Involvement of testosterone signaling in the integrity of the neurovascular unit in the male: review of evidence, contradictions and hypothesis

Delnia Ahmadpour, Valérie Grange-Messent

Involvement of testosterone signaling in the integrity of the neurovascular unit in the male: review of evidence, contradictions and hypothesis

Delnia Ahmadpour[1] and Valérie Grange-Messent[1*]

[1] Sorbonne Université, CNRS, INSERM, Neuroscience Paris-Seine, Institut de Biologie Paris-Seine, 75005 Paris, France

Short title: Testosterone and the neurovascular unit in males

*Corresponding author

Valérie Grange-Messent

Sorbonne Université, INSERM U1130, CNRS UMR 8246, Neuroscience Paris Seine, Institut de Biologie Paris-Seine, 7 quai St Bernard, 75005, Paris, France.

Tel: +331 44 27 36 57

Fax: +331 44 27 25 08

e.mail: valerie.messent@sorbonne-universite.fr

Key words: Adult male brain, testosterone, Blood–brain barrier, Neuroinflammation, Neurovascular unit

Abstract

Age-related central nervous system function decline and increased susceptibility of females, compared to males, with respect to prevalence of several neurodegenerative and neuropsychiatric diseases, are both based on the principle that hormonal factors could be involved. These cerebral disorders are characterized by an alteration of the blood–brain barrier (BBB) properties and chronic neuroinflammation, which lead to disease progression. Neuroinflammation, in turn, contributes to BBB dysfunction. The BBB and its environment, called the neurovascular unit (NVU), are crucial for cerebral homeostasis and neuronal function. Interestingly, sex steroids influence BBB properties and modulate neuroinflammatory responses. To date however, the majority of work reported has focused on the effects of estrogens on BBB function and neuroinflammation in female mammals. In contrast, the effects of testosterone signaling on the NVU in males are still poorly studied. The aim of this

review is to summarize and discuss literature, providing insights and contradictions to highlight hypothesis and the need for further investigations.

Introduction

The reproductive effects of sex hormones, namely estrogens and androgens, are well known. Furthermore, it is increasingly apparent that these hormones have major actions on non-reproductive systems such as the central nervous system, controlling homeostasis, development and some behaviors for instance [1,2]. These effects can be relevant throughout the lifespan of an organism, and are not limited to the reproductive period [1–3]. Additionally, they have a profound influence on both sexes and not restricted to one sex or the other [1]. One such example of the effects that sex steroids have on non-reproductive target tissues is their impact on cerebral blood vessels. Estrogens and androgens both regulate vascular tone, angiogenesis and endothelial cell survival, oxidative stress, and inflammatory responses in cerebral blood vessels [1], but the actions of estrogens and androgens often oppose each other [4]. Moreover, the expression of the respective receptors of estrogens and androgens suggest that cerebral blood vessels are directly targeted by sex steroids [5]. Protective effects of other classes of sex steroids such as progestins, including progesterone, have also been documented [6].

Numerous therapeutic approaches aim to target the emerging effects of steroid deprivation and/or supplementation for amelioration of several CNS conditions [7,8]. Although many studies have investigated the therapeutic potential of estrogens and progesterone in blood–brain barrier (BBB) function in females [8,9], much less attention has been devoted to clarify the impact of estrogens and androgens in males. The mechanisms underlying the effects of testosterone signaling on the neurovascular unit (NVU) in males are complex and still poorly studied. Currently, no review is available on this subject to our knowledge and data reveal evidence but also contradictions. Thus, the intention of our review is to synthesize the data of the literature to bring out hypotheses and highlight the need for further investigations.

We present a summary of the distribution of sex steroid receptors, mediating testosterone effects, in the male brain. Then, we review and discuss data concerning the effects of testosterone on cerebral micro-vessels, focusing on BBB permeability modulation. We also present an overview of testosterone involvement in neuroinflammation and oxidative stress responses and their relation with BBB alterations.

- **Blood-brain barrier**

In the central nervous system (CNS), cells require a stable microenvironment, which is guaranteed by a complex cellular and molecular system involving brain micro-vessels and their close environment referred to as the BBB [10,11]. Indeed, the BBB is a highly regulated specific interface that maintains cerebral homeostasis to ensure neuronal activity. Indeed, the function of the BBB underlies the regulation of all vital functions of an organism. As such, BBB dysfunction can lead to many brain diseases and complicate recovery from injuries and insults [11,12]. The walls of cerebral blood vessels are formed of endothelial cells (ECs) that are sealed by continuous intercellular molecular binding systems including adherens junctions (AJs) and tight junctions (TJs) [13] that limit para-cellular diffusion [14] (Figure 1). The major transmembrane components of TJs are the claudins, occludin and junction adhesion molecules (JAMs) [15]. They interact with the actin cytoskeleton via cytoplasmic TJs accessory proteins, including zonula occludens (ZOs) proteins [16] (Figure 1). In both mice and humans, claudin-3 and -5, ZO-1 and occludin have been described as the predominant proteins found at the TJs [17]. Claudins are crucial for the para-cellular sealing function, hence, they directly determine barrier function [18]. But the presence of claudin-3 in TJs of mouse BBB endothelial cells has been recently called into question: immunodetection of claudin-3 in mouse brain endothelial cells is thought to be an artifact due to antibody cross-reactivity with an undisclosed endothelial junction antigen still present in mice deficient in claudin-3.[19]. C57BL/6J mice lacking claudin-3 expression display an intact BBB and do not show any sign of BBB dysfunction [19]**.** Claudin-5 knockout mice, however, have a significant para-cellular ionic selectivity defect [20,21]. A review published early in 2019 provides a comprehensive overview of the role of claudin-5 and its regulation in physiological and pathological conditions [18]. Likewise, disassociation of the ZO-1 junctional complex, which typically serves to stabilize TJs, leads to the breakdown of BBB integrity [22]. The AJs transmembrane components cadherin and nectin anchoring to cytoplasmic components such as catenin and afadin respectively, are also involved in the BBB integrity [23,24] (Figure 1).

