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Cellular Mechanisms of Estradiol-Mediated Masculinization of the Brain

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Abstract

The sexual differentiation of reproductive physiology and behavior in the rodent brain is largely determined by estradiol aromatized from testicular androgens. The cellular mechanisms by which estradiol masculinizes the brain are beginning to emerge and revealing novel features of brain development that are highly region specific. In the preoptic area, the major site controlling male sexual behavior, estradiol increases the level of the COX-2 enzyme and its product, prostaglandin E2 which promotes dendritic spine synaptogenesis. In the ventromedial nucleus of the hypothalamus, the major site controlling female reproductive behavior, estradiol promotes glutamate release from synaptic terminals, activating NMDA receptors and the MAP Kinase pathway. In the arcuate nucleus, a major regulator of anterior pituitary function, estradiol increases GABA synthesis, altering the morphology of neighboring astrocytes and reducing formation of dendritic spines synapses. Glutamate, GABA and the importance of neuronal-astrocytic cross talk are emerging as common aspects of masculinization. Advances are also being made in the mechanistic basis of female brain development, although the challenges are far greater.

Introduction

As with the bipotential gonad, many aspects of the brain are bipotential until secondarily differentiated into a male versus female phenotype in response to gonadal phenotype. This is most profoundly true in brain regions directly involved in reproductive functions. The default phenotype is feminine and will thereby support female associated endpoints such as ovulation, sexual receptivity, which in the rodent is characterized by the lordosis posture, and maternal behavior. Masculinization of the brain occurs secondarily to differentiation of the gonad into a testis in response to a molecular cascade initiated by the Sry gene on the Y chromosome (see for review[1]). The male brain will subsequently support expression of male sexual behavior, which in the rodent is characterized by mounting and intromitting, and generally shows little to no parenting behavior (with some exceptions) and high levels of aggression. Sexual differentiation of the brain occurs during a critical period of perinatal development, just before and after birth, in which hormone exposure permanently organizes the brain. Organization of the brain refers to specific patterns of synaptic connectivity, differential cell death and determination of neurochemical phenotype. This organization allows for activation of the brain

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later in life by circulating hormones to invoke the appropriate sex-typic response, be it behavior or control of gonadal function (see for review [2,3].

The Organizational / Activational Hypothesis of brain sexual differentiation

The study of sexual differentiation of the brain made a seminal advance in 1959, when Phoenix, Goy, Gerall, and Young [4] reported that female guinea pigs exposed to testosterone prenatally showed little or no female sex behavior as adults. This was the first evidence for the steroid-induced sexual differentiation of the brain and led to the still applicable Organizational / Activational Hypothesis of steroid hormone action. This is now viewed as the classic model of sexual differentiation and provides a useful heuristic framework for investigating the basis of sex differences in brain and behavior. As the study of sexual differentiation expands beyond those endpoints directly relevant to reproduction it is becoming increasingly apparent that the Organizational / Activational Hypothesis does not always apply. In particular there is emerging evidence for a critical role of chromosomal sex in either dictating brain sex differences or impacting on how the brain responds to gonadal steroids (see for review [5,6]. It is also apparent that gonadal steroids can induce sex differences in the adult brain that are independent of early organizational effects [7] and that there are several instances in which the sexes are actually striving to be the same, converging on the same endpoint via different mechanisms or strategies [8,9] and in these instances the classic model clearly does not apply. The focus of this review, however, is the classic model because it provides us robustly sexually dimorphic endpoints with clear functional significance. Moreover, the central role of gonadal steroids in determining the sexual phenotype of the brain provides us a tool with which to initiate the process of masculinization in the genetic female simply by providing her male hormones during the critical developmental period. The technical advantage of simply injecting a new born female with testosterone, or its aromatized by-product estradiol, and then monitoring the process of masculinization, allows for the discovery of novel cellular processes impacting on brain development. It is these processes, as we understand them to-date, that we will review here.

The importance of aromatization in brain sexual differentiation

Subsequent to the establishment of organizational effects of male gonadal hormones on brain development were attempts to determine the specificity of the hormones involved. One of the surprises was that treatment of neonatal females with exogenous estradiol, originally administered as a control for testosterone, *also* induced complete masculinization of brain and behavior [10,11]. We now understand that most sexually dimorphic areas of the brain contain substantial levels of both aromatase cytochrome P450 (CYP19), the enzyme responsible for the conversion of testosterone to estradiol, and high densities of estrogen receptors [12–17]. Maternal estrogens are sequestered in the peripheral circulation of the fetus by alpha-fetoprotein, a steroid-binding glycoprotein that has high affinity for estradiol but little affinity for androgens, allowing the testicular testosterone to reach and influence fetal target tissues, including the brain.

