

Aromatase in the Brain: Not Just for Reproduction Anymore

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Aromatase, the enzyme that synthesises oestrogens from androgen precursors, is expressed in the brain, where it has been classically associated with the regulation of neuroendocrine events and behaviours linked with reproduction. Recent findings, however, have revealed new unexpected roles for brain aromatase, indicating that the enzyme regulates synaptic activity, synaptic plasticity, neurogenesis and the response of neural tissue to injury, and may contribute to control nonreproductive behaviours, mood and cognition. Therefore, the function of brain aromatase is not restricted to the regulation of reproduction as previously thought.

Key words: behaviour, cognition, neurogenesis, neuroprotection, oestrogens, synaptic plasticity.

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Aromatase in the brain: new unexpected roles for an old enzyme

Testosterone and other C19 steroids are converted to oestradiol by aromatase (Fig. 1), which is an enzyme that consists of two components: a cytochrome P450 (P450 aro), the product of the *cyp19* gene, and the ubiquitous flavoprotein NADPH (reduced nicotinamide adenine dinucleotide phosphate)-cytochrome P450 reductase (1, 2). Aromatase activity in the brain was first detected by Naftolin *et al.* (3–5) in the foetal human limbic system and in the rat hypothalamus. After these pioneering findings, numerous studies have shown the expression, activity and distribution of aromatase in the central nervous system of several species of vertebrates (6–12), including humans (13–18). Brain aromatase is thought to be involved in the regulatory effects of androgens, via conversion to oestrogens, on reproductive neuroendocrine development. Thus, by the regulation of local oestrogen levels, aromatase activity participates in the sexual differentiation of brain regions involved in the control of gonadotrophin secretion and sexual behaviour (19–22). During adult life, brain aromatase activity also controls local oestrogen levels within brain regions involved in the regulation of reproduction (23–25).

In addition to these classical reproductive roles of brain aromatase, its activity may also modulate mood and affective status (26). Thus, aromatase knockout (ArKO) female mice (27), but not ArKO male mice (28), show increased depressive-like behaviours and polymorphisms in the *cyp19* gene are associated with depressive symptoms in women (29). Furthermore, ArKO male mice develop

compulsive behaviours, such as excessive barbering, grooming and wheel-running (30). Modifications in brain aromatase activity may also play an important role in the regulation of aggressive behaviour (31, 32) and in its modulation by social experience (32). Some clinical and experimental studies suggest that aromatase activity also impacts on cognitive function. Two randomised, placebo-controlled clinical trials have assessed the effect of aromatase inhibition on cognition. In one of these studies, conducted in postmenopausal women, the aromatase inhibitor letrozole (Fig. 1) did not affect the improvements in visual and verbal memory caused by testosterone administration (33). By contrast, another clinical trial demonstrated that aromatase inhibition in healthy older men prevents the improvement in verbal memory produced by testosterone (34). Other studies suggested that aromatase inhibitors, used as a treatment for breast cancer, may impair verbal and visual learning in women (35, 36). Studies in animals also suggested that aromatase activity may interfere with cognitive processing. Local aromatisation of testosterone to oestradiol within the brain of songbirds enhances hippocampal function, including spatial memory performance (37). By contrast, in male rats, inhibition of brain aromatase counteracts spatial learning impairment induced by the injection of testosterone into the hippocampus (38) and the systemic administration of an aromatase inhibitor facilitates working memory acquisition (39). Aromatase activity may therefore improve or impair specific cognitive modalities, probably by the fine regulation of oestradiol levels at precise moments and in specific brain regions, because oestradiol exerts dose, time and region-specific actions on cognition (40–42).