In addition to the intercellular binding systems that limit para-cellular diffusion, cerebral ECs also lack fenestration and exhibit extremely low rates of transcytosis, both of which greatly limit trans-cellular diffusion [25]. Both features arise from several changes to ECs morphology and biochemistry that occur during embryonic development and allow the transition from a permeable to a continuous vessel [26]. BBB is present throughout the CNS except in certain unique regions of the brain, the circumventricular organs. The circumventricular organs are characterized by fenestrated capillaries permeable to blood-borne molecules, and the interface ensuring the

cerebral homeostasis, in these cerebral areas, is constituted by specific cells called tanycytes. Those cells, lining cerebral ventricles, are sealed by tight junctions preventing the extravasation of molecules from fenestrated capillaries to the parenchyma via the cerebro-spinal fluid, forming thus the blood-CSF barrier (see for review [27]). Control of substance exchange between blood and cerebral parenchyma is also mediated by specific carrier systems present in EC abluminal or luminal plasma membranes (Figure 1). As such, the ability of macromolecules, fluids, and cells to cross endothelial barriers depends on charge, size, and binding characteristics [28]. Instead of passive diffusion, the supply of nutrients into the CNS occurs via active influx transporters such as glucose transporter 1: GLUT-1, whereas elimination of metabolites occurs via transporters of two major membrane transporter families, the ATP-binding cassette (ABC) family and the solute carrier transporter superfamily (SLC) [29]. Notably, efflux transporters (e.g., P-glycoprotein: P-gp, Breast Cancer Resistance Protein and the Organic Anion Transporter 3) are also responsible for the transport of several classes of pharmaceutical drugs [30,31]. Due to their restrictive nature, those efflux transporters can also be major obstacles in central distribution of many therapeutic agents for treating CNS diseases. Many lipid-soluble molecules, such as certain therapeutic drugs, are the substrates of efflux transporters, but some of them limit their absorption in order to protect the brain against potentially harmful exogenous substances [31,32].

In addition to active protein transporters, endocytic vesicles serve as another major trans-cellular pathway accounting for BBB permeability in brain injury models [33, 32] (Figure 1). Caveolae are non-clathrin-coated vesicles with plasma membranes enriched in caveolin (Cav) proteins, involved in receptor-independent transport of blood-borne molecules such as albumin [32, 34]. To date, Cav-1, Cav-2 and Cav-3 isoforms have been identified in murine, bovine, and human cerebral endothelial and astroglial cells [35].

The BBB represents a complex cellular system that consists not only of ECs, but also of pericytes embedded in basal lamina surrounding ECs, and astrocytic end-feet anchored to this basal lamina. ECs, pericytes, basal lamina, astrocytic end-feet and their neighboring microglial cells and neurons are collectively named the NVU (Figure 1); they govern BBB development, along with its maintenance and integrity [13].

Pericytes cover a large part of the abluminal surface (between 22% and 99% [10]) of the endothelium and contribute to the stability of micro-vessels [36]. Their position and abundance within the cerebral capillaries suggest that these cells play a major role in BBB integrity. To date, it has been hypothesized that pericytes would deliver angiogenic factors that regulates permeability and vascular remodeling [37,38], and that they would have contractile

capabilities to allow them to regulate blood flow through the capillaries [39]. Moreover, pericytes play a major role in the differentiation of ECs during fetal life, even before the appearance of astrocytes [40]. The recruitment of pericytes to endothelium and the interaction between the two are essential factors for the formation, maturation and maintenance of the BBB [40–42].

The basal lamina forms a continuous network of extracellular matrix (ECM) composed of type IV collagen, laminin, nidogen, heparan sulfate, elastin, and various proteoglycans and glycoproteins [43]. The interaction between endothelial integrin, laminin, and other ECM proteins allows ECs to be anchored onto this basal lamina [44]. In addition to its structural role, ECM degradation is associated with an increase in para-cellular permeability of the BBB [45].

Glial cells including microglial and astrocytes also represent an important element of the NVU. Microglial cells were described for the first time in 1919 by Del Rio-Hortega as a specific type of glial cell (see for review [46]). When circulation begins to form at the CNS, at a time when the BBB is still permissive, some microglial cells precursors enter the CNS through the vascular network (see for the different hypotheses about the origin of microglia [47]). Those microglial cells that enter the brain parenchyma during embryonic development, branch out then and remain quiescent [48]. Microglial cells are activated during inflammatory reaction or immune challenge in the CNS and then differentiate into resident CNS macrophages [45,49]. Upon immune challenge or following microglia activation, astrocytes also produce inflammatory cytokines [50,51]. In addition, astrocytes are pivotal in the formation of tight junctions of the ECs, the promotion of the enzyme system γ-glutamyltranspeptidase involved in amino acid transport across the BBB, the differentiated distribution of transporters present in the luminal and abluminal plasma membrane of the ECs and ionic concentrations at the BBB [51–53].

A dysfunction of the BBB is associated with neurological damage and disease [54]. Disruption of the BBB is also associated with peripheral diseases such as diabetes [55] and non-neural, inflammatory-related pathologies [56]. Alteration of the cells of the NVU upon aging, inflammatory activation, oxidative stress, or neuropsychiatric and neurodegenerative diseases, leads to BBB dysfunction [44], which, in turn, contributes to the progression of neurological diseases [17,44].

- **Testosterone**

Testosterone was classically delineated as male-specific hormone according to its marked synthesis in the testes and its contribution to phenotypic and reproductive sex differences [2].

The biosynthesis of testosterone from cholesterol has been demonstrated in many tissues in addition to Leydig cells of the testes (in males). These include the brain (hippocampus) and liver and, to a lesser extent, the adrenal cortex zona reticularis and zona fasciculata in both males and females [57]. Testosterone, which is a precursor of ovarian estrogens, is also presents in females but at lower concentration (0.5–1 ng/ml in adult women vs 3-8 ng/ml in adult men) [58]. Testosterone levels are sensitive to many internal and external factors, and mainly fluctuate with aging [58,59]. Indeed, aging in men is accompanied by a progressive lowering of the serum testosterone level, in parallel with a decline in several CNS function [60–63]. This provides a logical reason to increase research to determine whether testosterone acts as a protective factor against several CNS disorders [64]. In addition to age-related circulating testosterone changes, there are obvious gender differences in the prevalence of neuropsychiatric (e.g. depression, anxiety disorder, anorexia nervosa) and neurodegenerative (e.g. cognitive decline, autoimmune diseases, Alzheimer's (AD) and Parkinson's (PD) disease) diseases, with a higher incidence in females than in males [8,64]. The reasons for gender differences, and a better understanding of how the state of being male limits these CNS disorders, are still unclear. It appears that sex bias is partly due to the protective effect of testosterone, or to the presence of the Y chromosome in males [58,65]. A recent review has finely summarized the sex differences in cerebrovascular function, highlighting the evidences for sex steroid hormone-mediation in numerous cerebrovascular regulation and pathology [66].