Masculinization and Defeminization

It is now apparent that estradiol during the critical period is responsible for much of the sexual differentiation of the male rat brain. This sensitive period for steroid-induced sexual differentiation is operationally defined by the onset of testicular androgen secretion in males (approximately embryonic day 18) and by the loss of sensitivity of the female to exogenous androgen treatment (approximately postnatal day 10). During this time, normal development of the male rodent brain requires completion of two distinct processes: masculinization and defeminization (Figure 1). Masculinization is the organization of a neural substrate permissive to the adult expression of male sexual behavior. Defeminization is the loss of capacity as an adult to respond to the activational effects of estradiol and progesterone to induce female sex

behavior. Both processes oppose the process of feminization, which occurs in the absence of critical levels of neuronal estradiol as a neonate and is the pathway leading to adult female-typical behavior [18,19]. There is no naturally occurring demasculinization, although development of the male brain can certainly be disrupted by various means. Studies attempting to elucidate the mechanistic basis of sex differences benefit from a robust and reliable endpoint, sexual behavior in the albino laboratory rat. Male sexual behavior, including mounting, intromitting and ejaculating, is opportunistic and readily expressed whenever a receptive female is present. Female sexual behavior, including lordosis, is physiologically constrained in order to be expressed only in proximity to ovulation, and requires a threshold level of estradiol sequential with progesterone. This dichotomy in the nature of the behavior between the sexes suggests a dichotomy in the neuronal circuitries underlying these behaviors between the sexes. This model, therefore, provides a natural system in which to study the organization and plasticity of these circuits and the subsequent effect on adult behavior.

Sex differences in the rodent brain

The rodent brain has many types of sex differences, which are determined by early hormone exposure. These include projections from one brain region to another, such as the projection from the bed nucleus of the stria terminalis to the anteroventral periventricular nucleus, which is denser in males than females [20]. There also exists a sex difference in the volume of certain brain nuclei. This includes the sexually dimorphic nucleus of the preoptic area, which is 3 to 5 times larger in the male, [21] or the medial nucleus of the amygdala, also larger in males than in females [7,22]. Other dimorphisms include differences in dendritic spine density [23–28] neurite branching [29] or astrocyte complexity [30–32]. Many of these sex differences are localized to regions that are necessary for adult sex behavior, including the arcuate nucleus, the ventromedial nucleus of the hypothalamus, and the preoptic area. Testosterone (aromatized to estradiol) exerts region-specific effects on dendritic spines and branching in the developing hypothalamus. Specifically, testosterone exposure decreases the dendritic spine density and axodendritic spine synapses in the arcuate nucleus [27,31,32]. In the preoptic area (POA), a region necessary for male sex behavior, estradiol has the opposite effect and increases dendritic spine density [33]. In the VMN, a region necessary for female sex behavior, estradiol has no effect on dendritic spine density [27] but does increase the number of dendritic spines per neurite (Todd et al., 2006). Thus, while the effects of estradiol in differentiating the developing brain (i.e. organizing) are irreversible and easily quantifiable, careful attention to regional analysis reveals that despite high levels of estrogen receptors in many subnuclei of the diencephalons, the actions of estradiol are highly region specific [34]. This suggests separate and different processes by which estradiol permanently masculinizes the developing brain, but what are those processes? We will review the mechanisms as currently understood in three separate subnuclei, each believed to subserve a distinct sexually dimorphic process.

The Medial Preoptic Nucleus

The medial preoptic nucleus (MPN) is the major brain region controlling male sexual behavior. Lesions of this brsin region render animals asexual and hormonal, chemical or electrical stimulation of the MPN will induce or enhance male sexual responding [35]. Proper sexual differentiation of the MPN during the sensitive period is a prerequiste for expression of male sexual behavior in adulthood and a critical component of the differentiation of this brain region is the induction and stabilization of dendritic spine synapses. Compared to females, males have 2–3 fold more dendritic spines, which are the major sight of excitatory synapses in the brain. The induction of the dendritic spine patterning is mediated entirely by estradiol and occurs during the perinatal sensitive period. Administering exogenous estradiol to a newborn female will both induce the male pattern of dendritic spines and permit the expression of male sexual behavior in adulthood if she is supplied with testosterone [36]. So how does estradiol do that?

Early studies focused on the usual suspects, the classic neurotransmitters such as serotonin, noradrenaline, dopamine and sometimes GABA. Manipulation of any these neurotransmitter systems disrupts normal masculinization, but none of them can mimic the actions of estradiol in the absence of estradiol itself. Thus the cellular messenger that estradiol was invoking to induce masculinization was not identified and it was generally assumed the steroid acted in a multifactorial multiplicative manner and that there was no one cellular mediator. In hindsight one can appreciate this view but we now know that a very unusual agent is actually the mediator of estradiol-induced masculinization, the prostaglandin PGE2 [36]. There are at least eight prostanoids, of which PGE2 is one, and synthesis begins with the oxygenative cyclization of arachidonic acid by cyclooxygenase. The inducible isoform of cyclooxygenase, COX-2, is an immediate early gene responsive to a variety of stimuli including fever, injury and stimuli associated with neuronal plasticity [37–39]. COX-2 mRNA and protein are higher in the POA of newborn males than females and treating females with estradiol increases COX levels to that of males. Although there are numerous prostanoids, an increase in COX-2 is yoked with an increase in PGE2 via the specific synthetic enzyme PGEsynthase. Blocking COX-2 activity (there are no specific PGEsynthase inhibitors) in a newborn male will permanently disrupt synaptic patterning of the MPN and impair the ability of the male to show normal sexual behavior in adulthood. More importantly, administrating PGE2 to newborn females induces a two to three fold increase in dendritic spines (i.e. the male pattern) in the MPN, and results in the expression of masculine sexual behavior in adulthood [33,36]. Thus, PGE2 satisfies the criteria of being both necessary and sufficient to mediate steroid hormone induced masculinization of sexual behavior in the rat.

