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The cerebellum as a target for estrogen action

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Abstract

This review focuses on the effects of estrogens upon the cerebellum, a brain region long ignored as a site of estrogen action. Highlighted are the diverse effects of estradiol within the cerebellum, emphasizing the importance of estradiol signaling in cerebellar development, modulation of synaptic neurotransmission in the adult, and the potential influence of estrogens on various health and disease states. We also provide new data, consistent with previous studies, in which locally synthesized estradiol modulates cerebellar glutamatergic neurotransmission, providing one underlying mechanism by which the actions of estradiol can affect this brain region.

Keywords

estrogen receptor; glutamate; ataxia; Purkinje neuron; neuroprotection; estradiol; motor control; alcohol; hormone therapy

Introduction

Studies characterizing the effects of steroid hormones on neural function have often taken a logical and programmatic approach. By first utilizing methods to identify brain nuclei which contain steroid hormone receptors, researchers would then examine the cellular mechanisms of steroid action on those nerve cells. The work would culminate in an understanding of the physiological and behavioral functions nested within the neural circuitry encompassing the steroid responsive neurons [102]. For estradiol, the classic autoradiographic studies (e.g., [101]) highlighted the basal forebrain, including the hypothalamus, as the primary region in which cellular uptake of radioactive estradiol occurred. Many studies followed, demonstrating the importance of estradiol on a variety of behaviors related to reproduction [26, 39, 66, 101, 102].

As valuable as the initial autoradiography technique was to identify brain regions sensitive to estradiol, it became clear that this method is useful primarily in identifying areas in which estrogen receptor expression is abundant. The natural tendency by investigators, often stated explicitly, was to suggest that brain regions exhibiting lower radioactive signals were less, or not at all influenced by the hormone [3, 120, 155, 168]. In a simple system, in which estradiol had just one receptor and one mechanism of action, such an assumption could be easily justified. We now know, however, that there are multiple estrogen receptors, each of

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which can affect numerous cellular signaling pathways [79, 86, 87, 88, 109]. Many of these actions require only a few receptors to produce pronounced changes in cellular function. Thus, the conclusions drawn from these foundational autoradiography discoveries have led to many false negatives regarding neuronal tissue sensitive to hormonal manipulation.

One such brain area considered insensitive to estradiol has been the cerebellum. This structure, with unique cell types and cytoarchitecture [32, 60, 98], regulates a variety of behaviors including motor control, language, attention, and memory [16, 151]. Over many years, several key findings have emerged that redefine the cerebellum as a target of estradiol action. More sensitive detection methods have determined a much wider distribution of estrogen-responsive brain regions, including the cerebellum. Another critical observation has been that estrogens guide cerebellar development. Additionally, the cerebellum itself may be a source of estradiol, through local steroid hormone synthesis. Estradiol also appears to directly affect cerebellar glutamatergic neurotransmission, provides protection against toxic insults, and impacts a variety of health related cerebellar functions. Yet, remarkably, the canon that the cerebellum is unresponsive to estradiol remains. This review examines each of these topics in turn. In addition, we will offer new *in vivo* data demonstrating that estradiol modulates glutamate neurotransmission in the cerebellum through locally synthesized estradiol. We conclude that the coalescence of data in support of estradiol regulation of cerebellar functioning is persuasive. That said, our understanding of the roles of estrogen signaling in the cerebellum is still in its infancy.

Estrogen Receptors and Estradiol Synthesis in the Cerebellum

Before the first cloning of estrogen receptor α (ER α), estrogen responsive neurons were detected by examining for radiolabeled estradiol accumulation. As mentioned in the Introduction, this led to the identification and characterization of hypothalamic and other diencephalic structures involved in reproduction. Interestingly, estradiol uptake was also observed in the cerebellum, albeit to a far lesser extent [81, 82, 83]. This surprising finding led to the cerebellum being considered a target of estrogen action for the first time. However, the stated implications were extremely limited. The hypothesis put forward was that steroid hormones acting within the cerebellum could affect signaling to other brain regions more traditionally considered to be involved in reproduction [81, 83]. The idea that estradiol affects cerebellar function to influence a variety of non-reproductive behaviors was yet to be explicitly considered.

