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REVIEW ARTICLE

# Sex differences in dopamine release regulation in the striatum

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The mesolimbic dopamine system—which originates in the ventral tegmental area and projects to the striatum—has been shown to be involved in the expression of sex-specific behavior and is thought to be a critical mediator of many psychiatric diseases. While substantial work has focused on sex differences in the anatomy of dopamine neurons and relative dopamine levels between males and females, an important characteristic of dopamine release from axon terminals in the striatum is that it is rapidly modulated by local regulatory mechanisms independent of somatic activity. These processes can occur via homosynaptic mechanisms—such as presynaptic dopamine autoreceptors and dopamine transporters—as well as heterosynaptic mechanisms, such as retrograde signaling from postsynaptic cholinergic and GABAergic systems, among others. These regulators serve as potential targets for the expression of sex differences in dopamine regulation in both ovarian hormone-dependent and independent fashions. This review describes how sex differences in microcircuit regulatory mechanisms can alter dopamine dynamics between males and females. We then describe what is known about the hormonal mechanisms controlling/regulating these processes. Finally, we highlight the missing gaps in our knowledge of these systems in females. Together, a more comprehensive and mechanistic understanding of how sex differences in dopamine function manifest will be particularly important in developing evidence-based therapeutics that target this system and show efficacy in both sexes.

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## INTRODUCTION

For many psychiatric disorders, such as anxiety, depression, and substance use disorder, sex is a critical biological variable and women represent a particularly vulnerable population [1, 2]. While sex differences in the pervasiveness and prognosis of these disorders have long been known to exist, we still lack a complete understanding of why these differences emerge. Many of these disorders are characterized by dysfunction in the dopamine system in reward-related brain regions [3, 4], and as such, there has been considerable interest in outlining the sex differences in dopaminergic anatomy, regulation, and function in preclinical models. Many sex differences exist independent of ovarian cycle fluctuations and reflect differences in basal dopamine system organization/neuroanatomy [5–14]. In addition, there is also robust dopamine system regulation by ovarian hormones where hormones, such as 17β-estradiol (E2), increase dopamine cell activity and release from dopamine terminals in the striatum [15]. Together, several physiological differences, including disparate neuroanatomical distribution of dopamine neurons in the midbrain, increased basal dopamine levels, and ovarian hormone regulation, combine to enhance dopamine system responsivity in females [5, 6, 15].

While the aforementioned studies have laid the groundwork for understanding basal sex differences in dopamine release, many of these studies have conceptualized dopamine along a linear continuum where function is either increased or decreased. This viewpoint overlooks a complex system of factors, both at the terminal and in the local microcircuitry, that can shape the magnitude, regional spread, and duration of dopamine in the synapse (For a comprehensive review, see Nolan et al. [16]). In the mesolimbic dopamine system, dopamine release from terminals in the striatum is maintained and controlled by regulatory proteins, including DATs [17], dopamine type-2 autoreceptors (D2Rs) [18, 19], heteroreceptors [20], channels regulating ion flux [21, 22], and steroid and ovarian hormones [23] (Fig. 1). These mechanisms can both elicit and inhibit dopamine release as well as modulate the timing and magnitude of release, independent of axonal input from midbrain cell body activity [24]. As such, these systems represent a biological substrate where sex differences in expression, function, and hormonal regulation may act to alter both the dopamine signal and ultimately dopamine-dependent behaviors.

Understanding how these sex differences fit into a comprehensive framework of dopamine regulation is critically important to our understanding of the basic mechanisms that govern neurotransmission in both sexes, as well as evidence-based interventions for diseases that are characterized by dysregulation of this system. Here we highlight the literature outlining these mechanisms and identify targets that represent important sites for sex-specific dopamine regulation, as well as discuss gaps in the existing literature that preclude our understanding of how sex differences in this circuit manifest. We focus first on the existing literature on global hormonal regulation of dopamine release, which has provided the framework for understanding the mechanisms governing dopamine release (see section "Hormone dependent effects on dopamine release in the striatum"). Next, we