The discovery that PGE2 is the cellular mediator of estradiol-induced masculinization of synaptic patterning in the MPN merely begs the question of how does PGE2 induce the formation of dendritic spines? The answer appears to be at least in part via glutamate, since pretreatment with an AMPA receptor antagonist will reduce the level of spine induction by ~50% [33], and in other brain regions PGE2 releases glutamate from astrocytes [40,41]. These observations have led to the working hypothesis that estradiol up regulates COX-2 in neurons, increasing PGE2 production and release which then acts on neighboring astrocytes to induce glutamate release, and this then communicates back to the neurons inducing the formation of dendritic spines [42]. While appealing, several key steps in this model have yet to be confirmed (Figure 2).

The Ventromedial Nucleus of the Medial Basal Hypothalamus

During development and in adulthood, the medial basal hypothalamus (MBH) is a key target for estradiol. A central region in the MBH, the ventromedial nucleus (VMN) is necessary for female sex behavior. The VMN is characterized by its well-defined oval shape and sparse thin dendrites [43] Injection of estradiol directly into or electrical stimulation of the VMN in adulthood facilitates female sex behavior, while lesions of the VMN prevent the expression of female sex behavior [44] . In males, the VMN is only slightly larger than in females yet contains more than three times as many dendritic spine and shaft synapses as females [45]. These sex differences in synaptic patterning are detected as early as postnatal day 2 and are still present at postnatal day 100 [26,46], consistent with the classic organizational / activational model. The male pattern can be induced in females by treatment with either testosterone or estradiol within the first few days of life. Blocking estradiol production in males using the aromatase inhibitor, Letrozole, blocks the sexual differentiation of the VMN – thereby inducing the female phenotype for spines and behavior during development and adulthood [47]. In adulthood, the female estrous cycle or exogenous estradiol treatment causes changes in the dendritic spine density and patterning of the VMN, which correlates to changes in female sex receptivity [48]. Recalling that there is no reported sex difference or hormonal modulation of dendritic spine density in the VMN, it was surprising that males had higher levels of spinophilin than

females in the MBH (including the VMN), and that treatment of females with estradiol increased spinophilin levels to levels seen in males [49]. As a result of this discrepancy, we reanalyzed the Golgi-impregnated sections from 1999 and found that neurons in the male VMN and regions just outside, had more branches than female VMN neurons, and there is an increase in the overall number of spines on these highly branching dendrites. However, there was no sex difference in spine density.

Unlike in the MPN, treatment of females with PGE2 has no effect on the dendritic morphology in the developing VMN and does not induce defeminization of sex behavior [50]. Therefore, females treated with PGE2 during development express both male and female sex behavior. We can therefore conclude that the VMN is not the site of estradiol-induced masculinization of the brain. However, the question still remains: How does estradiol induce normal development of the male hypothalamus and possibly defeminization of behavior in adulthood?

Initial experiments investigating the mechanism underlying the effect of estradiol on the developing hypothalamus focused on the amino acid neurotransmitter GABA, with good reason. Estrogen sensitive GABAergic neurons are found in the hypothalamus [51]. Within the MBH, newborn males have twice as much glutamic acid decarboxylase (GAD – the rate-limiting enzyme in GABA synthesis) and GABA as females [52,53]. This sex difference is hormonally modulated and decreasing GAD in males with an antisense oligonucleotide disrupts sexual differentiation of sex behavior [54]. The $GABA_A$ receptor is an ionotropic receptor permeable to chloride. During development an increase in the intracellular chloride concentration allows chloride ions to flow out of the cell upon $GABA_A$ receptor activation to depolarize the neuron. As the brain matures, GABA gradually switches from being depolarizing and excitatory to hyperpolarizing and inhibitory. Estradiol delays this switch, extending the duration of time during which GABA is excitatory [55]. In doing so, estradiol mediates sex differences in intracellular signaling, including the phosphorylation of the cyclic AMP response element binding protein (pCREB) [56,57]. These findings led to the hypothesis that depolarizing GABA may be the mediator of estradiol's induction of dendritic spines in the MBH. However, treatment of females with muscimol, a $GABA_A$ receptor agonist, has no effect on spines in the MBH and pretreatment of females with bicuculline, a $GABA_A$ receptor antagonist, does not block the estradiol-induced increase in spines (Todd et al., *in press*). Thus there is currently no evidence that GABA mediates the estradiol-induced increase in dendritic spines in the developing VMN.

The classical model of estradiol action involves binding to the nuclear estrogen receptor (ER) and regulation of gene transcription via its interactions at estrogen response elements (EREs) located in the promoter region of genes modulated by estradiol. This is considered the direct mechanism of estradiol on transcription, via an ERE. Estradiol also has the ability to act indirectly via intracellular signaling pathways to act at other promoter elements of genes. Many of these indirect effects of estradiol are initiated at the neuronal membrane and include the activation of protein kinase A (PKA) and CREB [58–60], mitogen-activated protein kinase (MAP kinase) [61–63], and phosphatidylinositol 3-kinase (PI3 kinase) [62,64]. We have recently determined that estradiol can activate MAP kinase in the developing MBH within 6 hours of treatment. Using PD98059 to block the activation of MAP kinase we have also found that this activation of MAP kinase is necessary for the estradiol-induced increase in spinophilin.