Detection of estrogen receptors is the current means for defining estrogen-responsive cells. ER α and estrogen receptor β (ER β) are the two most studied receptors [47, 80, 86, 170]. GPR-30 (also termed GPER) is another estrogen-responsive receptor [51], and there are several more putative (not yet verified through genetic cloning) receptors (i.e., ER-X and the STX-sensitive ER-Gq) [106, 154]. Modern and more sensitive methodological techniques demonstrate that the adult cerebellum expresses both ER α and ER β [58, 90, 105, 137, 138, 141]. ER α expression in the adult is localized primarily to granule cells, albeit at low concentrations [14, 58]. In contrast, the adult cerebellum expresses high levels of ER β , primarily in Purkinje cells, but in cerebellar granule cells as well [14, 45, 47, 58, 75, 76, 90, 91, 101, 105, 138, 154, 175], but see [148]. GPR-30 is also expressed throughout the cerebellum, with expression most abundant within Purkinje neurons [51]. To date the cerebellum has not been examined for ER-X and the STX-sensitive ER-Gq.

Estrogen receptors can affect neuronal function through a variety of mechanisms. For a detailed review see [87]. Originally defined as ligand-gated transcription factors, both ER α and ER β can also act at the surface membrane, triggering various intracellular signaling pathways. In comparison, GPR-30 was initially described as a membrane receptor, although

recent evidence suggests it may primarily regulate signaling in the endoplasmic reticulum. Both ER-X and the STX-sensitive ER-Gq are believed to act solely as membrane receptors.

With the discovery that steroid hormones are not only produced by the gonads, but can also be produced *de novo* within the brain, a series of studies have investigated whether the cerebellum contains the appropriate machinery to produce neurosteroids. Purkinje cells express various hormone synthesizing enzymes, including the key enzyme in estradiol formation, cytochrome P450 aromatase [6, 124]. Importantly, experiments have not just been limited to rodent and avian models. A positron emission tomography (PET) study using a radiolabeled aromatase inhibitor tracer found elevated levels of aromatase within the human cerebellum [15]. Aromatase immunostaining of post-mortem cerebellar tissue further confirmed that aromatase is found within human Purkinje cells and interneurons [6].

Estrogens and Cerebellar Development

The development of the cerebellum has been detailed in several excellent reviews [2, 22, 89, 140]. In regard to the actions of estrogens, a different pattern of estradiol binding is observed during development compared to the adult, beginning in the late postnatal period [161] and continuing up until weaning [76]. Ikeda and Nagai [58] demonstrated that ER α mRNA is significantly higher in the cerebellum of neonatal rat pups compared to adults. Measurements of ER α protein were consistent with the PCR data. Elevated ER α protein and mRNA during development is mainly confined to Purkinje cells during dendritic growth and synapse formation that occurs around postnatal day 7, suggesting a role for ER α in Purkinje cell differentiation. ER β expression in the cerebellum also varies with age. In the first postnatal week, ER β immunoreactivity was localized to both granule and Purkinje cells. Similar to ER α , ER β expression reaches peak levels during the initiation of Purkinje cell dendritic growth, slightly decreasing over time to the level maintained throughout adulthood [62].

The best described role for estrogens in the developing cerebellum involves Purkinje cell dendritic growth, and spine and synapse formation, based on a series of compelling studies by Tsutsui and colleagues. Initial discoveries found that estradiol promotes dendritic growth and spine formation, effects that were blocked by the estrogen receptor antagonist tamoxifen [124]. Interestingly, these phenomena were observed in the developing cerebella of both male and female rats. Additionally, this paper demonstrated mRNA expression of aromatase within the Purkinje cell, suggesting that locally synthesized estradiol may be responsible for these effects.

In a follow up study [129], work from this laboratory demonstrated the importance of endogenous estrogens in not only dendritic growth and spine density, but in synaptogenesis as well. Specifically, aromatase knockout mice exhibited a reduction in Purkinje cell dendritic length, spines and synapse number. Each of these effects in the knockout mice are reversed following administration of estradiol. Mechanistically, the actions of estradiol are less clear. The current model posits that locally derived estradiol activates nuclear ER β , resulting in increased expression of the neurotrophin BDNF, which ultimately leads to changes in Purkinje cell structure and function [158]. Evidence in support of each step in the proposed signaling pathway varies. Local synthesis of estradiol is suggested not only because of the measured mRNA expression of aromatase within Purkinje cells [124], but also because there are no other obvious sources of estrogens in the male and female rodent during this age. Whether ER β is the (sole) receptor responsible is less clear. A role for ER β is based primarily on expression studies in the developing cerebellum that looked for this particular receptor. Additional support is derived from the ER β knockout mouse that exhibits deficits in spatial learning, neuronal migration, and apoptosis [163]. However, while