Previously, testosterone research was almost entirely focused on reproductive issues. However, it is now known that testosterone is a regulator of fundamental physiological processes of non-reproductive tissues such as cardiac and skeletal muscle, or of development and function of the immune and nervous systems [1], including cerebrovascular tissue [67–70]. Outside the brain, testosterone has a key effect on the cardiovascular system by the regulation of the vascular tone [71]. The greater incidence of clinical manifestations of heart disease in men and postmenopausal women compared with premenopausal women, translates a sex steroid hormones-induced gender difference for the vascular tone with a possible vascular protective effect of female sex hormones. It has been suggested that this gender difference in vascular tone is mediated at least in part by specific sex hormone receptors[68]. Nevertheless, the role of testosterone in vascular contractility is debated. A number of studies have shown that testosterone facilitates vasoconstriction in rat (*in vivo* and *in vitro*) [72,73] while other data have reported a vasodilator effect in rat and rabbit [74,75]. Vascular mechanisms of testosterone have been thoroughly reviewed very recently [76]. In brain, the regulation of cerebrovascular items is mediated through several mechanisms such as for instance, angiogenesis, vascular contractility, and control of the BBB permeability.

Distribution of sex steroid receptors, mediating testosterone effects, in the male central nervous system

Sex steroids, mediate their action via members of the nuclear receptor superfamily. These are expressed widely throughout the CNS by multiple cell types, but especially in brain areas involved in cognitive and reproductive behavior, and in neuroendocrine function [2]. Testosterone exerts its effects on the organization and function of the CNS by either androgen receptors (ARs) or estrogen receptors (ERs) after undergoing neural aromatization to estradiol (E2; about 0.3%) [1,77]. The reductase enzyme catalyzes the conversion of about 7% of testosterone to 5-α dihydrotestosterone (DHT), which is 2.5 to 10-fold more potent than the former [78,79]. ERs- and ARs-mediated signaling pathways modulate several CNS activities by both genomic and non-genomic actions [5,79]. To describe sex steroid receptor localization within the adult CNS, mRNA expression patterns of ARs and ERs were investigated in adult rats [79–81] (Figure 2). In males, nuclear ARs are expressed in the cortex and hippocampus (CA1 and CA2/CA3 regions), which control the regulation of cognitive behavior. However, ARs are also widely expressed in the olfactory bulb, the medial amygdala (MeA), the bed nucleus of stria terminalis (BNST), and the preoptic region (POA) of the hypothalamus involved in neuroendocrine functions and reproductive behavior [79] (Figure 2). It is also known now that nuclear ARs are present in neurons and glial cells as well as in cerebral blood vessels at the level of smooth muscle and the endothelium [5].

Neural estrogens exert their effects through interaction with two nuclear ERs: estrogen receptor alpha (ERα) and beta (ERβ). A comparative study provided evidence that nuclear forms of both ERα and ERβ are expressed in the male rat CNS; some regions express exclusively ERα, for example at the level of the ventromedial hypothalamic nucleus (VMH), while others express only ERβ, for example paraventricular nuclei. In contrast, other regions like BNST, medial and cortical amygdaloid nuclei, POA, periaqueductal gray and spinal trigeminal nuclei contain both forms of ER mRNA [79,80]. The cerebral cortex and hippocampus also contain both ER mRNAs, giving a lower hybridization signal for ERα than for ERβ. This suggests the involvement of ERβ in cognition and memory [2,79,80] (Figure 2). It is noteworthy that the highest level of ERα mRNA expression is in the hypothalamus and amygdala is related to regulation of sexual behavior and the hypothalamic-pituitary-gonadal (HPG) axis [82].

In addition to their neural expression, specific receptors for gonadal steroids are expressed in the cerebrovascular tissue [5]. They can thereby control the integrity of the BBB by modulation of membrane transporters, intercellular molecular binding systems and neuroinflammatory responses [67,69,83], as we will discuss further below.

Additionally to their specific receptors, the metabolic enzymes for gonadal steroids have been found in cerebral blood vessels [5].

Testosterone -induced neuroprotection in the male central nervous system

As evoked above, gender differences in the prevalence of neurological disorders and diseases can be explained by neuroprotective effects of testosterone in males. Recently a pivotal role of androgenic signaling in modulation of neural activity within the CNS through myelination and re-myelination of neurons and synaptic plasticity has been documented [68,84].

The decreased susceptibility of men, compared to women, to inflammatory autoimmune disorders such as Multiple Sclerosis (MS) is also described in some mouse models like the SJL/J mouse. This exhibits spontaneous myopathy, associated with an increased susceptibility to autoimmune diseases. Castrated SJL/J mice showed greater demyelination and inflammation within the spinal cord than sham-operated males [1,8,58,64]. Furthermore, recent data obtained in a mouse model of chronic demyelination [85] and lysolecithin-induced demyelination [64] have allowed the effects of androgenic pathway on myelin repair and re-myelination processes to be established. It is also well known that synaptic changes are involved in memory impairment occurring in the hippocampus of patients with AD [86]. A beneficial role of testosterone in improving cognitive performance in mice and rats by enhancing the hippocampal protein network which is critical for synaptic transmission and plasticity has been described [87,88]. Furthermore, the expression of ARs, not only in nuclei [89] but also at extra-nuclear sites of hippocampal neurons [90], has revealed the involvement of testosterone and DHT in neural activity and synaptic function. Gonadectomy also decreases density of spine synapses in the male rat hippocampus [91]. Replacement therapy by added DHT or testosterone restores spinogenesis through AR signaling, which rapidly promotes kinase networks in neurons [92]. In addition, despite the fact that there are no ARs in the dentate gyrus of the adult male rat hippocampus, it has been demonstrated that AR signaling induce neurogenesis via modulation of the survival of new neurons in this area of the brain [93].

In many studies concerning psychiatric disorders, women are reported to have a higher incidence of depression than men [90]. Aging is also associated with an increased prevalence of depression in both sexes [95]. Depression can be associated with alterations in the proliferation and survival of hippocampal neurons: evidence indicates a role for androgenic signaling in the survival of new neurons in the hippocampus of young adult males [64].