Dendritic spines are the major site of excitatory synapses. Activation of the AMPA subtype of the glutamate receptor at these sites provides a majority of the synaptic current mediating excitatory postsynaptic potentials (EPSPs) seen in neurons. In addition, activation of the NMDA subtype of the glutamate receptor induces a large influx of calcium, which is necessary for many cellular processes. The effect of estradiol on glutamatergic neurotransmission has been widely studied in the adult hippocampus. Estradiol regulates hippocampal dendritic spine

formation in CA1 neurons by increasing NMDA receptor sensitivity, thereby increasing the size of calcium transients in these neurons [65–67]. Estradiol also differentially affects both Type I and Type II metabotropic glutamate receptor signaling, thereby increasing pCREB in the hippocampus [68]. Though the effects of estradiol on glutamate neurotransmission are well studied in other regions of the brain, its potential role in developing the sexually dimorphic hypothalamus has not been explored. In the preoptic area, blocking AMPA glutamate receptors using NBQX partially blocks the estradiol-induced increase in spinophilin [33]. However, recently, we have found that NBQX completely blocks estradiol-induced increases in spinophilin protein in the adjacent hypothalamus. Surprisingly, blocking AMPA receptors, in addition to preventing increases in spinophilin, also completely blocked estradiol-induced activation of MAP kinase in the developing hypothalamus. This pivotal observation suggests that rather than ER interacting directly with MAP kinase, as previously described, in the developing MBH there is a requirement for glutamate activation of AMPA receptors prior to MAP kinase activation to increase spinophilin. Based on these observations, one might predict that direct AMPA receptor activation would be able to both activate MAP kinase and increase spinophilin in the developing hypothalamus. Though treatment of females with AMPA activates MAP kinase, it had no effect on spinophilin levels in the developing hypothalamus. We conclude that AMPA receptor activation is necessary, but not sufficient, for estradiol-induced increases in spinophilin in the developing MBH.

Glutamate also acts at the NMDA receptor, a calcium permeable ionotropic receptor. We next hypothesized that NMDA receptor activation might be necessary for estradiol-induced increases in spinophilin in the MBH. Treatment of females with MK801, an open channel blocker of the NMDA receptor, blocked the estradiol-induced increase in spinophilin protein in the MBH to the same degree as seen with NBQX. In the same animals, treatment with MK801 had no effect on estradiol-induced spinophilin in the POA, consistent with previous studies. These preliminary data indicate that glutamate acting at the NMDA receptor is a possible mechanism by which estradiol sexually differentiates the MBH. There are two potential mechanisms by which estradiol can affect glutamatergic transmission; **1)** estradiol acts presynaptically to increase glutamate release and/or **2)** estradiol alters the number or functionality of AMPA or NMDA receptors postsynaptically. If the effect of estradiol on glutamate transmission was postsynaptic, we might expect a change in the number or subunit composition of AMPA or NMDA receptors, an increase in the number of cells that respond to glutamate, or an increase in the intracellular calcium response to glutamate. We tested this possibility by measuring protein levels of specific receptor subunits and quantifying calcium influx into neurons in response to glutamate and could find no evidence of changes in the number or sensitivity of the post-synaptic receptors. We next hypothesized that the effect of estradiol on glutamatergic transmission is presynaptic. The simplest mechanism by which estradiol could act presynaptically is to enhance glutamate release. Synapsin I is a protein located on synaptic vesicles and is associated with increased neurotransmitter release [69]. We have recently found that treatment of females with estradiol increases levels of synapsin I in the developing MBH within 6 hours. There are a number of cellular mechanisms by which estradiol could increase neurotransmitter release, but we must first determine if this in fact actually happens. Using the fluorescent dye FM4-64, we labeled presynaptic boutons of cultured hypothalamic neurons. Upon depolarization of these neurons, the FM4-64 is released into the medium where it is washed away. Neurons pretreated with estradiol show increased rates of FM4-64 release following KCl-induced depolarization. These data indicate that estradiol may enhance glutamate release from presynaptic terminals in hypothalamic neurons, thereby acting on NMDA receptors of neurons in the VMN and surrounding hypothalamus to increase dendritic branching and spines on these neurons to induce male neuronal patterning in the hypothalamus (Figure 3). Further experiments will determine whether these effects may in fact constitute defeminization of the male brain, or the loss of capacity to express female sex behavior.

The Arcuate Nucleus

The arcuate nucleus is also found in the MBH but subserves a different function than the VMN. There appears to be no significant role for the arcuate in control of reproductive behavior, but instead it is a major regulator of the anterior pituitary. The LHRH (leutinizing hormone releasing hormone) neurons of the preoptic region project toward the anterior pituitary via the arcuate nucleus. They terminate in the median eminence which contains a portal plexus that delivers this releasing hormone to the gonadotrophs containing the gonadotrophin hormones, LH and FSH, in the anterior pituitary. Once released into the general circulation the gonadotropins act on their target organ, the gonads, and regulate a variety of cellular activities. One of the more dramatic cellular activities being regulated is ovulation, and this requires a massive surge in LH at the appropriate time in the reproductive cycle. The LH-surge is induced by synchronized firing of the LHRH neurons, and this activity is a function of the positive-feedback effects of estradiol (see for review [70]. The masculinized brain does not respond to estradiol with positive feedback and there is therefore no LH surge. Glutamatergic neurons of the aneteroventral periventricular nucleus project to and excite the LHRH neurons, making them the surge generators. There are more of these neurons in females, and this is one component of the lack of an LH surge in males [3].

