger. Consistent with retrograde signaling, endocannabinoid action mediates estradiol modulation of MSN dendritic spine density in the nucleus accumbens core and associates with membrane ER and mGluR action.[113] GPER-1 is also expressed in the nucleus accumbens core and other striatal regions and could also potentially play a role.[14,19,131] There is evidence

from the hippocampus that sex-specific ERs can play key roles in regulating both presynaptic and postsynaptic glutamatergic transmission,[132] and this may be occurring in the nucleus accumbens core, given that both mEPSC frequency and amplitude are modulated by estradiol in distinct sex-specific manners. ERα, ERβ and GPER1 expression are present in the nucleus accumbens core on terminals and dendrites of MSNs of female rats.[7,10,14,19] Sex differences in ER expression on glutamatergic terminals may mediate the sex differences in the estradiol sensitivity to presynaptic excitatory synapse properties. To date, these sex-specific comparisons have not been conducted.

6 | ESTRADIOL DID NOT RAPIDLY MODULATE EXCITATORY SYNAPSE PROPERTIES IN CAUDATE-PUTAMEN MSNS

It is highly significant that estradiol application does not rapidly modulate mEPSC properties in either female or male caudate-putamen MSNs.[112] This negative finding does not mean that caudate-putamen MSNs are insensitive to acute estradiol exposure. Indeed, at this point, it is still the case that the majority of studies of striatal estradiol action have been performed in the caudate-putamen, although this situation is changing as the sensitivity of the nucleus accumbens core to estradiol becomes more prominent.[17] There is one broad finding from this body of research in the caudate-putamen that is consistent with our work on rapid estradiol modulation of nucleus accumbens core MSN: female MSNs appear to be more sensitive to acute estradiol action than are male MSNs.[12,18,133] Two different studies dating from the 1980s demonstrate differences in the in vivo spontaneous action potential generation rates of MSNs across different phases of the estrous cycle and in response to longer-term exposure to exogenous estradiol exposure in ovariectomized female rat caudate-putamen.[134,135] Arnauld et al.[135] made the initial discovery that estradiol exposure augmented dopamine sensitivity and in vivo spontaneous action potential firing rates in adult female ovariectomized rat caudate-putamen. However, this finding can not be classified as rapid estradiol action per se given the timing of the exogenous estradiol exposure. Tansey et al.[134] followed up on this study to show that the in vivo spontaneous action potential firing rates of identified striatonigral MSNs are augmented in the caudate-putamen of adult female rats during the high-estrogen phases of the estrous cycle or in response to estradiol implants compared to the low-estrogen phases of the estrous cycle or males. The study by Tansey et al.[134] is the first to demonstrate a sex-specific estradiol action upon striatal MSNs and is also the first to show that the electrophysiological properties of MSNs could change within hours as part of the estrous cycle.

The field of estradiol action upon striatal neuron electrophysiology then lay dormant for over a decade, until the mid-1990s. In a groundbreaking study, Mermelstein et al.[12] showed that, in female but not male rat caudate-putamen MSNs, estradiol decreased L-type calcium channel currents in a membrane-associated ER. The effect of estradiol on the L-type calcium channel is likely not directly responsible for the changes in caudate-putamen MSN excitability noted in the

studies conducted in the 1980s[134,135] or more contemporary research from our own laboratory.[69,73,136] This conclusion is a result of the L-type calcium channel's primary role in triggering internal signaling cascades typically activated by sustained depolarization.[137] Alterations in L-type calcium channel conductance did not robustly alter intrinsic excitability in a computational study of MSN biophysics conducted in our laboratory. Grove-Strawser et al.[11] later linked this decrease in L-type calcium channel currents to modulation of a signal transduction cascade that modulated CREB phosphorylation in MSNs, along with identifying the specific activated estrogen receptor as membrane ERβ. This membrane ERβ activates a mGluR, which is important given that membrane ERβ itself is not a GPCR. Other studies demonstrate that acute exposure to estradiol can induce not only behavioral changes, but also decreases in GABA release and increases in dopamine transmission in the caudate-putamen after an acute estradiol injection.[56,57,138]