Chronic stress is also associated with reduced neurogenesis and expression of synaptic proteins in the CA1 and CA3 regions of the adult hippocampus [96]. The stress response is mediated through the production of glucocorticoids [97] and it is well known that testosterone reduces glucocorticoids release [95,98].

In men, the relatively high levels of circulating testosterone, compared to women, is correlated with a higher incidence of strokes [99]. It was shown that the high levels of testosterone and DHT, may contribute toward sensitivity to cerebral ischemia in young males [100,101]. Emerging experimental studies, however, have indicated that the decline of testosterone level upon aging in males can be associated with an increased risk of stroke [102]. These data describe testosterone dose-dependent neuroprotective and neurodamaging effects in experimental models of cerebral ischemia. Finally, recent experiments *in vitro* have shown that gonadal hormones may be also involved in the suppression of neuronal apoptosis during ischemic disorders: testosterone and DHT suppress oxygen-glucose deprivation/re-oxygenation induced neuronal cell death in hippocampal slice cultures [103].

In many brain diseases, disorders, trauma or stroke, neuronal damage is associated with an inflammatory response [104] and a dysfunction of the BBB [105]. However, it has been shown in a model of MS a partial uncoupling of inflammation and neurodegenerative process[106]. This study also suggests that a BBB dysfunction may persist regardless of inflammatory signals.

Recent data have suggested that the cross-talk between testosterone and glial cells, astrocytes or microglia, participates in the neuroprotective effect. This is achieved by attenuating neuroinflammation and oxidative damage during acute traumatic brain injuries such as stroke and hypoxia [58,107–109]. However, it has been hypothesized that the control of reactive gliosis such as an increase of astrocyte and microglia activation, a key event in neuroinflammation, is part of the testosterone neuroprotective mechanism [110]. We will devote another part of this paper to a detailed discussion of testosterone effects upon neuroinflammation (see below).

Testosterone and cerebral blood vessels

Testosterone modulates vascular function through several mechanisms including, but not limited to, angiogenesis and BBB permeability.

- ### Impact of testosterone on angiogenesis and vasculature formation

The development of the BBB occurs in several steps, beginning with angiogenesis from preexisting vessels around embryonic day E10 in mice [111] (Figure 3A). It has been demonstrated that testosterone modulates vasculature function and integrity via several mechanisms that involve vasculature formation, e.g. proliferation, migration,

and differentiation [112]. The patterns of circulating levels of testosterone in the male mouse from birth to adulthood are illustrated in figure 3B [113]. Several researchers have focused their activities on testosterone impact on angiogenic mechanisms in cardiovascular regeneration and cerebral angiogenesis in adults [1,62,114]. However, the role of testosterone in CNS angiogenesis during fetal life has remained largely unexplored. In cultured human umbilical endothelial cells, testosterone induces endothelial cell migration through myosin activation and actin cytoskeleton remodeling [115]. It also increases formation of endothelial cell tubes, by stimulating Smad1 phosphorylation. This represents one possible mechanism for testosterone-enhanced angiogenesis [116]. An *in vitro* study using human dermal fibroblasts revealed that , DHT is a regulator of vascular endothelial growth factor (VEGF) secretion, the key mediator of angiogenesis in both CNS and non-CNS tissues [62]. In addition, VEGF has a therapeutic potential for the induction of neuroprotection, neurogenesis, and cerebral angiogenesis after ischemic disorders such as stroke [117]. Furthermore, histological and transcriptomic analyses of adult female robins and canaries treated with testosterone, reveal that this hormone induces angiogenesis in song control nuclei. This is associated with up-regulation of VEGF, VEGF receptors, and brain-derived neurotrophic factor (BDNF) [118–120].

- **Impact of testosterone on cerebral endothelial cells and blood-brain barrier**

The impact of sex steroid hormones as therapeutic agents has received special attention with emphasis being placed on their neuroprotective effects. However, only a few studies have focused on the influence of sex steroid on the BBB and the NVU in both physiological and pathological conditions. Relatively little is known about testosterone compared to estrogens and progesterone due to: a) controversial results concerning its involvement on vascular endothelium function, b) debate as to therapeutic aspects - dose, duration, and health status, c) misuse or abuse for non-medical purposes, d) complicity of action due to interaction with multiple ERs. The maintenance of the BBB as a crucial interface, separating peripheral circulation and the CNS, requires perfect interaction between all components of the NVU. Additionally, altering critical properties of the BBB including, but not limited to, membrane transporters, vesicular pathways, basal lamina and TJ proteins of ECs could lead to a modification of microvascular integrity, hence, brain homeostasis. Testosterone modulates cerebral endothelial cells and blood-brain barrier function through the modulation of key elements of this complex cellular system.

  - **Endothelial membrane transporters**

To date, only very limited data exist concerning testosterone effects on the regulation of membrane transporters at the BBB. OAT3, which mediates the efflux transport of uremic toxin and neurotransmitter metabolites such as dopamine metabolite [121], is expressed at the BBB [13]. The expression of OAT3 in male rats is higher than in females [122], and ARs regulate the expression and uptake rate of OAT3 in BBB cells (Figure 4) [83]. It is noteworthy that the dopaminergic system participates not only in sexual behavior processes but also in regulation of cognitive behavior [123].

Sodium-dependent (SGLTs) and sodium independent (GLUTs) glucose transporters represent the two types of glucose transporter that regulate the uptake of glucose as a crucial energy source at the level of the BBB [124]. The activity of these transporters is subject to modulation in a variety of neuropathological and neuropsychiatric disorders, including AD, PD [125], and depression [126]. A study proposed that proinflammatory cytokine induce protein degradation of the number of glucose transporters, specifically, GLUT-1 and GLUT-3 in the BBB of AD patients (Figure 4) [127]. It is notable that ovarian steroids are essential modulators of glucose transporters [128,129]. To date, there has been no report concerning testosterone effects on these transporters' activities. Thus, the testosterone effects on membrane transporters at the BBB are not well understood, and this subject needs investigation. But it will be not surprising that testosterone could act as modulator of influx and/or efflux transporters partly through the modulation of inflammation mediators. Indeed, the impact of the Inflammatory mediators on BBB transporters has been described [130,131]. For instance, the inflammatory mediators, such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interferon gamma (IFNγ) can decrease the activity of P-gp at the level of the BBB (Figure 4) [132]. P-gp contributes to the efflux of brain-derived amyloid into blood. In addition, TNF-α reduces functional activity of BCRP (Figure 4) [133], which could alter the penetration of their substrates into the brain. Recently, evidence has shown that depletion of gonadal testosterone by castration of male mice induces up-regulation of IL-1β and TNF-α in the preoptic area [67] (Figure 4), although the effects of these shifts upon membrane transporters were not evaluated.