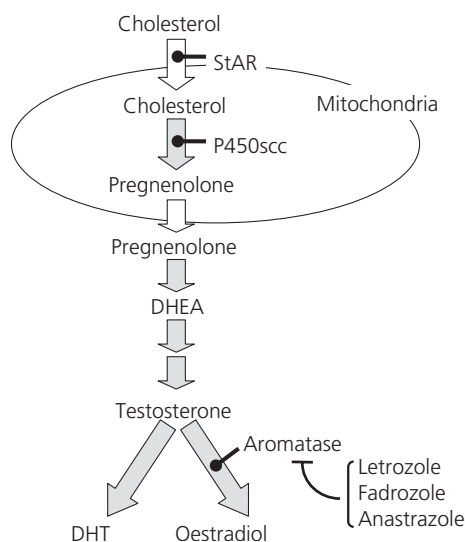


Fig. 1. Simplified representation of the pathways of synthesis and metabolism of testosterone that are mentioned in the text. Cholesterol is converted to pregnenolone by the enzyme cytochrome P450 side chain cleavage (P450scc), which is located in the inner mitochondrial membrane. Steroidogenic acute regulatory protein (StAR) is one of the proteins that facilitate the transfer of cholesterol within the mitochondria through the hydrophilic space between the outer and inner mitochondrial membranes. Pregnenolone formed in the mitochondria is metabolised in the endoplasmic reticulum to dehydroepiandrosterone (DHEA), which is a precursor of testosterone. Testosterone is metabolised to dihydrotestosterone (DHT) or to oestradiol. The conversion of testosterone into oestradiol is catalysed by the enzyme aromatase. Several pharmacological inhibitors of the enzyme, such as letrozole, fadrozole and anastrozole, have been used to determine the function of brain aromatase and are mentioned in the text. Filled arrows represent enzymatic steps and empty arrows represent intracellular transport of steroids.

Brain aromatase regulates brain plasticity

The role of aromatase on cognition may be related with its regulatory actions on brain plasticity. Rune and colleagues have provided solid evidence indicating that local oestradiol synthesis in the hippocampus by the enzyme aromatase regulates synaptic contacts in CA1 pyramidal neurones, showing that the pharmacological inhibition of aromatase activity in hippocampal slices decreases the number of dendritic spines and the number of dendritic spine synapses (43–46). In addition, aromatase inhibition reduces axon outgrowth in developing hippocampal neurones (47). Therefore, oestradiol synthesised by hippocampal cells via aromatase is an endogenous regulator of synaptic formation and synaptic plasticity.

Evidence for a role of aromatase in the regulation of hippocampal synaptic plasticity *in vivo* has been obtained by Leranth and colleagues, who found that testosterone and the non-aromatisable androgen dihydrotestosterone (DHT; Fig. 1) increased the number of synapses in the dendritic spines of CA1 pyramidal cells and that treatment of ovariectomised females with the aromatase inhibitor letrozole significantly reduced the effect of testosterone on the number of CA1 spine synapses (48, 49). As expected, the aromatase inhibitor did not affect the response of synapses to DHT (48, 49).

Further studies assessing the effect of the adrenal androgen dehydroepiandrosterone (DHEA; Fig. 1) confirmed the role of aromatase on hippocampal synaptic plasticity because letrozole abolished the induction of CA1 spine synapses by DHEA in females (50, 51). Interestingly, testosterone and DHEA do not need to be converted into oestradiol to exert an effect on spine synapses in males (49, 51), suggesting a sex-specific role of brain aromatase on synaptic plasticity. These findings indicate that brain aromatase modulates synaptic plasticity in brain regions related to cognition, such as the hippocampus. The enzyme may also influence synaptic development and plasticity in other nonreproductive regions of the central nervous system because cerebellar Purkinje cells in ArKO mice show decreased dendritic growth, and decreased formation of dendritic spines and synapses (52).

An important form of brain plasticity regulated by oestradiol is adult neurogenesis. New neurones are generated throughout the lifespan in several regions of the vertebrate brain. These newly-generated neurones migrate to their final destination and are incorporated into functional synaptic circuits (53–56). Data are available indicating that adult neurogenesis is of functional significance. For example, changes in the rate of adult neurogenesis in the brain of songbirds are correlated with the manifestation of song behaviour (53) and changes in the rate of neurogenesis in the dentate gyrus of the hippocampus in mammals are correlated with modifications in cognition and affection (54, 57–59). The role of adult neurogenesis is not simply to add new neurones to pre-existing neuronal circuits but to induce a reorganisation of these circuits by the substitution of cellular elements. The reorganisation of the synaptic circuits may be transient because many newly-generated neurones will finally die. However, these transient synaptic circuits may be functionally relevant and there is evidence indicating that both the addition and removal of new hippocampal neurones is necessary for spatial learning (58). Oestradiol is known to regulate adult neurogenesis in the hippocampus (60, 61) and recent studies suggest that aromatase may be involved in the division of progenitor cells in mammals (62). In addition, the constitutive expression of aromatase in radial glia in the brain of teleost fish (63, 64) is suspected to be related to the continuous process of neurogenesis that occurs in the brain of these animals throughout their lifespan. Indeed, aromatase-expressing radial glial cells in the ventricular layer of the forebrain in zebrafish have been identified as progenitor cells (65). Further evidence for a role of aromatase in neurogenesis has been obtained in rat hippocampal cultures (66). Treatment of these cultures with letrozole decreases the number of proliferative cells and increases the number of apoptotic cells. Similar effects have been observed after the inhibition of steroidogenesis in cultures using small interfering RNA against steroidogenic acute regulatory protein (StAR; Fig. 1). Application of oestradiol to the medium had no effect on proliferation and apoptosis whereas the antiproliferative and pro-apoptotic effects of StAR knockdown and letrozole treatment were counterbalanced by treatment of the cultures with oestradiol (66). These findings suggest that local oestradiol formed within the hippocampus regulates neurogenesis.