tamoxifen reduces the effects of both endogenous and experimentally administered estrogens, this anti-estrogen does not completely block the actions of these hormones on Purkinje cell development [48]. Whether incomplete inhibition by tamoxifen is due to a concentration/blockade issue, or because there are tamoxifen-insensitive estrogen signaling pathways present is unknown. Finally, BDNF expression is decreased in tamoxifen-treated and aromatase knockout animals; knockout animals exhibit increased BDNF expression following administration of estrogen benzoate [48]. The importance of BDNF on Purkinje cell development is well established [33, 118, 136]. Therefore, estradiol promotion of BDNF expression is likely to play an important role. That said, multiple mechanisms are involved in the complex process of Purkinje cell development. As an example, development and maintenance of excitatory synapses is an activity-dependent process. In other brain regions, estradiol-mediated changes in spine density require glutamate receptor activation [84, 172]. And as discussed below, locally synthesized estradiol promotes glutamatergic neurotransmission between parallel fibers and Purkinje cells in the adult cerebellum. Hence, BDNF signaling is most likely only one component of the many effects of estradiol on cerebellar development.

A series of other studies examined the biochemical changes elicited by neonatal estrogens acting in the cerebellum by analyzing the levels of free amino acids in the developing rat brain [57]. Administration of estradiol from postnatal day 6 through 10 eliminates the normal developmental increase of glutamic acid and GABA in the cerebellum, as well as the typical decrease in glutamine [57]. Cavallotti et al. [23] focused on the effect of estradiol on the enzymes that degrade GABA, the principal neurotransmitter of Purkinje cells [32]. Immunohistochemical analysis revealed that estradiol treatment stimulates GABA transaminase and succinic semialdehyde dehydrogenase activity. Together, these studies suggest that estradiol may affect the balance between excitatory and inhibitory neurotransmission within the cerebellar cortex. This idea will be discussed again later in this review.

Estrogen Facilitation of Cerebellar Glutamatergic Signaling

While it has become clear that estradiol affects cerebellar function, the mechanisms of action are not well understood. Sheryl Smith and colleagues pioneered the concept that of estrogens augment glutamatergic neurotransmission in the adult. In their initial studies, estrogens applied either directly to the cerebellum or *i.v.* in anesthetized adult (ovariectomized) rats resulted in an increase in Purkinje cell responsiveness to microionotophoresis-applied glutamate. The local effects of estradiol enhancing cerebellar glutamate responsiveness were determined to be stereospecific [146]. Additionally, estradiol potentiates both AMPA and NMDA receptor-mediated excitation [144, 147].

While these studies were transformative, criticism regarding the use of anesthetized animals and a lack of behavioral relevance followed. To address these concerns, Smith and colleagues monitored the firing of individual Purkinje cells while female rats walked on a treadmill [147]. Subcutaneous injection of estradiol increases locomotor-induced Purkinje cell activity within 15 minutes of steroid administration [147], demonstrating effectiveness in an intact, awake animal and implicating estradiol in cerebellar locomotor function. These data parallel the finding that estradiol enhances striatal-mediated motor function [13].

In female rodents, ovarian estrogens enhance cerebellar Purkinje cell responsiveness to glutamate. More recent studies utilizing wire arrays have found augmented single unit activity with locomotor activity in multiple cells across consecutive estrous cycles coinciding with increased circulating estrogens [145]. Moreover, these effects of estradiol are not limited to locomotion. In female mice, estradiol enhanced long-term potentiation at

the parallel fiber to Purkinje cell synapse, increased the density of these same glutamatergic synapses, and improved vestibulo-ocular reflex adaptation via ER β signaling [4].

As we gain appreciation for the cellular actions of estradiol within the cerebellum, it is also important to consider the role the cerebellum plays in behavior. While originally believed to be involved solely in motor-related behaviors, recent findings have shown that the cerebellum influences language, attention, and memory [16, 151]. Hence, it would be unsurprising to find out that estrogen signaling in the cerebellum impacts such diverse functions as memory [103, 104], cognition [119, 130, 131, 132], locomotor activity [94] and mood [107].