○ Trans-endothelial vesicular transport pathway

As already mentioned caveolae are known as a major trans-cellular pathway in brain injury models. It was shown that the components of the caveolae plasma membranes, Cav proteins, play a critical role in signal transduction [77]. In addition to brain endothelial cells, the Cav-1 protein has been described in vascular pericytes [134], while both Cav-1 and Cav-3 have been found *in vivo* and *in vitro* in reactive astrocytes, (Figure 4) [134,135].

It is important to address the controversy concerning the role of Cav-1 in regulating vascular permeability. An *in vivo* study in rats showed an enhancement of permeability of the BBB through up-regulation of the expression level of Cav-1 [34]. Nonetheless, data obtained from Cav-1 and Cav-2 knockout (KO) mice have shown a higher vascular permeability and lower lesion volume, respectively, after a stroke, compared to wild-type mice [136,136]. This suggests a positive role for Cav-1 in the maintenance of the integrity of the BBB, in contrast to Cav-2. Cav-1 KO ischemic brains are also characterized by impaired angiogenesis and increased cell death [137]. The role of Cav-1 in BBB integrity in neuropathology context and neural recovery after ischemic stroke was reviewed very recently, and the discrepancies in the literature pertaining to the role of Cav-1 were discussed [138].

Data based on rat immunohistochemistry analyses showed that an increase in Cav-1 expression at the cortical lesion site of cold-injury blood vessel model, is associated with loss of occludin and claudin-5 TJ proteins [139]. However, it has been suggested that Cav-1 expression contributes to the recovery of TJ proteins and BBB function after injury. There are clear results indicating that TJ proteins are either bonded or localized in caveolae, and internalization, but not degradation, of occludin and claudin-5 through Cav-1 is responsible for alterations in brain endothelial barrier permeability during inflammation [140]. Recycling of internalized TJ proteins is crucial for recovery of the brain endothelial barrier integrity [140] (Figure 4).

Despite confusion in the current literature concerning the roles of Cav-1 in modulation of the BBB integrity, thus far studies have provided evidence that Cav-1 could prevent oxidative and inflammation injury, and extracellular degradation of TJ proteins and matrices [140,141]. The increase in Cav-1 upon brain injury may reflect its beneficial involvement in the recovery of BBB integrity in damaged CNS but would not explain a detrimental role. We can also suppose that the effects of Cav-1 may be isoform, tissue, state of health and dose dependent. Further, Cav-1 may have different functions in different neurological dysfunctions. The exact mechanisms and explanations for these controversial results remain unclear and this is an issue that needs further investigation.

There is evidence showing that non-genomic ARs can interact with numerous signaling molecules at the plasma membrane. Thus, ARs interacting with caveolae, trigger numerous kinase-signaling pathways involving in particular the Src kinase, extracellular signal-regulated protein kinase, the phosphoinositide 3-kinase and the protein kinase B [77]. Cav-1 protein is known as ARs co-activator [142,77], allowing this nuclear receptor to efficiently mediate gene expression. Thus, it cannot be excluded that AR non-genomic activity indirectly influences activity of either genomic ARs or other nuclear receptors. Furthermore, a recent study showed a positive correlation

between the level of Cav-1 and ARs transactivation and sensitivity of ARs to ligand-dependent activation, confirming the role of Cav-1 as a convergent point for ARs cross-talk with other cellular signal transduction pathways (Figure 4) [143]. Thus, given the importance of Cav-1 in modulation of the BBB integrity and its links with ARs, study of the molecular mechanisms underlying the interaction between the Cav-1 protein and the androgen receptor requires further investigation.

○ Basal lamina and the immediate surroundings of endothelial cells

The basal lamina as an acellular membrane surrounding ECs has a pivotal role in BBB integrity [144]. Loss of the basal lamina stability triggered by metalloproteases or collagenases is frequently observed in pathological manifestations involving the BBB breakdown [145].

Nitric oxide (NO) is a diffusible gas, which at a basal concentration is dose-dependently capable of regulating numerous physiological functions (neuronal communication, vascular tone regulation etc.), and at higher concentrations can cause disease (cell death) through multiple mechanistic pathways [146]. The basal concentration of NO derives from endothelial nitric oxide synthase (eNOS) enzyme activity, whereas deleterious effects are due to the activity of neuronal and inducible NOS isoforms (nNOS and iNOS respectively) [146].

NO mediates its physiological effects at least in part by the activation of matrix metalloproteinases (MMPs) during matrix reorganization [146]. MMPs 2, 3, and 9 are the main forms found in the brain, and are the prime proteolytic enzymes particularly involved in the degradation of the ECM [147] and in neuron damage [148–150]. Activated MMPs also lead to BBB leakage by catalyzing hydrolysis of the TJ proteins, thereby inducing their degradation [150–152] (Figure 4). In addition, both MMP2 and MMP9 contain Cav-binding motifs and there is direct evidence that Cav-1 can down-regulate MMP activities and decrease BBB permeability in brain microvascular ECs during cerebral ischemia-reperfusion injury [153]. Moreover, Cav-1 protein regulates the activation of NOS by binding to all NOS isoforms that contain the Cav-binding motif, thereby inhibiting NO production (Figure 4) [153]. In a recent investigation, the Cav1/NO/MMP pathway has been suggested as a therapeutic target to ensure protection against cerebral ischemia-reperfusion injury (Figure 4) [141]. Given the importance of the links between ARs and Cav-1, as mentioned previously, it could be hypothesized that AR would be another important partner of the Cav1/NO/MMP pathway, ensuring BBB integrity.

Pericytes are located around the cerebral capillaries, and they share the basal lamina with ECs. Although a decrease in pericyte numbers and ultrastructural changes in aging and age-related brain diseases such AD, in both human and preclinical models [154], there is no data concerning the effects of sex steroid hormones on pericytes. Further investigations in this regard are clearly required.