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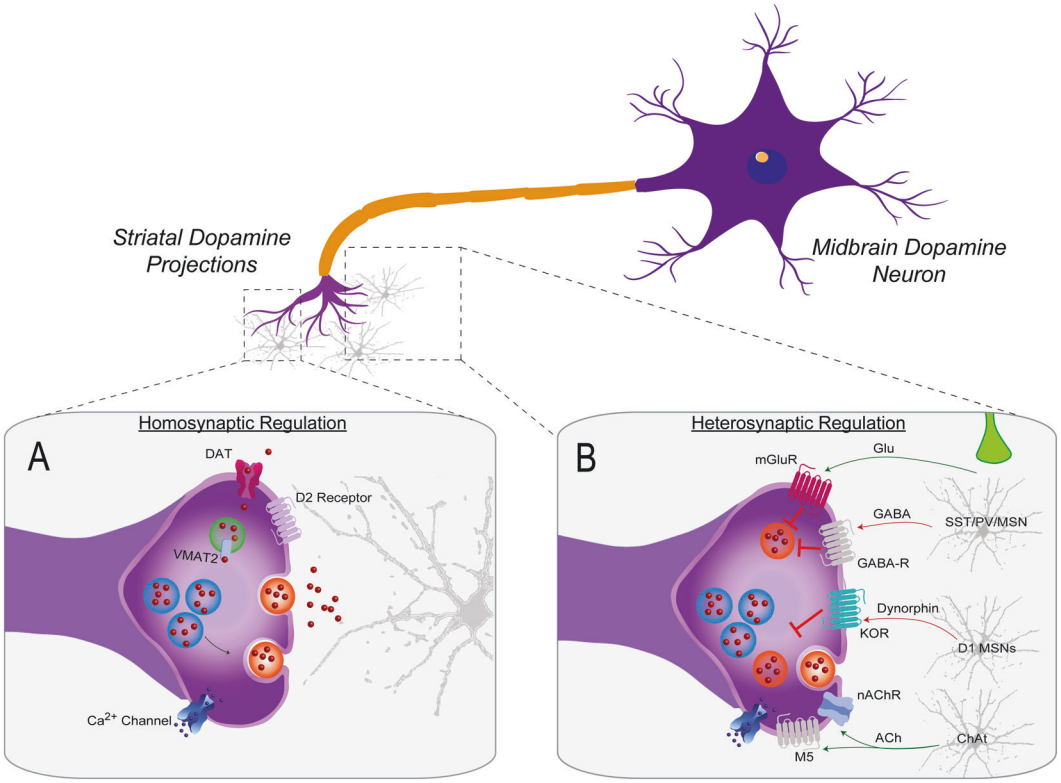


**Fig. 1 Homosynaptic and heterosynaptic regulation of striatal dopamine release from axon terminals.** Dopamine release at the terminal can be elicited and modulated through mechanisms intrinsic to the cell itself, as well as via substrates released from postsynaptic cells and presynaptic inputs from non-dopaminergic systems. **a** Activity and expression of transporters and autoreceptors—defined as homosynaptic regulators due to their expression directly on the terminal—can alter dopamine release. **b** Several neurotransmitters from local microcircuitry, such as glutamate, GABA, dynorphin, and acetylcholine, can modulate dopamine release at the terminal via heterosynaptic mechanisms. ACh acetylecholine, ChAt choline acetyltransferase, DAT dopamine transporter, GABA-R GABA receptor, Glu glutamate, KOR kappa opioid receptor, nAChR nicotinic acetylcholine receptor, M5 type muscarinic acetylcholine receptor, mGluR metabotropic glutamate receptor, MSN medium spiny neuron, SST somatostatin, PV parvalbumin, VMAT2 vesicular monoamine transporter 2. For a comprehensive review of these factors and some that have not been identified here, see Nolan et al. [16].

discuss how basal sex differences in homo- (see section "Mechanistic insight: Homosynaptic regulation of dopamine release via hormonal and non-hormonal factors") and hetero-synaptic (see section "Mechanistic insight: heterosynaptic regulation of dopamine release") regulatory mechanisms in the striatum interact with hormonal regulation to lead to differential regulation of dopamine release in males and females. We end by outlining why understanding these precise regulatory mechanisms within the local striatal microcircuit is critical to understanding female neurobiology.

## HORMONE-DEPENDENT EFFECTS ON DOPAMINE RELEASE IN THE STRIATUM

### Interactions between the estrous cycle and dopamine release

During development, X-chromosome genes play a role in developmental programming of the dopamine system via genetic and hormonal factors that can lead to sex differences in both basal function and the subsequent hormonal control over these processes in adulthood [25–27]. In adulthood, many basal sex differences in the dopamine system exist independent of ovarian cycle fluctuations and thus reflect differences in basal dopamine system organization/neuroanatomy. For example, in adult rodents, the substantia nigra contains more dopamine neurons in males than females [5]; however, the opposite is true in the VTA [6]. Further, in the VTA, dopamine neurons make up a larger percentage of the cellular population in females [7]. Finally, morphologically, VTA soma in females are larger compared to males [14]. These neuroanatomical differences serve as a critical substrate upon which subsequent hormonal regulation or stimulus-specific excitation can act to elicit and regulate dopamine release in males and females.