The arcuate nucleus exerts a modulatory effect on the LH surge. The neurons of this nucleus exhibit striking plasticity in synaptic patterning across the estrus cycle, and this is largely driven by changes in the morphology of neighboring astrocytes [71,72]. Hormonally mimicking the estrus cycle in males does not induce the same plasticity, and females masculinized at birth by exogenous androgen also do not respond to steroids in adulthood [73]. In neonates, estradiol induces a dramatic morphological shift in astrocytes towards an increased stellation with more primary processes and branches [31,32]. However, there is no evidence of estrogen receptors in astrocytes of the arcuate nucleus [74]. We now know that the primary site of estrogen action is neurons, where it increases the synthesis and release of GABA which then acts on neighboring astrocytes to induce a stellate morphology [75]. In ways that remain poorly understood, the astrocytes then modulate the formation of dendritic spine synapses by the neurons, reducing their formation in males [27] (Figure 4). This is an example, similar to that in the preoptic area, where cross-talk between neurons and astrocytes is an essential component of establishing sex differences in the brain and likely contributes to the ability of the entire population of cells within a nucleus to respond to the steroid, not just those containing estrogen receptors.

What are the mechanisms establishing a female brain?

A disproportion amount of research energy is directed towards determining the cellular mechanisms establishing the male brain because of the clear advantage of having a triggering event, the onset of gonadal steroid synthesis, which can be mimicked by exogenous steroid administration to females. The female brain, by contrast, develops in the absence of such a trigger, making the identification of the key variables far more difficult. Any number of manipulations early in life might succeed in impairing female sexual behavior or reproductive physiology in adulthood, but this does not mean the targeted system is responsible for normal feminization. Is there a single, presumably genetic, trigger to feminization and if so, what is it? Alternatively, feminization may be an accumulation of unrelated developmental processes, the default. Given the complexity of female reproductive cyclicity and sexual responding, it seems clear the building of a female brain is an active and directed process that requires precision and coordination. Establishing how this occurs is one of the greatest challenges ahead for the field of sex differences in the brain. The lack of a clear trigger, such as testosterone/estradiol in males, makes the problem all the more challenging. One conceptual approach is to look for endpoints that are either higher in females and decreased by testosterone/estradiol.

Using a high throughput western blot analysis we identified two related proteins, focal adhesion kinase (FAK) and paxillin, which are higher in the developing female hypothalamus and decreased by exogenous testosterone or estradiol (Speert and McCarthy unpublished observation). FAK has been implicated in neurite outgrowth [76], making it an attractive candidate for mediating hormonally-induced sex differences in neuronal branching and connectivity. However, causally connecting FAX and paxillin expression to female brain development remains a goal unmet. An alternative conceptual approach can be based on emerging concepts of ovarian development. The ovary is the default fate of the undifferentiated mammalian gonad, and thus analogous to the female brain. But recent evidence indicates that DAX-1 gene expression serves to actively suppress male gonadal development [77], and is suppressed by factors that promote male development. Finding an analogous protein in female brain development would be a considerable break through.