- **Inter-endothelial tight junctions**

Cerebral ECs possess unique phenotypic properties due to the presence of continuous TJs, the lack of fenestration, minimal pinocytotic but more peptidase and mitochondrial activity [44,111]. Numerous biochemical components or pathophysiological states, can directly or indirectly disrupt the NVU at both the structural and functional level. Such disruption leads to an increase in leakage across the BBB [69].

Abnormal cerebrovascular TJs have been detected in a wide-range of diseases including ischemic stroke traumatic brain injury, AD and MS [44], as well as in physiological aging [13].

It is now well known that ovarian hormones influence the BBB integrity, with respect to limiting para-cellular permeability via the preservation of TJ proteins [69,155]. However, the contribution of testosterone to the BBB TJs response is poorly defined.

The first conclusive evidence that under physiological conditions, testosterone, impact TJ protein expression in males was obtained in our 2017 study. This showed that the castration of adult male mice significantly decreased the expression of TJ proteins (claudin-5 and ZO-1 but not occludin) in the hypothalamic POA [67], leading to changes in morphological arrangements and /or delocalization and disorganization of brain endothelial junction proteins. Additional results based on different molecular weight tracers, as a marker of BBB integrity, suggested that chronic depletion of testosterone induces defects in the BBB permeability in this region. The supplementation with testosterone after castration restores the BBB impermeability and TJs integrity [67]. In connection with this point, the specific roles of the neural ARs and their signaling pathways are being studied. These results can be compared to those obtained in mice with a selective deletion of ARs in Sertoli cells, which reveal that ARs in Sertoli cells are pivotal for timely and complete blood-testis barrier formation. The absence of functional ARs represents a potential cause for delayed and defective (leaky and incomplete) barrier formation [156]. Thus far, these results suggest that androgenic signaling supports the BBB integrity. However, this study does not shed light on whether, genomic or non-genomic steroid pathway, mediates androgen effects.

Testosterone and neuroinflammation: relationships with blood-brain barrier dysfunction

Inflammation and innate immunity are believed to contribute to the occurrence and severity of pathogenesis and neurodegenerative diseases. Furthermore, as already mentioned, microglial activation that is involved in neuroinflammation represents one of the most frequent clinical characteristics of these disorders. In addition, reports have already pointed out rapid and progressive alterations of the brain endothelium during such diseases. Interestingly, testosterone does not only regulate the development and function of the immune system, but also modulate neuroinflammatory responses. These should be neither underestimated nor neglected. In the context of neuroinflammation, further research has focused on the apparent roles of resident cerebral cells, including astrocytes and microglia.

This activation of microglia is directly associated with a dysfunction of the BBB [157,158]. Microglia express a cytoplasmic marker, the Iba-1 protein (ionized calcium binding adapter molecule 1), which is upregulated during microglial activation [159,160]. The degree of activation of microglia appears to be correlated with the type and severity of the brain disorder [161]. Thus, activation is part of their primary action as mediators of responses to pathogens and injury. They have either protective (housekeeping functions) or destructive (buffering action) impacts on the CNS, depending on their state and the duration of activation [162] . Microglia known as the source of pro-inflammatory cytokines (e.g. TNF-α, IL-1β, IL-2, IL-4, IL-6), prostaglandins, chemokines, enzymes associated with inflammation (e.g. iNOS and COX-2) as well as inflammatory cytokines production [163]. By releasing cytokines such as TNF-α activated microglia induces neurotoxic reactive astrocytes (termed “A1”) which might have harmful functions [164]. Numerous studies indicate that following CNS injury, reactive astrocytes upregulate both vimentin and glial fibrillary acidic protein (GFAP) [165,166]. Astrocyte-inducible NOS has been described to play a major role in human neuroinflammation [167–169].

Gliosis corresponds to the reactive change of glial cells (microglia and astrocytes) in response to damage [170,171]. This is conjugated with the modulation of local chemokines and cytokines, which govern inflammation (figure 4). Chronic gliosis may heighten the neurodegenerative process. Thus, neuroglia have been identified as a therapeutic target to halt inflammation in several neurodegenerative diseases [172].

The action of testosterone on reactive gliosis is not yet well-documented. However, available evidence indicates that administration of testosterone, but not DHT, down-regulated reactive gliosis after brain injury in male rats [110,173,174]. Recently, it has been shown that reactive astrocytes induce BBB disruption at least in part via the production of VEGF-A and thymidine phosphorylase (TYMP) in the case of inflammatory brain lesions [175].

Furthermore, we have shown that castration of male mice is associated with activation of neuroglial cells. This occurs via up-regulation of inflammatory proteins such as COX-2 and iNOS as well as pro-inflammatory cytokines such as TNF-α. Interestingly, supplementation of testosterone after castration suppresses these inflammatory features [67]. Data observed in a variety of cerebrovascular diseases and neurodegenerative disorders showed that the function of the BBB is compromised once a state of neuroglia activation is attained. This is accompanied by an increase in the expression and release of several pro-inflammatory molecules such as TNF-α and IL-1β [176]. In addition, it has been proposed that in turn, BBB dysfunction contributes to the progression of neurodegenerative diseases [14]. However, due to lack of a key pathway, the link between neuroinflammation and disruption of the BBB is still poorly understood, and it remains difficult to determine whether BBB breakdown is a cause or a consequence of the inflammatory status.

Testosterone and mitochondria-derived oxidative stress: relationships with blood-brain barrier dysfunction

The sensitivity of the BBB to oxidative stress is due to a high mitochondrial content (8 to 11% of the endothelial cytoplasmic volume) in cerebral endothelial cells, which is pivotal to maintain active transport [177]. Nevertheless, information concerning testosterone impact upon mitochondrial reactive oxygen species (ROS) generation in the BBB is still scarce.