Extracellular dopamine concentrations in the striatum vary with estrous cycle stages in females [28], with highest levels occurring during proestrus and estrus (when ovarian hormones are highest (proestrus) and immediately after (estrus)) and lower levels during metestrus and diestrus (See Fig. 2b–d for hormone cycles and phases), indicating that hormonal cycles are critical regulators of dopamine release. This phenomenon has been observed across a variety of recording techniques, including temporally slow techniques like microdialysis [12] and faster sampling methods, such as fast-scan cyclic voltammetry (FSCV) [29, 30]. In addition, dopamine neuron action potential activity is increased in the VTA during estrus [30, 31]. Interestingly, ex vivo voltammetry experiments in isolated striatal terminals have shown enhanced evoked release—despite the fact that VTA cell bodies are not present in the preparation—highlighting that vesicular release of dopamine is enhanced in addition to enhanced VTA cellular activity [32]. These effects likely combine to augment extracellular dopamine levels in vivo and enhance stimulus-dependent release during this period.

### Synaptic regulation of dopamine release via exogenous hormone application

A large amount of work has aimed to understand which ovarian hormones contribute to these effects. While multiple ovarian

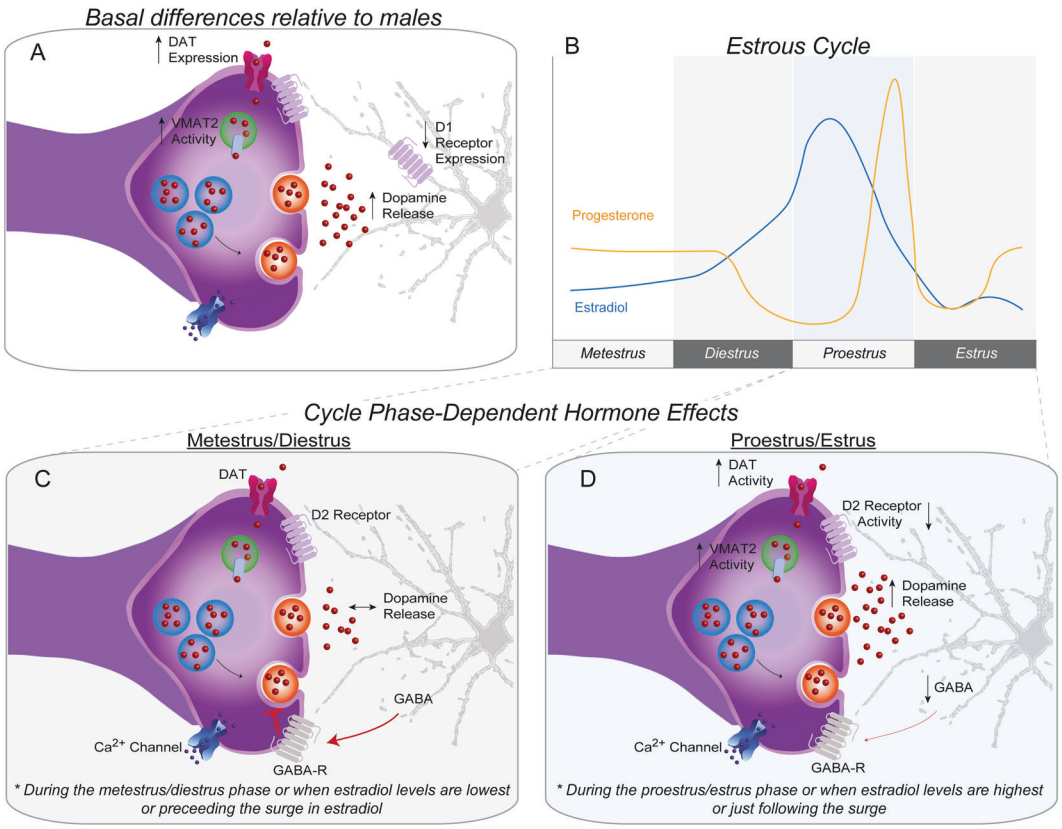


**Fig. 2 Sex differences in regulation of dopamine release at dopamine terminals in the striatum. a** Basal differences in females relative to males. Females show lower expression of D1 receptors on GABAergic MSNs as well higher DAT expression and increased VMAT2 activity, indicating greater regulation of dopamine release and reuptake. **b** Estradiol (E2) and progesterone are known to regulate dopamine release in striatal brain regions. Their levels are dependent on the phase of the estrous cycle in intact, cycling females. **c** In metestrus/diestrus, when ovarian hormone levels are low or preceding the surge and dopamine release is at basal levels (equivalent to OVX females and males). **d** During proestrus/estrus, when hormone levels are at their highest, dopamine release is enhanced via increases in active release, reduced D2 autoregulation, increased DAT and VMAT2 activity, and reduced GABA release and associated receptor activation. Many of these effects have been linked to E2, specifically. Further, similar changes have been observed following E2 administration to OVX rodents. As a note, some of the changes identified (GABA) are following E2 application.

hormones have been implicated in dopamine regulation, the most robust and well-studied regulator of these processes is E2; however, it is important to note that E2 does not act in isolation. Indeed, treatment with E2 or progesterone alone only slightly increased the release of dopamine from striatal tissue. However, when ovariectomized (OVX) females were treated with E2 followed by progesterone—to mimic endogenous hormone release during estrus—stimulus-dependent dopamine release was augmented to levels seen in intact animals [33, 34]. Thus, the interaction between hormones during the endogenous estrous cycle plays a key role in dopamine release regulation. However, previous work has shown that E2 is more tightly correlated with dopamine release effects than progesterone [30] and E2 replacement alone is capable of recapitulating many of these effects. For example, E2 administration to OVX mice and rats increases dopamine release, enhances VTA cell firing, and enhances amphetamine-induced dopamine efflux [35, 36]. Therefore, below, we will focus on how E2 functions as a critical mediator of site-specific dopamine release regulation in the striatum.