While the number of laboratories examining the direct effects of estradiol on cerebellar function is limited, the work by Sheryl Smith and others demonstrate an enhancement of glutamatergic neurotransmission between the granule cell parallel fibers and Purkinje cells. Estradiol enhancement of glutamatergic neurotransmission has been described in additional brain regions, including the hippocampus and cerebral cortex [35, 67, 68, 123, 150, 162, 177].

Utilizing novel *in vivo* imaging techniques, we find evidence in support of previous work, where locally- and gonadally-derived estrogens promote glutamatergic neurotransmission at the parallel fiber-Purkinje cell synapse. Here we will describe the first published data using this technique. Activity-dependent optical imaging experiments in the intact cerebellum have elucidated numerous mechanistic models to explain both normal cerebellar functioning as well as abnormal functioning in cerebellar ataxia [10, 31, 112, 164, 165]. This is an ideal approach to decipher the role of estradiol regarding its influence on glutamatergic neurotransmission at the parallel fiber-Purkinje cell synapse. Specifically, the imaging of mitochondrial flavoproteins allows the monitoring of parallel fiber-Purkinje cell synaptic transmission *in vivo* [110, 111]. Methodologically, upon exposing the cerebellum of an anesthetized mouse, a water-tight optical chamber is constructed and filled with artificial cerebrospinal fluid (aCSF). A microelectrode is placed just below the surface of the cerebellar cortex to stimulate parallel fibers (Figure 1A). The evoked action potentials along the parallel fibers promote the release of glutamate, which activate the post-synaptic Purkinje cells. The increase in flavoprotein fluorescence in response to parallel fiber stimulation is due to the *postsynaptic activation* of many hundreds of mediolaterally aligned Purkinje cells and is principally dependent upon AMPA receptors, with a smaller contribution from mGluR1 [110, 164, 165]. This activity response is imaged as a beam-like increase in intrinsic fluorescence (Figure 1B) and is stable over many hours of recording.

Using this technique, we find glutamatergic neurotransmission between the parallel fiber-Purkinje cell synapse to be reliant upon estrogen signaling. Application of estrogen receptor blockers to the aCSF resulted in a profound decrease in beam fluorescence (not shown). Electrophysiological recordings determined that estradiol directly impacts the efficacy of the glutamate synapse, and not the excitability of parallel fibers. Furthermore, locally synthesized estradiol appears to maintain robust neurotransmission. Applying the aromatase inhibitor fadrozole (1 μ M) to the exposed folia diminished the beam amplitude within 30 minutes (Figure 2), supporting the hypothesis that local estrogen synthesis is essential for glutamatergic transmission within the parallel fiber-Purkinje cell network. This effect is not completely surprising, as the cerebellum is capable of synthesizing steroids. The rapid effects of fadrozole suggest estradiol is synthesized on demand for maintenance of parallel fiber-Purkinje cell neurotransmission.

In a larger context, evidence is rapidly accumulating that estradiol supports neurotransmission, directly affecting the electrochemical state of the cell [8, 9]. Not only can

estradiol immediately influence the membrane physiological properties of a neuron [65], but synthesis of estradiol from aromatase localized to presynaptic boutons appears to place estradiol in the category of a neurotransmitter [56, 96, 133, 157]. Interestingly, aromatase expression in glia may be an equally viable alternative [41, 125], as glial cells play an essential role in the support of neurotransmission [36]. While much of the work regarding local estradiol neurotransmission began in avian systems [126, 134], there is evidence of similar mechanisms in the mammalian brain, in which ER β plays a principal role [67, 149, 150]. With ER β expressed within the adult cerebellum, the study of estrogen support of cerebellar neurotransmission provides a tractable model in which to gain additional insights into this expanding field.

Health Implications

An important extension of the observations that estradiol affects cerebellar development and promotes neuronal excitatory neurotransmission in the adult, is the functional significance for human health. While these types of studies typically use systemic estrogen treatment, and thus the observed effects cannot be definitively attributed to the hormone acting directly and solely upon this brain region, the impact of estrogen treatment on cerebellar functioning is unequivocal.