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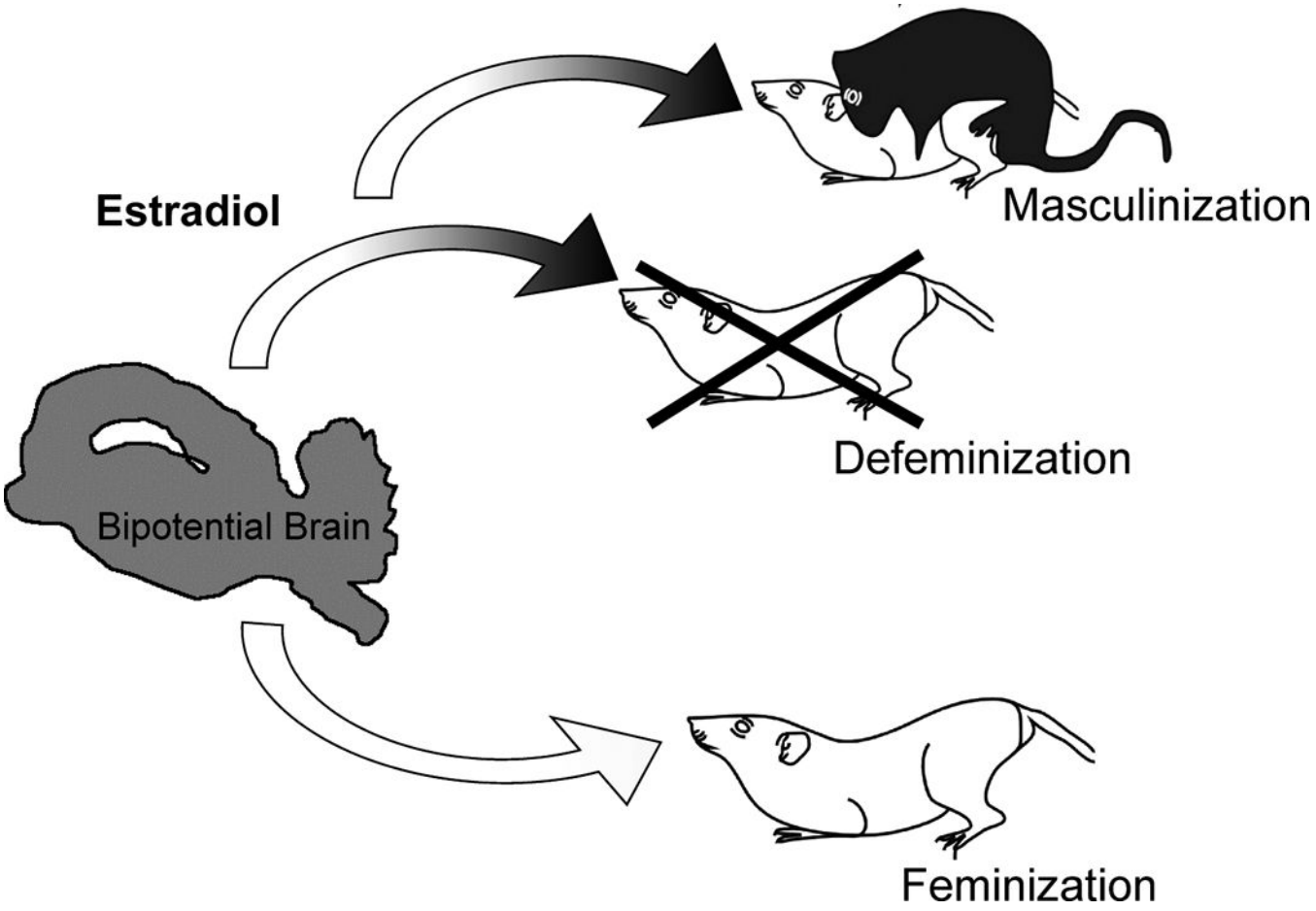

Figure 1. Schematic representation of the three processes of brain sexual differentiation

These include estradiol-mediated masculinization, an active process of building a neural network for the expression of male sexual behavior, and estradiol-mediated defeminization, an active process that results in the lack of expression of female sexual behavior in adulthood. The process of feminization occurs in the presence of either no or very low levels of estradiol, resulting in an adult animal that expresses female sexual receptivity. There is no naturally occurring process of demasculinization.