Rapid changes in brain aromatase activity are linked to rapid changes in synaptic function and behaviour

The effects of aromatase on behaviour and brain plasticity suggest that the enzyme may affect synaptic function and information processing by neuronal circuits. Interestingly, studies performed by Balthazart and colleagues demonstrated that brain aromatase is rapidly modulated by afferent synaptic inputs, including glutamatergic afferents, by a mechanism involving K⁺-induced depolarisation, increased intracellular Ca²⁺ levels and Ca²⁺-dependent phosphorylation (67–70). These studies show that brain oestrogen levels can be regulated within minutes by changes in aromatase activity. The rapid modulation of aromatase activity by synaptic inputs has implications for the processing of information by neuronal circuits. Thus, acute inhibition of aromatase activity in the dorsal horn of the spinal cord in quail results in a rapid (i.e. within 1 min) reduction in the response to a thermal painful stimulus (71, 72). Therefore, oestradiol produced by local aromatase activity exerts rapid effects on neuronal physiology, probably by nongenomic mechanisms of action. This role of aromatase as an acute modulator of neuronal information processing has also implications for behaviour. Indeed, Balthazart and colleagues have shown that rapid modulation of aromatase activity and the consequent rapid fluctuations in brain oestrogen concentrations are followed within minutes by changes in male sexual behaviour (67–70, 73). Therefore, synaptic activity may quickly regulate brain aromatase activity and, in turn, local oestrogen formation by aromatase may quickly regulate synaptic function and behaviour. In addition, Zhou *et al.* (74) have shown that the aromatase inhibitor fadrozole (Fig. 1) decreases GABA synthesis in hippocampal cultures whereas bicuculline, a GABA_A receptor blocker that induces overexcitation of hippocampal neurones, decreases both the number of dendritic spines and the synthesis of oestradiol in hippocampal slices, suggesting that aromatase may play an important role in brain function by linking neuronal activity and synaptic plasticity. Because testosterone may be synthesised in the brain from endogenous cholesterol (75), the neuromodulatory role of aromatase may be at least in part independent from exogenous testosterone. However, brain aromatase may be subjected to endocrine regulation and a recent study by Prange-Kiel *et al.* (76) indicated that gonadotrophin-releasing hormone regulates, in a dose-dependent manner, oestradiol synthesis and spine synaptic density in hippocampal cells by a mechanism mediated by aromatase. Therefore, the neuromodulatory and neuroplastic actions of brain aromatase may play an important role by coupling neuronal and hormonal signalling with behaviour and cognition.

The subcellular localisation of aromatase within the brain suggests that its neuromodulatory function may be exerted, at least in part, directly on synapses. In the brain of birds and mammals, aromatase immunoreactivity is located in the cytoplasm of neuronal perikarya and neuronal processes (9, 11, 12, 17, 18, 21, 75). In addition, aromatase is present in pre-synaptic terminals. The localisation of aromatase on synapses was indicated by early studies showing aromatase activity in synaptosomal preparations (77, 78). Immunohistochemical localisation of aromatase in brain sections from

quails, rats, monkeys and humans and studies by immunoelectron microscopy have confirmed that the enzyme is located in pre-synaptic terminals (18, 79–82). Therefore, oestrogen formed on pre-synaptic terminals may be potentially released to activate oestrogen receptors located in postsynaptic structures (83, 84) or may also potentially act within the pre-synaptic terminals, targeting synaptic vesicles expressing oestrogen receptors to regulate neurotransmitter release (85, 86).

Brain aromatase is neuroprotective

Another unexpected finding on brain aromatase is its role during pathological conditions. In addition to the physiological regulation of its activity in association with modifications in synaptic function and behaviour, the expression and activity of aromatase is also altered after brain injury. Different forms of neurotoxic and mechanical lesions in the brains of zebra finches, rats and mice and experimental stroke in rats, provoked by the occlusion of the middle cerebral artery, increase aromatase activity and induce *de novo* expression of the enzyme in reactive astroglia (87–91). The induction of aromatase expression in astrocytes after brain injury is accompanied by a significant increase in aromatase enzymatic activity (87) and increased levels of oestradiol within the brain (92). Aromatase-expressing astrocytes are observed in both sexes and in all injured brain areas, including the cortex, corpus callosum, striatum, hippocampus, thalamus and hypothalamus (87). This indicates that astrocytes from most, if not all, brain regions have the potential for expressing aromatase, and therefore to produce oestradiol, in response to injury.