Estradiol action has been implicated in neuroprotection from many diseases [28, 69, 93]. Recently, an emphasis has been placed on the role of estradiol in aging and neurodegenerative diseases such as Alzheimer's disease (AD) [52]. Although not often associated with this disorder, the cerebellum influences cognitive function. Furthermore, estradiol has been consistently found to affect the cerebellum in afflicted/vulnerable humans as well as in animal models of AD.

Estrogen therapy initiated at the onset of menopause reduces the risk or delays the onset of AD in women [175, 176]. Likewise, administration of the androgen/estrogen precursor dehydroepiandrosterone (DHEA) improved memory performance in both normally aged mice [37, 38] and in a mouse model of AD [78]. Interestingly, the conversion of DHEA into the high affinity estrogen receptor agonist, Δ^5 -androstene-3 β , 17 β -diol occurs in abundance within the cerebellum compared to the frontal cortex and hippocampus of animals modeling AD [167].

Hormone therapy remains a controversial treatment for cognitive impairment associated with age-related cognitive decline or pathological aging, such as AD in post-menopausal women. Meta-analysis of clinical trials have not generally supported the use of hormone therapy to treat cognitive decline in post-menopausal women [74]. However, these findings cannot be extended to specific subgroups of women, such as young menopausal women, who could potentially still benefit from such therapies. A longitudinal study was conducted comparing cognitive performance of healthy post-menopausal women with risk factors for AD who had been receiving either estradiol hormone therapy or conjugated equine estrogen therapy for least one year [173]. Women who received estradiol displayed better verbal memory performance when compared to women given conjugated equine estrogens, independent of age, IQ, years of education, risk factors for AD, duration of estrogen exposure, concurrent progesterone use, or menopause status (natural or surgically produced) [173]. Additionally, fMRI studies demonstrate that gray matter volumes increase in the cerebellum of post-menopausal women given estrogens, and are greater in women who underwent treatment in the past versus women who were currently undergoing treatment [17, 42]. Further, gray matter volume is decreased in the cerebellum of females who had never been given hormone therapy compared to both young females and older females on hormone therapy, suggesting that estradiol is protective for cerebellar neurons [117].

Estradiol also impacts vasodilatation, affecting both cerebral and cerebellar circulation. In adult rats, estradiol increases blood flow within 10 minutes of administration to many areas of the brain, including the cerebellum [43]. It has been suggested that estradiol exerts these effects through a nitric oxide and cGMP-dependent mechanism [99, 169]. Interestingly, post-menopausal women exhibit approximately a 5% increase in cerebellar blood flow following estrogen replacement therapy compared to their pre-therapy blood flow measurements [143]. Further, this cerebellar blood flow increase correlates with an increase in serum levels of estradiol [143]. Intranasal administration of estrogens also results in an increase in cerebellar blood flow in post-menopausal women [64].

Endogenous estrogens may also provide a neuroprotective role in the cerebellar ataxias [139]. Chemically-induced cerebellar ataxia in castrated male rats was mitigated by estrogen implants. Specifically, estradiol significantly decreased neuronal death. This study also established that the inferior olivary nucleus of adult male rats express aromatase, the enzyme responsible for the conversion of testosterone to estradiol. Correspondingly, inhibition of aromatase using this model led to an increase in cell death within the inferior olive [139], demonstrating that the estradiol produced within the inferior olive provides neuroprotection. As a side note, the targeting of aromatase has been a treatment for various reproductive cancers. As an example, administration of the aromatase inhibitor aminoglutethimide is beneficial in the treatment of breast carcinomas in post-menopausal women [127]. However, one of the major side effects is a significant increase in the observance of ataxia [127].

Not surprisingly, balance and coordination, two known functions of the cerebellum, have also been studied in post-menopausal women with regard to the potential therapeutic effects of estrogen therapy [21]. Postural stability and balance are known to decrease with age. Impaired balance and mobility are risk factors in post-menopausal women and contribute to at least one fall per year in 33% of women aged 60 or older [5, 11]. Trans-dermal estrogen treatment in post-menopausal women increased balance performance [46], potentially implicating a neuroprotective role of estradiol in cerebellar function.

Friedreich's ataxia is the most common form of inherited ataxia in the world, affecting primarily spinocerebellar and other spinal neurons. The disease shows no sex bias in incidence [18, 49, 73, 100, 135]. However, epidemiological studies indicate a better prognosis in female patients [73], suggesting a possible role of estradiol in the prevention and treatment of Friedreich's ataxia [30]. Consistent with this hypothesis, estradiol protects against the effects of oxidative damage in fibroblasts of patients with Friedreich's ataxia [115]. It is not known whether estradiol acting within the cerebellum impacts the progression of Friedreich's ataxia or whether this is due to estradiol action within the spinal cord and/or another site.