The ROS and reactive nitrogen species (RNS) free radicals are derived from both endogenous and exogenous sources. They are well-recognized for dual roles, acting as both deleterious and beneficial species. The mitochondrial respiratory chain is an important endogenous source and target of ROS [178] (figure 4). It is noteworthy that the intrinsic apoptotic pathway is mediated by mitochondria through ROS production. Increased ROS production induces oxidative stress, which has been reported to be involved in several pathological conditions such as cardiovascular disease. Furthermore, ROS activates inflammation factors [179]. Interestingly, the mitochondria impairment disrupts EC TJs and increases BBB permeability, which is accompanied by deterioration as an outcome of neurodegenerative disorders (e.g. stroke, PD and AD) [180]. In addition, oxidative stress induces inflammation, which can further increase oxidative stress activity (Figure 4) [181]. Low testosterone levels [182,183], aging [184], and testosterone-deprivation [185] are risk factors for oxidative stress. It was shown that there is an inverse association between testosterone concentrations and oxidative stress markers in adult men [186]. In the CNS, there is evidence to indicate that testosterone improves mitochondrial membrane potential, and reduces nuclear fragmentation and ROS generation in a human astrocyte cell model, acting via positive regulation of neuroglobin. The latter represents a key factor involved in cellular oxygen homeostasis [187]. To date, the

involvement of testosterone in oxidative stress at the level of the NVU is still not well-established, and needs further investigation. Effects of testosterone upon the oxidative status are still controversial, ranging from ROS generation to scavenging or chain-breaking of ROS and RNS [188–191]. Numerous studies have indicated that the effect of testosterone in this regard is tissue, sex and dose dependent [192]. In prostate cancer cell lines, androgen signaling regulates the cytoplasmic dynamin-related protein, a key protein of the mitochondrial fragmentation machinery, that leads to cell death but not to cell division [193]. Moreover it was shown that, in these cell lines, testosterone causes the ROS and H_2O_2-induced apoptosis [194]. Like cerebral endothelial cells, gastric parietal cells contain many mitochondria. Data based on gonadectomized male rats, following testosterone supplementation, suggested that testosterone could mediate mitochondria-associated apoptotic signaling in the gastric mucosa by reducing ROS generation and by impairing apoptosis processes [195]. However it has been reported that androgens induce ROS generation through AR signaling in cultured vascular smooth muscle [196] and renal interlobar arteries [197].

Conclusion

The aim of this review was to present an overview of the data of the literature concerning the effects of testosterone on the NVU in males. It mainly emerges from these studies that i) testosterone promotes cerebral angiogenesis and vasculature formation, ii) testosterone supports the integrity of BBB TJs and, iii) regulates some endothelial membrane transporters although very little data is available yet. The effects of testosterone are mediated by either androgen or estrogen receptors and the mechanisms by which it regulates these features are usually not described. Nevertheless, ARs and their interaction with the Cav-1 protein would be involved in the BBB integrity by participation in Cav-1/NO/MMPs pathway.

A dysfunction of the BBB is often associated with an inflammatory status inducing a neurodegenerative process. But the link between neuroinflammation and disruption of the BBB is still poorly understood. Data suggest that testosterone could prevent from gliosis reaction and up-regulation of inflammatory proteins, and could participate in reducing ROS generation and limiting oxidative stress.

To conclude, data from literature show an undeniable role of testosterone on brain capillaries guaranteeing the cerebral homeostasis in males but they reflect complex actions of hormones which appear to be brain region,

state of health and/or dose-dependent. The cellular and molecular mechanisms underlying the maintenance of the BBB integrity and the associated signaling pathways remain to be analyzed in greater details.

Understanding the role of testosterone and testosterone signaling in the functions of cerebral blood vessels and the BBB could lead to the exploration of the therapeutic potential of testosterone in several CNS disorders. Indeed, this could answer partly, the enigma of gender differences and in age-related emergence and progression of some CNS pathologies or dysfunctions.

It is also important to continue to explore this area of research, as this will lead to a better understanding of the action of environmental contaminants such as endocrine disruptors that interfere with normal hormonal balance and/or pathway and thus exert potentially deleterious effects on health. Considering the abundance of environmental contaminants that have anti-androgenic and/or anti-estrogenic properties, the consideration should be given to the effects of these components on brain micro-vessels.

Acknowledgments

We thank Dr. Sakina Mhaouty-Kodja (Neuroscience Paris-Seine, Sorbonne University, Paris, France) for the attentive reading and her pertinent advice.

Declaration of conflicting interests: The authors declare no competing interests with respect to the research, authorship, and/or publication of this article.

Author contributions: D.A. wrote the manuscript with significant input from V.G.M.

Figure Legends

Figure 1. The major cellular elements of blood-brain barrier localized at central nervous system micro-vessels are comprised of a continuous layer of endothelial cells, basement membrane cells, pericytes, microglial cells, neurons and astrocyte end-feet. BCRP, breast cancer resistance protein; GLUT-1, glucose transporter 1; JAMs,

junction adhesion molecules; OAT-3, organic anion transporter 3; P-gp, P-glycoprotein; ZOs, zonula occludens proteins.

Figure 2:-Overview of a whole male brain with a close-up view of the position of hypothalamic nuclei pointing out the relative location and density of androgen receptor (blue circle) and estrogen receptor (ERα, green square; ERβ, pink diamond) mRNA distribution using in situ hybridization analysis in male rat [79–81]. AC, anterior commissure; AHN, anterior hypothalamic nucleus; AMe, medial amygdala; ARC, arcuate nucleus; BNST, bed nucleus of stria terminalis; DHN, dorsomedial hypothalamic nucleus; Hip, hippocampus; Hyp, hypothalamus; L, lateral; LN, lateral nucleus; LPN, lateral preoptic nucleus; M, medial; MPN, median preoptic nucleus; OB, olfactory bulb; OC, optic chiasma; PN, periventricular nucleus; POA, preoptic area; PPN, periventricular preoptic nucleus; PVN, paraventricular hypothalamic nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; TH, thalamus; TMN, tuberomammillary nucleus; VMN, ventromedial nucleus.

Figure 3: A: Developmental timeline of the blood-brain barrier set up in the murine central nervous system [111]. B: pattern of circulating level of testosterone in the male mouse from birth to adulthood [113]. E: embryonic days.

Figure 4: State of the art schematic representation of testosterone signaling-induced cellular and molecular interactions in the blood-brain barrier system, highlighting in particular the Cav-1/NO/MMPs pathway involvement. Testosterone controls the integrity of the BBB by preserving the intercellular molecular binding proteins degradation, by regulating endothelial transporters expression or activity, and by decreasing inflammatory response. Cav-1 protein is known as an ARs co-activator. Cav-1 protein is expressed by brain endothelial cells and pericytes, while reactive astrocytes express both Cav-1 and Cav-3. Cav-1 is responsible for 1) internalization and recovery of TJ proteins during a brain insult 2) inhibition of NO production and consequently inhibition of MMPs activity, thereby preventing TJ protein hydroxylation, ECM degradation and neuronal loss.