Several studies have analysed the role of aromatase after brain injury by using aromatase deficient mice or aromatase inhibitors. Such studies have shown that ArKO male mice and intact male rats chronically treated with fadrozole have an increased vulnerability of hippocampal neurones to excitotoxic injuries (91, 93). Similar results were obtained when fadrozole was administered systemically and when it was infused in the lateral cerebral ventricle to specifically inhibit aromatase within the brain (91, 93). Fadrozole also increases excitotoxic neuronal death in ovariectomised rats (94), indicating that extragonadal aromatase is also involved in neuroprotection in females. In addition, the neuroprotective effect of testosterone and of other steroids upstream in the synthetic pathway of testosterone, such as pregnenolone (Fig. 1) and DHEA, are also impaired in the hippocampus, cerebral cortex and the olfactory bulb in animals treated with fadrozole or letrozole (93–97). Interestingly, the increased expression of aromatase in the injured brain is also accompanied by the upregulation in the expression of other steroidogenic molecules, such as StAR and cytochrome P450 side chain cleavage (P450_{sc}; Fig. 1) (98), suggesting that the nervous tissue responds to damage with an increased steroidogenesis and that the aromatisation of steroid precursors to oestradiol may be part of an endogenous mechanism activated in the brain tissue to cope with neurodegeneration (99).

Another experimental model of neurodegeneration in which the neuroprotective actions of aromatase have been assessed is the ischaemia induced by reversible middle cerebral artery occlusion

(100). Female wild-type mice that were chronically treated with fadrozole, as well as female ArKO mice, showed more cortical and striatal damage in this model than wild-type animals treated with vehicle. Furthermore, female ArKO mice showed more brain damage than ovariectomised wild-type animals, indicating that extragonadal synthesis of oestradiol is involved in neuroprotection in this model too (100). Aromatase is also protective for the nigrostriatal system. ArKO female mice are more vulnerable to nigrostriatal damage than wild-type females (101) and the intracerebral infusion of the aromatase inhibitor anastrozole (Fig. 1) in male rats has been reported to increase striatal dopamine loss after the partial neurotoxic injury of substantia nigra pars compacta (102). The neuroprotective effect of aromatase has been also assessed in a model of cerebellar ataxia produced by the degeneration of rat inferior olivary nucleus after treatment with 3-acetylpyridine (3AP), an antimetabolite of nicotinamide. Exogenous oestradiol is neuroprotective for inferior olivary neurones (103) and this hormonal action may be important for the maintenance of motor function that is under the control of the olivo-cerebellar system (104, 105). Interestingly, the inhibition of aromatase with fadrozole enhanced the injury produced by 3AP in the inferior olive of male rats, decreasing the number of neurones that survived to 3AP and increasing the number of dying cells (103).

The zebra finch brain comprises another excellent model with which to test the neuroprotective effects of aromatase. As previously mentioned, penetrating mechanical brain injury induces aromatase expression in astroglia in the zebra finch brain (88, 106–108). Wynne and Saldanha (106) injected fadrozole at the site of injury in one hemisphere and vehicle in the other hemisphere and found an increased number of apoptotic nuclei and a larger injury size in the hemisphere injected with fadrozole compared to the hemisphere injected with vehicle. Furthermore, Wynne *et al.* (108) recently demonstrated that intracerebral injection of fadrozole at the site of injury increases reactive gliosis and results in the appearance of a wave of apoptotic secondary degeneration that is not detected after the injection of vehicle. This indicates that endogenous brain aromatase activity is exerting a neuroprotective role, modulating gliosis and preventing secondary neuronal death. Further important information, as revealed by Schlinger and colleagues using this animal model, is that aromatase regulates cell proliferation after brain injury. A penetrating lesion in the zebra finch hippocampus induces the accumulation of newly-generated cells around the lesion site (109–111). These new cells are generated within the hippocampus and in neuroproliferative regions, such as the subventricular zone, located in proximity to the injury. Newly-generated cells are associated with radial glial cell processes expressing aromatase (109) and there is a positive correlation of the number of aromatase immunoreactive astrocytes and the number of newly-generated cells (111). Furthermore, the administration of fadrozole reduced cell proliferation after injury (110, 111). These findings suggest that oestradiol released by glial cells after injury may sustain the proliferation and migration of new cells and may therefore play an essential role in the reorganisation and repair of the damaged brain.