The dopamine system contains a high density of estrogen receptors (ERs), suggesting that the basal differences in anatomy, as well as release and regulatory mechanisms, likely serve as important substrates through which ovarian hormones exert their influence on dopaminergic function. ERs (both alpha and beta subtypes) as well as membrane bound G-protein estrogen receptor-1 receptors are all expressed in both VTA dopamine neurons and in local microcircuitry in the NAc [37–39]. As such, estradiol exerts its effects via both genomic and non-genomic mechanisms. Interestingly, ERs are localized on both dopamine terminals and local GABA circuitry in the ventral striatum, while in the dorsal striatum ERs are localized on GABA neurons but not expressed on dopamine terminals [37, 40, 41]. Importantly, these GABAergic neuronal populations can regulate dopamine release through indirect mechanisms providing multiple avenues for dopamine release regulation through hormonal regulation of the striatum (outlined in section “Mechanistic insight: heterosynaptic regulation of dopamine release”).

While basal and hormonal factors, such as E2, appear to have a fundamental role in the expression of sex differences in dopamine release, understanding the mechanism by which they exert these effects is key to understanding their impact. Elucidating these mechanisms requires rapid sampling techniques like FSCV that allow for the dissociation of vesicular release from other factors that affect dopamine levels on both rapid and prolonged timescales, such as transporter-mediated dopamine clearance. Indeed, voltammetry studies have shown that females have increased evoked dopamine release as well as enhanced clearance rates, suggesting that differences in both release regulation and clearance mechanisms contribute to overall differences in basal synaptic dopamine levels [13, 30, 36]. E2 has also been shown to regulate both release and clearance mechanisms in females [36]. First, changes in subsecond dopamine release over the estrous cycle correlates with the levels of circulating E2, but not

progesterone [30]. In addition, E2 administration increases evoked dopamine release (electrical or K+ stimulated), increases dopamine turnover and metabolism, and enhances DAT activity [31, 42–47]. Furthermore, E2 facilitates the ability of stimulants to increase dopamine levels through multiple mechanisms related to enhancing releasable pools [23, 30]. Thus, while it is clear that there are both basal differences in terminal regulation (through vesicular release and transport mechanisms) it will be important to further define how sex differences in terminal regulatory mechanisms contribute to and control these effects at the mechanistic level.

## MECHANISTIC INSIGHT: HOMOSYNAPTIC REGULATION OF DOPAMINE RELEASE VIA HORMONAL AND NON-HORMONAL FACTORS

The work above has highlighted clear sex differences that are characterized by two factors: (1) basal differences in dopamine system organization/function that exist independent of hormonal control, and (2) estrous cycle/hormonal regulation of terminal effectors that ultimately alter dopamine release (Fig. 2). The interaction between these two factors combines to control how dopamine is regulated at the terminal in females and set the dynamic range by which signaling and regulation can occur. Here we outline the mechanisms by which effectors located on dopamine terminals respond to dopamine itself (autoreceptors and transporters) to dictate release probability and timing.

### Autoreceptor regulation

D2Rs are located at the center of feedback regulation on dopamine terminals and as such serve as potent regulators of release. D2Rs are expressed at the cell body in the VTA, as well as on the terminals in striatal regions (Fig. 1), and serve to decrease dopamine production and release when extracellular dopamine is elevated at the synapse by reducing tyrosine hydroxylase expression, reducing cell body/terminal excitability, and reducing release pools [18, 19, 48–54]. In female rats, the D2 antagonist haloperidol—which removes feedback inhibition—increased dopamine release to a greater extent (twofold greater than males). However, quinpirole—A D2 agonist—was less effective at inhibiting dopamine release in females, suggesting that these receptors are near-maximally activated at baseline and thus subsequent increased receptor activation cannot further reduce release [55]. While the previous study did not explicitly assess hormonal/estrous cycle regulation of D2 function, this process was shown subsequently to be regulated over the estrous cycle. During estrus, quinpirole was 10-fold less effective at reducing dopamine release through D2Rs as compared to both males and females in diestrus [30]. Interestingly, there were no differences between diestrus and male mice, suggesting that the effects are hormone-mediated, rather than basal sex differences. Similar effects have been observed at the cell body in the VTA where there is a decreased ability of applied dopamine to reduce the firing activity of these dopamine neurons during estrus [56]. Thus, along with enhanced release, there is also a reduction in the ability of D2Rs to reduce release in the presence of dopamine—an effect that would increase release magnitude and reduce feedback mechanisms, further promoting stimulus-dependent dopamine release and sensitivity to stimuli that act on this system.