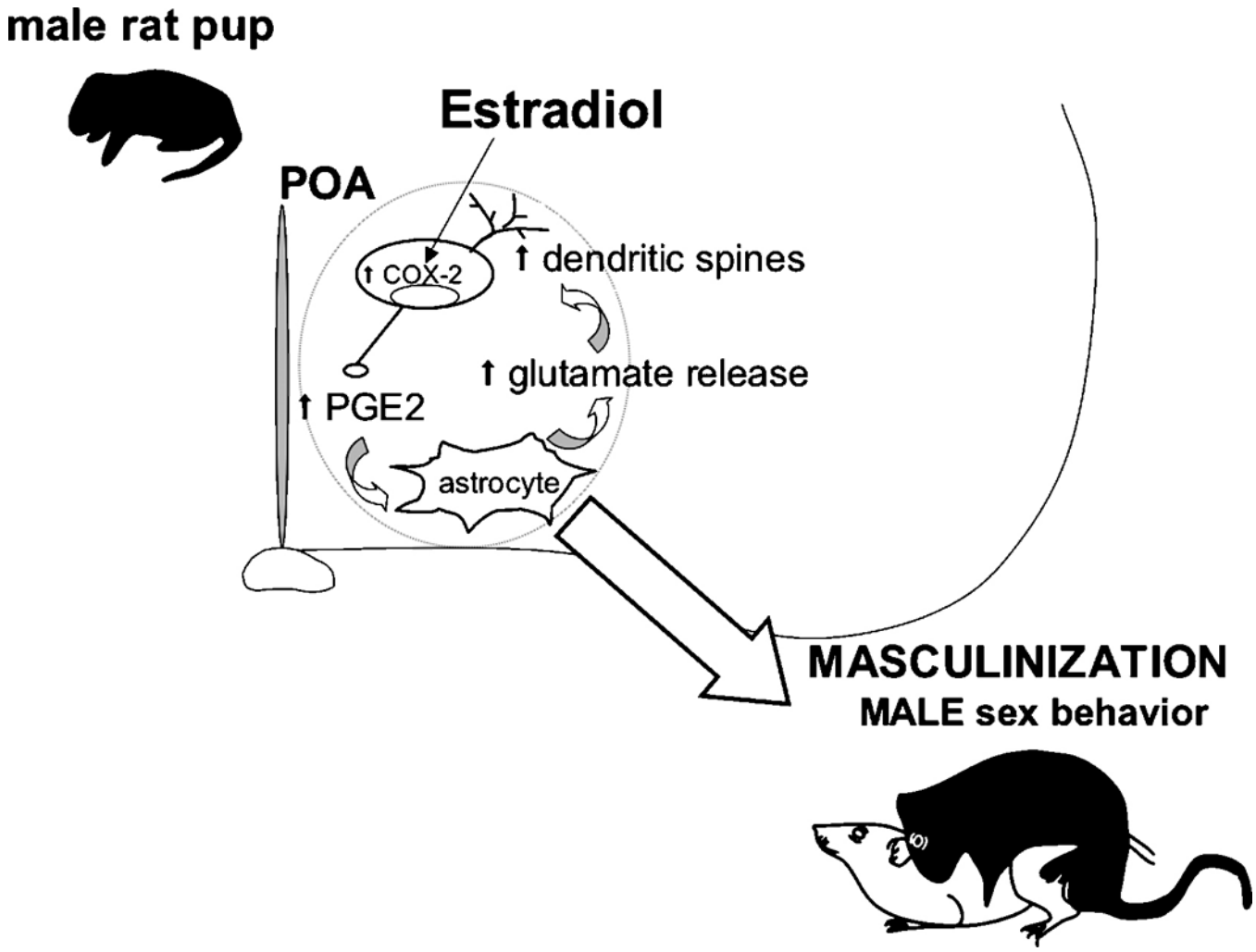

Figure 2. A working model of estradiol-induced masculinization of preoptic area neurons

Estradiol up regulates the COX-2 enzyme in neurons, leading to increased PGE2. This prostaglandin is then believed to act on neighboring astrocytes, inducing the release of glutamate which then acts back on neurons to induce the formation of dendrtic spines, resulting in male dendrites with a 2–3 fold greater density of spine synapses than females and male sexual behavior in adulthood.

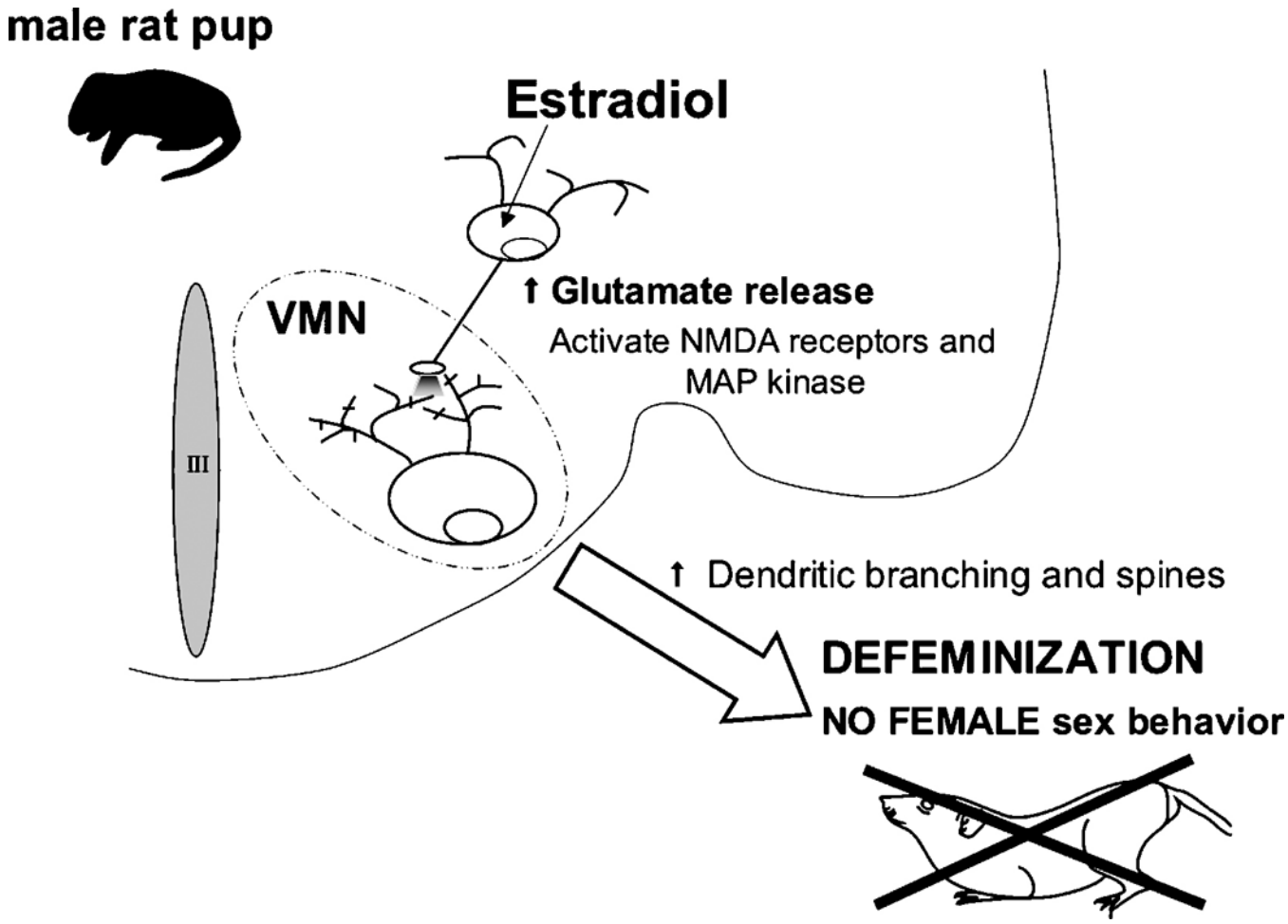

Figure 3. A working model of estradiol-induced masculinization, or possibly defeminization, of neurons in the ventromedial nucleus of the hypothalamus

Estradiol enhances the release of glutamate from neurons. Glutamate activates both AMPA and NMDA receptors and activates MAP Kinase to increase spinophilin and the total number of dendritic spines on male VMN neurons. Functionally this is predicted to result in an adult animal that does not display female reproductive behavior.