Additional evidence points to cerebellar involvement in a psychoneuroendocrine disorder, premenstrual dysphoric disorder (PMDD). Affective symptoms of PMDD include irritability, anger, depression, mood swings, anxiety, fatigue, and difficulty concentrating (DSM-IV). Although triggered by ovulation [7], these symptoms cannot be attributed to differences in circulating hormone levels of women with PMDD compared to non-affected women [121, 122]. A PET study mapping the functional brain abnormalities associated with PMDD across the menstrual cycle found an increase in cerebellar activity, particularly in the right cerebellar vermis. This increase in cerebellar activity correlated with worsening of mood in the same PMDD subjects, suggesting a role of the cerebellum in the affective state of patients with PMDD [107]. The mechanisms for estradiol action on cerebellar function in these and other health related findings, remains unknown.

An ongoing dilemma regarding the effects of estrogens on human health is the apparent duality regarding hormonal benefit versus detriment. None is probably more intensively studied or debated than the use of estrogens regarding stroke [95, 97, 116]. Various animal models found that estrogens ameliorate the effects of stroke [24, 25, 29, 55, 59, 71, 85, 108, 142, 156]. Yet, hormonal therapy in post menopausal women increases the likelihood of stroke and worsens symptoms [12, 53, 128, 166]. Some of these discrepancies across animal models/human subjects may be related to age [72, 95] and duration of hormone deprivation [6], but these findings also highlight potential differences between the actions of endogenous estrogens and those exogenously administered. Concentration and duration-dependent factors in estradiol treatment may also play a role. Hence, it becomes increasingly difficult to integrate various findings into a simplified model of estradiol action. Within the cerebellum, estrogen-mediated neuroprotection from ethanol toxicity falls within this category. Ironically, this is probably the most studied area regarding the cerebellum and estrogens.

Ethanol use and abuse profoundly affects many areas of the brain, though some regions are more susceptible to ethanol's toxic actions. The cerebellum is particularly sensitive to ethanol [61]. The effects of chronic ethanol use and withdrawal on the cerebellum can be so profound that ataxias are common signs in alcoholics (e.g. [152]). In long-term alcoholics, a characteristic pattern of cerebellar degeneration is seen, particularly within the vermis [61]. Many neuronal cell types are affected, though most of the focus has been on the atrophy of Purkinje cell dendrites, the loss of Purkinje neurons, and shrinkage of cerebellar white matter [61, 174]. While in humans it is difficult to disentangle whether these neuropathology's result from alcohol use or dietary (especially thiamine) deficiency [34, 77], the observation that similar cerebellar pathology occurs in controlled animal studies [34] suggest that these degenerative processes are due to ethanol use itself.

Ethanol use in adults would be expected to have potential toxic actions, just as it does during fetal and early neonatal development [61]. However, much of the adverse neurological effects in adult animals result from the consequences of ethanol withdrawal. Withdrawal following ethanol use produces a characteristic pattern of symptoms in both animals and humans [44]. Studies in rats use a paradigm of chronic ethanol administration, typically as part of the animal's diet, followed by a transfer to an ethanol-free diet (e.g. [63]). In this paradigm, estradiol is administered chronically during ethanol administration and withdrawal. Animals withdrawing from ethanol model human disease by exhibiting a widespread reduction in Purkinje cells, particularly in the vermis, i.e. the midline subdivision of the cerebellum separating the left and right hemispheres [1, 63, 159, 160]. Concurrent estradiol treatment to rats counteracts the effects of ethanol and ethanol withdrawal, maintaining control numbers of Purkinje neurons [63].

The neurodegeneration produced by ethanol withdrawal is accompanied by ataxia. Ethanol withdrawal produces a decrease in accelerated rotarod performance consistent with cerebellar ataxia [63, 113, 114]. Degraded rotarod performance is typically associated with Purkinje cell loss [19]. As estradiol protects against Purkinje cell loss during withdrawal, the corresponding maintenance of rotarod performance by estradiol treatment is predictable [63, 113, 114].