### Changes in the expression and function of transporters

*Dopamine transporter*. The primary mechanism by which dopamine is cleared from the synaptic and extra-synaptic space is via DAT (Fig. 1) [57]. DAT is a membrane-spanning transporter located on dopamine cell bodies, their dendrites, and their axonal projections [58, 59]. DAT plays both a critical role in the timing and duration of dopamine release events as well as allowing for effective repackaging into vesicles for re-release [60–62]. It thus represents a likely mechanism underlying sex differences in the temporal dynamics of stimulus-dependent dopamine release [63]. Previous work has shown that uptake rates are enhanced in the striatum of female rats compared to males [13] and that drugs that block dopamine clearance are more effective at increasing dopamine levels in females suggesting that synaptic dopamine is more tightly regulated by DAT [55]. While these functional differences in clearance rate highlight that there are differences in DAT regulation, this can occur via both changes in DAT levels as well as changes in orthosteric DAT function independent of relative expression—both of which have been shown to be differentially regulated between males and females.

First, males and OVX females show lower DAT expression than intact females [64], suggesting that DAT regulation between males and females is, at least in part, controlled by ovarian hormones. However, there are conflicting reports about changes in DAT expression over the estrous cycle in intact females with some reports showing that expression levels within intact females are highly regulated by estrous cycle phase [64] while others have shown no change in total DAT expression over the cycle [30]. Regardless of whether the total DAT protein levels are changed, there are significant alterations in clearance rates—mediated by DAT—that occur over the estrous cycle. Multiple studies have shown enhanced dopamine clearance rates in females [30, 47] and that clearance rate is highest during proestrus/estrus as compared to met/diestrus. Interestingly, there are cycle-dependent post-translational modifications of DAT that could explain increases in clearance rates, independent of changes in transporter levels. Specifically, during estrus, there is an increase in the phosphorylation of DAT at threonine 53 [30]. This site has specifically been shown to regulate the speed of clearance as well as the ability of psychostimulants to bind to the transporter [65, 66]. Indeed, multiple studies have shown that the ability of cocaine and amphetamine to bind to DAT and/or increase dopamine levels in striatal regions is highest during estrus [8, 30, 67].

There are also some reports in cell culture that E2 itself may block the DAT and reduce clearance rates [47]. However, studies in intact slice preparations observed no effects of E2 on clearance rates [30]. Alternatively, steroid hormones have been shown to have actions on organic cation transporter-3 which is a secondary high capacity, low-affinity transport system for dopamine [68]. Thus, this provides an additional unstudied mechanism by which hormonal regulation could influence overall dopamine clearance independent of direct effects on DAT.

Together, the literature suggests tighter regulation of clearance by DAT and increased clearance rates in females. While this may seem counterintuitive to enhanced synaptic dopamine levels recorded in females, it is important to note the critical role that DAT plays in repackaging and release. For example, knocking out DAT significantly reduces dopamine release and shifts release to a synthesis-dependent mechanism which is easily depleted [60]. Indeed, female heterozygous DAT knockout mice (which express ½ DAT levels as WT littermates) show decreased striatal tissue dopamine content as compared to wild-type controls and heterozygous males. Thus, females rely more on repackaging mechanisms for continued release than males [69].

*Vesicular monoamine transporter (VMAT) 2*. Giving further support to the idea that females rely more on dopamine repackaging for re-release is data showing enhanced vesicular monoamine transporter 2 (VMAT2) function in females. VMAT2 transports dopamine from the cytosolic space into synaptic vesicles in preparation for future release events at the terminal [70, 71]. A number of pharmacological agents can be used to probe the contribution of VMAT2 to release—including reserpine which is a potent and selective VMAT2 blocker. In mice treated with reserpine, extracellular striatal dopamine concentrations were more drastically reduced in females than males showing that

dopamine levels are tightly regulated by VMAT2 and to a greater extent in females [47]. Further, application of reserpine to striatal tissue also had a greater effect on extracellular dopamine levels in females suggesting they having more active/efficient VMAT2 function, which aids in repackaging and re-release. However, there is only limited data on VMAT2 expression and function in intact cycling females. Interestingly, in a study that looked at genes upregulated in proestrus in antero-ventral periventricular zone and medial preoptic area, the gene encoding VMAT2 was upregulated [72], suggesting potential for estrous-cycle dependent regulation in the striatum as well. However, in studies that have explicitly tested the effect of E2 (and ovariectomy) on VMAT2 regulation the effects were less clear. In rats, E2 treatment in OVX rats downregulated [$^3$H]TBZOH (a marker for VMAT2) binding in the striatum, suggesting that E2 does act to regulate VMAT2 protein expression. However, ovariectomy alone did not affect the density of [$^3$H]TBZOH binding sites in the striatum [73]. Thus, exactly how hormonal regulation in intact cycling females regulates VMAT2 expression and function requires further study.