7 | ENDOGENOUS ESTRADIOL PRODUCTION IN THE NUCLEUS ACCUMBENS

Although this review focuses upon gonadal estradiol acting in the context of the estrous cycle, the production of endogenous estradiol within the nucleus accumbens core may also play a prominent role. A previous review provides sufficient evidence to conclude that the striatal regions are potentially capable of producing local estradiol,[36] consistent with supporting data of aromatase protein expression across striatal brain regions including nucleus accumbens core.[19] Functionally, Tozzi et al.[139] detected that aromatase activity is necessary for at least one type of long-term potentiation (LTP) in adult male caudate-putamen MSNs. Female and nucleus accumbens core MSNs were not tested. Tozzi et al.[139] found that additional estradiol application did not augment LTP, suggesting that there may already be enough endogenously produced estradiol in the striatal regions to modulate neural physiology. Aromatase inhibition experiments will be an important avenue of future experiments for the nucleus accumbens core in both males and females, reminiscent of previous experiments in the songbird model organism. Work in the zebra finch, an oscine songbird, indicated that brain regions featuring enriched aromatase exhibit sensitivity to rapid estradiol modulation of neural electrophysiology.[140] Remage-Healey[140] have also demonstrated that neurons in brain regions that receive projections from areas with enriched aromatase can also be rapidly modulated. This indirect mechanism could be occurring in the nucleus accumbens core, as trans-synaptic hormone action occurs from cortical-like brain regions to striatal regions in white-crowned sparrow songbird brain.[141,142] However, the cell bodies of the neurons providing the glutamatergic synaptic terminals are not present in the employed nucleus accumbens core brain slice preparation, suggesting that estradiol action is specific to either the presynaptic terminal or triggers retrograde signals originating from the MSN (or potentially some other neuron). Related to this point, multiple types of membrane ERs are present on both the

pre- and postsynaptic sides of glutamatergic synapse onto striatal MSNs in general, and also in striatal cholinergic and dopaminergic synapses.[7,10,14] There is evidence that estradiol can modulate striatal cholinergic systems.[10,143] In any case, inhibiting aromatase action and ER blockade both comprise critical future experiments with the aim of determining the underlying mechanism of rapid estradiol action upon nucleus accumbens core MSNs.

8 | FUTURE DIRECTIONS

The mechanisms underlying all of these estradiol actions and their interrelations between themselves and the action of other hormones are all clear and important avenues for future investigations. The identity of the underlying sex-specific ERs is a key question, as are the consequential signaling cascades. Males and females may utilize different ER types and these ERs may differ in mediating either presynaptic or postsynaptic changes, as has been shown in the adult hippocampus[132] and prepubertal caudate-putamen.[136] How ER expression changes in response to the adult female hormone cycle and to relevant environmental and pathological contexts remains unexplored.[19] Whether ER expression and consequent action differs between MSN subtype remains an open question, as is estradiol action upon striatal neuron subtypes such as the cholinergic interneurons. A differential distribution of ERs by either sex, endocrine status or subtype would exert profound effects regarding nucleus accumbens function and relevant disorders.

9 | CONCLUSIONS

It is clear that the estrous cycle modulates nucleus accumbens core MSNs and that estradiol is an important component of this action. As befits a complex, natural process, select aspects of changes induced by the estrous cycle regarding MSN intrinsic excitability are mimicked by estradiol action over a slower, more persistent time course, and some aspects regarding MSN excitatory synapse properties are mimicked by estradiol action over a rapid time course. Given the strong influence of sex and hormones upon nucleus accumbens core-relevant behaviors and disorders,[34,144,145] the role of estradiol in modulating nucleus accumbens core neuron function in all its respects represents an important avenue of future research.

AUTHOR CONTRIBUTIONS
Amanda Krentzel: Visualization; writing – original draft; writing – review and editing. **Stephanie Proano:** Visualization; writing – original draft; writing – review and editing. **David Dorris:** Visualization; writing – review and editing. **Beverly Setzer:** Formal analysis; investigation; methodology; writing – review and editing. **John Meitzen:** Conceptualization; funding acquisition; project administration; supervision; visualization; writing – original draft; writing – review and editing.

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The authors declare that they have no conflicts of interest.

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