Underlying ethanol-induced toxicity and estradiol-mediated neuroprotection is the balance between GABAergic and glutamatergic neurotransmission. Glutamate-induced cell death is a proposed mechanism for cerebellar neuronal toxicity during ethanol withdrawal, as the magnitude of elevated extracellular glutamate levels measured during withdrawal correlates with the severity of behavior [34]. To the degree that GABA neurotransmission can modulate glutamate-induced neuronal excitability, ethanol withdrawal can be modulated

through pharmacological manipulation of GABA-A receptor activity [70]. Specifically, GABA-A agonists reduce the severity of ethanol withdrawal, whereas antagonists potentiate withdrawal. Notably, increased GABAergic signaling, in addition to acting as a counterbalance to excitatory stimuli, can also directly inhibit glutamatergic neurotransmission, [20, 32, 40, 50].

How does estradiol signaling within the cerebellum fit within this model? This question may be hard to answer unequivocally. Chronic estradiol treatment mimics the action of GABA-A agonists by reducing the severity of withdrawal symptoms [113, 114]. Additionally, the GABA-A antagonist bicuculline counteracts the effects of estradiol on preventing Purkinje cell loss following ethanol withdrawal. Bicuculline also eliminates the ability of estradiol to maintain rotarod performance following withdrawal. Based on these data, the assumption is that GABA is the common mechanism of action mediating estradiol protection against neurotoxicity and motor loss. In numerous brain regions, estradiol promotes GABA synthesis [54, 92, 171], suggesting a potential mechanism. However, the effects of estradiol and the GABA-A agonist muscimol are additive [114], suggesting estradiol may recruit additional signaling mechanisms independent of GABA-A receptors.

Another potential mechanism is estradiol regulation of glutamatergic neurotransmission. At first this appears counterintuitive, since as previously described, endogenous estradiol potentiates glutamatergic neurotransmission. Thus, the straightforward assumption would be that estradiol would exacerbate ethanol withdrawal. However, down-regulation of excitatory synapses may be a case of compensatory action following chronic estradiol treatment. Within multiple brain regions, chronic activation of ER β leads to down-regulation of glutamatergic neurotransmission (in parallel to increased GABAergic signaling) [153]. Hence, if similar mechanisms occur within the cerebellum, we predict that while chronic estradiol administration is neuroprotective, acute estradiol would worsen the effects of ethanol withdrawal. Future research could test this hypothesis.

Conclusions and Future Directions

Once thought to be extraneous regarding estradiol action, the cerebellum is now known to be a brain structure affected by this hormone, leading to alterations in a variety of behaviors. Although not described in detail here, there is also growing evidence that the cerebellum contributes to sex differences in the pathology of autism, attention-deficit hyperactivity disorder, schizophrenia, and depression (for a review see [27]). Whether this is due to sex differences in cerebellar physiology and/or differences in hormone production between males and females remain to be fully determined. Additional studies determining the mechanism by which estradiol acts upon the cerebellum and the ultimate functional impact estrogens have on cerebellar-mediated behaviors are clearly warranted.

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Highlights

Estrogen action in the cerebellum has been observed for years, but often ignored

Estradiol affects cerebellar development

Estradiol promotes glutamate neurotransmission within the cerebellum

Cerebellar estradiol impacts a variety of health issues

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Figure 1.

Optical imaging of the parallel fiber-Purkinje neuron synapse. (A) Schematic diagram of cerebellar anatomy. The stimulating electrode is placed on the surface of the cerebellum after removal of the skull to activate multiple parallel fibers, leading to activation of Purkinje cells aligned in a mediolateral orientation. (B) Change in flavoprotein fluorescence within Cerebellar Purkinje neurons following parallel fiber activation. Changes in fluorescence are directly due to glutamatergic neurotransmission between the parallel fiber and the Purkinje neuron. Due to the circuitry of the cerebellum, electrode stimulation results in a “beam” of synaptic activity.

Figure 2.

Local estrogen synthesis maintains glutamatergic neurotransmission between the parallel fiber-Purkinje cell synapse. (A) Bright field image and changes in fluorescence following parallel fiber activation. Fadrozole ($1\mu\text{M}$) added to the recording chamber surrounding the cerebellum decreased synaptic activity. (B) Fadrozole produced an approximate 50% reduction in synaptic beam fluorescence ($n=4$).