Together, these data highlight potent regulation of dopamine release in females by transporters and other homosynaptic mechanisms. Enhanced release, paired with reduced autoregulation through D2 receptors and more effective repackaging of dopamine into vesicles for re-release through DAT and VMAT2 combine to allow more dopamine release following both low frequency and high-frequency stimulations in females [30]—both at baseline and to an even greater extent during and immediately after circulating E2 is elevated (during proestrus and estrus).

## MECHANISTIC INSIGHT: HETEROSYNAPTIC REGULATION OF DOPAMINE RELEASE

Dopamine terminals in the striatum express several classes of heteroreceptors that can affect the magnitude and frequency of release (Fig. 1). Ligand-gated receptors—such as nicotinic acetylcholine receptors (nAChRs) [74] and GABA-A receptors [75]—allow for the passage of ions that can increase or decrease release probability at terminals. Similarly, G-protein coupled receptors (GPCRs) located on terminals can also alter release probability and dopamine synthesis through initiation of intracellular cascades [76]. Dopamine terminals express many classes of GPCRs, including: D2Rs [48, 77], kappa opioid receptors (KOR) [78, 79], GABA-B receptors [79, 80], as well as metabotropic glutamate receptors (mGluR) [81, 82], and metabotropic acetylcholine receptors [83, 84]. We outline here known sex differences in local microcircuitry and what is still unknown.

### GABA circuitry

GABA likely plays a critical role in the expression of sex differences in dopamine release across the striatum [85]. Locally released GABA reduces dopamine release, either through Gi-coupled GABA-B receptors or ionotropic GABA-A receptors [75, 79, 86]. However, there is some debate as to the localization of these receptors on local circuitry and terminals. While the effects of GABA-B on dopamine release are through direct mechanisms, GABA-A receptor-mediated reductions in dopamine release can be blocked with a GABA-B receptor antagonist, suggesting either interactions between the two receptors or a polysynaptic mechanism [75]. Regardless, both receptors are capable of robustly regulating dopamine release on a rapid time scale.

There are a variety of sources of GABA within the striatum such as parvalbumin and somatostatin interneurons, and GABAergic medium spiny neurons MSNs [87, 88]. MSNs make up ~90% of the cells in the striatum and are divided into two, mostly non-overlapping, cellular populations defined by their expression of D1 and D2-type dopamine receptors [89]. Through these receptors, dopamine can also regulate the activity of these GABA cell populations—providing a loop by which dopamine signaling and subsequent release can be finely tuned. Therefore, sex differences in GABA receptor expression on terminals, cellular excitability of local GABAergic populations, or dopamine receptor expression on GABA cell populations can all contribute to sex differences in local dopamine release regulation via GABA.

Currently, sex differences in direct GABA receptor regulation of dopamine terminals have not been studied; however, there are well-reported differences in the activity and regulation of the GABAergic cells in the striatum—which provide the substrate for these receptors. First, there are basal differences in D1—but not D2—receptor expression on GABAergic MSNs, with female rats expressing 10% fewer D1 receptors in the striatum [90–92]. D1 receptors are $G_q$ coupled receptors that activate signaling cascades that have been shown to enhance the excitability of the MSN populations that express them [93]. Thus, these reductions in dopamine receptor expression could reduce GABA feedback onto dopamine terminals and lead to enhanced dopamine release in females. In addition, in the NAc, MSN intrinsic excitability does not differ between males and females at baseline, although increased excitatory synaptic input onto MSNs has been observed in females [94]. Together, it is likely that these differences provide the baseline upon which hormones act to robustly regulate GABA signaling—and, accordingly, dopamine release at terminals.

In females, E2 decreases dendritic spine densities on MSNs and decreases MSN excitability [95], which reduces GABA release [96, 97]. This rapid reduction in GABA release likely leads to disinhibition of dopamine terminals and renders them more responsive to stimulus-dependent dopamine release, which would explain data showing that E2 in the striatum enhances dopamine release indirectly, but through presynaptic mechanisms [96, 98]. While E2 stimulation of dopamine release has been observed in both the ventral and dorsal striatum, in the dorsal striatum ERs are not expressed on striatal dopamine terminals. This would likely necessitate an indirect mechanism by which dopamine is increased, such as through GABA-mediated disinhibition [37]. Together, disinhibition of GABA regulation of dopamine terminals provides another regulatory mechanism that is hormonally mediated and also likely underlies enhanced dopamine release in females.