The above mentioned experimental studies clearly indicate that astroglial cells express aromatase after brain injury and that the

enzyme exerts a neuroprotective action. Furthermore, in all the experimental models studied, the neurodegenerative effect of aromatase deficiency was counterbalanced by the administration of oestradiol (93, 100, 103, 107), indicating that the neuroprotective properties of aromatase lie in its ability to catalyse the formation of oestradiol rather than in reducing oestradiol precursor levels. Thus, the question arises on the possible relationship between brain aromatase expression, brain oestradiol levels, oestrogen therapy and human neurological and affective disorders. This is a field that remains almost completely unexplored; although alterations in aromatase immunoreactivity have been detected in the brain of Alzheimer's disease patients (17) and genetic variation of the aromatase gene in humans may increase the risk of Alzheimer's disease (112). Another question that needs to be addressed is the possible existence of sex differences in the neuroprotective activity of brain aromatase. We have seen previously that brain aromatase may have a sex-specific role on synaptic plasticity, behaviour and cognition. In addition, although aromatase expression in astrocytes after brain injury is observed in both sexes (87), it has been reported that astrocytes from females are less susceptible than male astrocytes to oxygen-glucose deprivation *in vitro* and that this endogenous protection is associated with enhanced aromatase activity (113). On

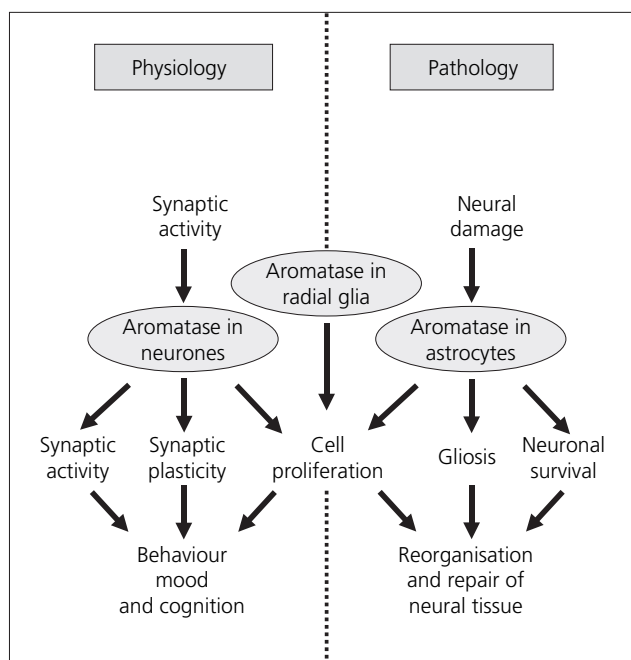


Fig. 2. Summary of nonreproductive actions of brain aromatase. Under physiological conditions, aromatase activity in neurones is rapidly regulated by synaptic activity. In turn, aromatase regulates synaptic function, synaptic plasticity and neurogenesis. Brain aromatase, conceivably via its actions on synapses and neurogenesis, affects behaviour, mood and cognition. Under pathological conditions, neural damage induces aromatase expression in astroglia. Aromatase activity in glial cells down-regulates gliosis, enhances cell proliferation and promotes neuronal survival, contributing to the reorganisation and repair of neural tissue. Aromatase activity in radial glia of nonmammalian vertebrates may be involved in the regulation of cell proliferation under physiological and regenerative conditions.

the other hand, apoptosis of dopaminergic neurones has been detected in the medial preoptic area and arcuate neurones of 1-year-old male, but not female, ArkO mice (114) and sex differences have been observed in oestrogen levels in post-mortem Alzheimer's disease brains (115). Therefore, it is possible that sex differences in the ability of astrocytes with respect to the production of oestradiol or in brain vulnerability to aromatase deprivation may be involved in a sexually dimorphic expression of some brain pathologies.

Conclusions

In summary, brain aromatase appears to be an important regulator of brain function both under physiological and pathological conditions (Fig. 2). Aromatase regulates and is regulated by synaptic activity, is involved in the control of brain plasticity and behaviour, and may affect specific cognitive abilities. In addition, brain aromatase is likely to play an important role in the protection of neural tissue by increasing local oestrogen levels. Therefore, local oestrogen synthesis within the nervous system is not restricted to the control of reproduction and emerges as a key regulator of brain function, plasticity and response to injury.

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