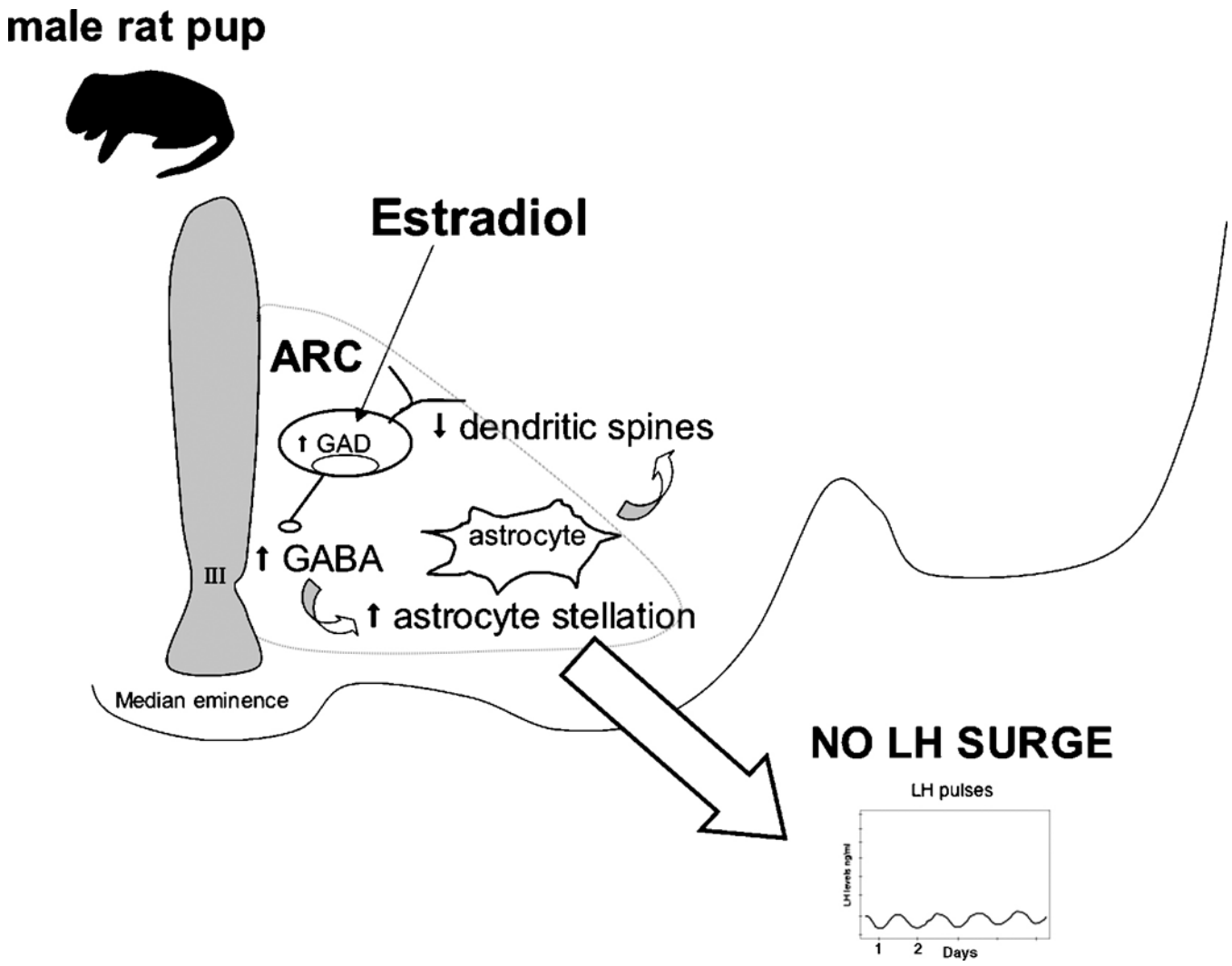

Figure 4. A working model of estradiol induced masculinization of neurons in the arcuate nucleus of the hypothalamus

Estradiol increases the enzyme glutamic acid decarboxylase (GAD), resulting in increased synthesis and release of GABA which acts on neighboring astrocytes causing them to differentiation and become more stellate. Masculinized astrocytes have longer processes with more frequent branching and are correlated with a decrease in the density of dendritic spine synapses on arcuate neurons of males. The arcuate exerts modulatory control of the anterior pituitary and this masculinization may contribute to the lack of positive feedback effects of estradiol and an LH surge in males.