### Metabotropic glutamate receptor (mGluRs): a critical role through indirect mechanisms

Another important regulator of dopamine release involves glutamatergic regulation—which can alter dopamine release at baseline and also play a critical role in the effects of E2 on the dopamine system [35]. First, while many ER effects are through genomic mechanisms, there are also rapid effects on membrane signaling that can alter the physiological properties of cells on a rapid time scale. Many of these effects have been shown to be mediated through the ability of ERs to functionally couple to mGluRs and activate their signaling cascades [99]. This has been well studied in MSN populations, where the rapid-acting effects of E2 on MSN activity are directly dependent on mGlu5 signaling [35]. Similarly, mGlu5 antagonism abolishes the ability of E2 to enhance amphetamine-mediated efflux showing that it plays a critical role in the direct effects of E2 in the striatum on dopamine release from terminals [35]. While there have been reports of mGluRs (mGluR1 and 5) directly located on dopamine terminals, these studies have shown that glutamate spillover from synaptic activity depresses—not increases—dopamine release [100]. Lastly, previous work has applied E2 directly to dissociated dopamine terminals in slice preparations and did not observe increases in evoked dopamine release, suggesting that the fast effects of E2 on dopamine release are through polysynaptic microcircuit mechanisms [30]. Thus, while mGluRs are inextricably linked to dopamine release effects when E2 is administered acutely these effects occur through indirect terminal regulatory mechanisms—likely through GABA effects [96].

Other circuitry that requires more research: kappa opioid receptors, cholinergic systems, other interneuron populations

*The cholinergic system*. Similarly, another potent dopamine terminal regulatory mechanism is through acetylcholine release from cholinergic interneurons and the associated activation of nAChRs. nAChRs are pentameric ligand-gated iononotropic receptors that are activated by the endogenous ligand acetylcholine. In the striatum, dopamine is released in tonic (slow and regular) and phasic (short, burst/spikes) frequency patterns [101] that are subject to heavy modulation by these cholinergic systems [102]. Normally, when increasing electrical stimulation frequencies are applied to dopamine terminals in brain slices, the total amount of dopamine release stays relatively stable; however, when acetylcholine is blocked by a nAChR antagonist, dopamine release is robustly responsive to stimulation frequency [102, 103]. These results have led to the hypothesis that acetylcholine in the striatum acts as a low-pass filter at dopamine terminals. In other words, in basal conditions where tonic acetylcholine is present, high frequencies stimulations are "filtered out", but when acetylcholine is blocked or reduced via endogenous mechanisms, this filter is lifted [102, 103].

Previous work looking at the relationship between tonic and phasic stimulation frequencies in females has shown that females are much more responsive to higher stimulation frequencies—i.e., have an enhanced tonic to phasic relationship—suggesting a potential role for cholinergic systems in sex differences in dopamine release [30]. Further, studies have shown that E2 has affinity for nAChRs and may serve a functional role in regulating their activity [104]—an effect that would alter dopamine release. However, while binding studies have shown E2 has affinity for nAChRs and is capable of inducing nAChR-mediated currents in cell culture, the functional consequences of these effects on dopamine release remain to be explicitly defined [104]. Further, receptor desensitization is an important mechanism by which these receptors are regulated [105] and it is possible that sex differences in desensitization at baseline—or in response to E2, which has been shown to alter their function—could lead to differences in the ability of this system to be recruited in females; however, this requires explicit testing to define how this would influence dopamine release regulation in different cases.

In addition to nAChR effects on dopamine terminals, there are also sex differences that suggest that acetylcholine signaling may be potentiated in females via multiple additional mechanisms. Choline acetyltransferase activity—the enzyme responsible for acetylcholine synthesis—fluctuates with the estrous cycle and in OVX animals, a single administration of estradiol significantly increases ChAT mRNA in the striatum for 1–3 days [106], suggesting a potential for increased stimulus-dependent acetylcholine release and/or elevated acetylcholine tone at baseline in females. In addition, there are increased levels of metabotropic muscarinic receptors in females [91], which are known to regulate dopamine release. Muscarinic receptor type 5 (M5) receptors are Gq coupled receptors expressed directly on dopamine terminals in the striatum and act to potentiate dopamine transmission [83, 84, 107]. However, currently it is not clear if there are sex differences in dopamine release regulation via these mechanisms.

Together, there are multiple avenues that may underlie sex differences in cholinergic regulation of dopamine release: acetylcholine synthesis and release, nicotinic receptors, and muscarinic receptors. While understanding these effects is important in general, this has wide-spread implications for sex differences in neural function and behavior. Any system that activates or regulates cholinergic interneurons, acetylcholine release, and subsequently acetylcholine receptors acts through this system and thus will likely also show sex differences. For example, glutamatergic inputs into the striatum (from motor cortex and other cortical areas) have been shown to evoke dopamine release directly at terminals—an effect that occurs through their ability to stimulate cholinergic interneurons [108–110]. Further, hormonal factors that have been shown to act through cholinergic interneuron regulation, such as corticotropin releasing factor, also likely show differences in their ability to regulate dopamine release and associated behaviors in females [111]. Moving forward, it will be important to specifically outline how sex differences in these systems manifest, given the central role that acetylcholine plays in the regulating striatal dopamine release by integrating information from a variety of sources across the brain.

*Kappa-dynorphin system*. A potent regulator of dopamine release in the NAc is through kappa opioid/dynorphin interactions. KORs are $G_i$ coupled receptors that, when activated, reduce dopamine release. Dynorphin is released following activity of D1 receptor containing MSNs and there are well-reported differences (outlined above) in both MSN excitability and D1 receptor expression that likely lead to differences in dynorphin released from these cellular populations [78, 79, 112]. While direct measurements of the effects of KOR-mediated dopamine terminal modulation have not been done, there is a large body of literature demonstrating sex differences in behavioral effects for KOR agonists [113, 114]—especially as they relate to dopamine-dependent behaviors—suggesting terminal regulation could be at play. Further, this is associated with a resistance to KOR-mediated dopamine reductions, suggesting that KOR effects on dopamine release are reduced in females [115]—an effect that would lead to enhanced dopamine release. Together, a large body of work on KOR-dopamine interactions as they relate to stimulant drugs (for comprehensive review see: Chartoff and Mavrikaki [114]) has highlighted robust sex differences in these processes; however, to date, it is unclear if these effects are occurring directly at dopamine terminals or are through circuit effects.

What do all of these regulators mean together?

The effectors described above represent a diverse set of regulatory mechanisms that can work either in concert or independently to alter when and where dopamine is released and determine the duration of dopamine's presence in the extracellular space. Through dynamic modulation of dopamine release, signaling, and clearance, temporal signals can be tightly regulated on a rapid time scale in both males and females. Further, feedback regulation can occur through retrograde signaling from microcircuitry, such as GPCRs and ligand-gated ionotropic receptors that regulate dopamine synthesis and release probability to alter future and ongoing dopamine release. Together, reduced feedback from D2 regulation, enhanced clearance and repackaging mechanisms, and reduced GABA inhibition combine to lead to increased basal dopamine levels and enhanced stimulus-driven dopamine release in females—an effect that is highly sensitive to circulating ovarian hormones.

A large majority of the work outlining striatal circuitry—and associated microcircuitry—was conducted in male animals, despite seminal work showing significant local effects of gonadal hormones within mesolimbic systems [8, 9, 13, 15, 55, 116–118]. Thus, beyond these sex differences in dopamine release magnitude there are likely important organizational differences in these systems that regulate their function through different effectors and circuitry. Along these lines, there is significant evidence for differences in distribution and size of dopamine cell bodies between the sexes, even early in development, suggesting that dopaminergic systems are at least in part differentially organized [119]. However, studies to explicitly test whether all terminal regulatory mechanisms identified in males [16] are present in females, whether the microcircuits are organized the same way between the sexes, or whether they affect dopamine release to a similar extent in both sexes have not been comprehensively and definitively determined.

Together, the data presented within this review may speak to more complex changes in sensitivity of dopamine terminals to sex differences in release regulation beyond just more or less dopamine at baseline. In females, dopamine release is more sensitive to lower stimulus magnitudes and feedback inhibition is reduced—effects that would lead to both the enhanced magnitude and timing of dopamine release in certain situations [30]. Further, in nearly all cases E2 augments these effects through a variety of potential circuit mechanisms, which may encode additional complex information depending on the effector and the signaling cascades maintaining these effects. For example, cholinergic regulation of dopamine release has been directly linked to specific aspects of cue-reward learning and motivation [120]; however, there have been limited studies about how sex differences in this specific type of regulation may underlie sex-specific behavioral strategies [121].

Taken together, this lack of understanding of how dopamine is modulated in both males and females will further impede our understanding of the basic mechanisms of neural control of behavior as well as the development of pharmaceuticals to many neuropsychiatric conditions involving dopamine dysfunction. Dopamine is at the center of a wide range of behaviors associated with mood regulation, motor control, motivation, drug effects, the development of substance use disorder, and adaptive learning and memory processes [122–124]. Thus, sex differences in this system likely underlie not only different behavioral strategies between the sexes that exist at baseline, but also the expression and trajectory of psychiatric disease states [121, 125]. Given the critical role that dopamine release plays in these processes, a more comprehensive and mechanistic understanding of how sex differences in dopamine function manifest will be particularly important in developing evidence-based therapeutics that target this system and show efficacy in both sexes.

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## AUTHOR CONTRIBUTIONS

ESC, JEZ, SON, and CAS conceptualized, wrote, and edited the manuscript. LJB and SJK wrote and edited the manuscript. ESC, JEZ, and SON made the figures.

## ADDITIONAL INFORMATION



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