Briefly, the CNS response to insult and injury is manifested by gliosis. The inflammatory responses are directly associated with a neuronal loss, degradation of TJ proteins, decrease of the number of GLUT-1 and decrease of the activity of P-gp and BCRP transporters. Testosterone, in turn, decreases inflammatory mediators. In addition, ARs regulate positively the expression and uptake rate of OAT3. Oxidative stress caused by an over-production

of ROS, activates inflammation factors. The cross-talk between oxidative stress and neuro inflammation causes BBB leakage. The involvement of testosterone in oxidative stress is not well-known.

ARs, androgen receptors; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; Cav, caveolin; ECM, extracellular matrix; GLUT-1, glucose transporter 1; IFNγ, interferon gamma; IL-1β, interleukin 1 beta; MMPs, matrix metalloproteinases; NO, nitric oxide; NOS, nitric oxide synthase; OAT-3, organic anion transporter 3; P-gp, P-glycoprotein; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha; ZOs, zonula occludens proteins.

References

1. Larson TA. Sex Steroids, Adult Neurogenesis, and Inflammation in CNS Homeostasis, Degeneration, and Repair. *Front Endocrinol* 2018; 9: 205.

2. Mhaouty-Kodja S. Role of the androgen receptor in the central nervous system. *Mol Cell Endocrinol* 2018; 465: 103–112.

3. Picot M, Naulé L, Marie-Luce C, et al. Vulnerability of the neural circuitry underlying sexual behavior to chronic adult exposure to oral bisphenol a in male mice. *Endocrinology* 2014; 155: 502–512.

4. Krause DN, Duckles SP, Gonzales RJ. Local oestrogenic/androgenic balance in the cerebral vasculature. *Acta Physiol* 2011; 203: 181–186.

5. Gonzales RJ, Ansar S, Duckles SP, et al. Androgenic/estrogenic balance in the male rat cerebral circulation: metabolic enzymes and sex steroid receptors. *J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab* 2007; 27: 1841–1852.

6. Liu M, Kelley MH, Herson PS, et al. Neuroprotection of Sex Steroids. *Minerva Endocrinol* 2010; 35: 127–143.

7. Gold SM, Chalifoux S, Giesser BS, et al. Immune modulation and increased neurotrophic factor production in multiple sclerosis patients treated with testosterone. *J Neuroinflammation* 2008; 5: 32.

8. Spence RD, Voskuhl RR. Neuroprotective effects of estrogens and androgens in CNS inflammation and neurodegeneration. *Front Neuroendocrinol* 2012; 33: 105–115.

9. Voskuhl R. Sex differences in autoimmune diseases. *Biol Sex Differ* 2011; 2: 1.

10. Herndon JM, Tome ME, Davis TP. Chapter 9 - Development and Maintenance of the Blood–Brain Barrier. In: Caplan LR, Biller J, Leary MC, et al. (eds) *Primer on Cerebrovascular Diseases (Second Edition)*. San Diego: Academic Press, pp. 51–56.

11. Abbott NJ, Patabendige AAK, Dolman DEM, et al. Structure and function of the blood–brain barrier. *Neurobiol Dis* 2010; 37: 13–25.

12. Archer DP, Ravussin PA. [Role of blood-brain barrier in cerebral homeostasis]. *Ann Fr Anesth Reanim* 1994; 13: 57–61.

13. Erdő F, Denes L, de Lange E. Age-associated physiological and pathological changes at the blood–brain barrier: A review. *J Cereb Blood Flow Metab* 2017; 37: 4–24.

14. Weiss N, Miller F, Cazaubon S, et al. The blood-brain barrier in brain homeostasis and neurological diseases. *Biochim Biophys Acta* 2009; 1788: 842–857.

15. Blanchette M, Daneman R. Formation and maintenance of the BBB. *Mech Dev* 2015; 138: 8–16.

16. Keaney J, Campbell M. The dynamic blood-brain barrier. *FEBS J* 2015; 282: 4067–4079.

17. Reinhold AK, Rittner HL. Barrier function in the peripheral and central nervous system-a review. *Pflugers Arch* 2017; 469: 123–134.

18. Greene C, Hanley N, Campbell M. Claudin-5: gatekeeper of neurological function. *Fluids Barriers CNS*; 16. Epub ahead of print 29 January 2019. DOI: 10.1186/s12987-019-0123-z.

19. Dias MC, Coisne C, Lazarevic I, et al. Claudin-3-deficient C57BL/6J mice display intact brain barriers. *Sci Rep* 2019; 9: 1–16.

20. Jia W, Jia W, Lu R, et al. The role of claudin-5 in blood-brain barrier (BBB) and brain metastases (Review). *Mol Med Rep* 2014; 9: 779–785.

21. Yang Y, Rosenberg GA. MMP-Mediated Disruption of Claudin-5 in the Blood–Brain Barrier of Rat Brain After Cerebral Ischemia. *Methods Mol Biol Clifton NJ* 2011; 762: 333–345.

22. Kook S-Y, Seok Hong H, Moon M, et al. Disruption of blood-brain barrier in Alzheimer disease pathogenesis. *Tissue Barriers*; 1. Epub ahead of print 1 April 2013. DOI: 10.4161/tisb.23993.

23. Chow BW, Gu C. The Molecular Constituents of the Blood–Brain Barrier. *Trends Neurosci* 2015; 38: 598–608.

24. Tietz S, Engelhardt B. Brain barriers: Crosstalk between complex tight junctions and adherens junctions. *J Cell Biol* 2015; 209: 493–506.

25. Ballabh P, Braun A, Nedergaard M. The blood–brain barrier: an overview: Structure, regulation, and clinical implications. *Neurobiol Dis* 2004; 16: 1–13.

26. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. *Nat Med* 2013; 19: 1584–1596.

27. Prevot V, Dehouck B, Sharif A, et al. The Versatile Tanycyte: A Hypothalamic Integrator of Reproduction and Energy Metabolism. *Endocr Rev* 2018; 39: 333–368.

28. Azzi S, Hebda JK, Gavard J. Vascular Permeability and Drug Delivery in Cancers. *Front Oncol*; 3. Epub ahead of print 2013. DOI: 10.3389/fonc.2013.00211.

29. Girardin F. Membrane transporter proteins: a challenge for CNS drug development. *Dialogues Clin Neurosci* 2006; 8: 311–321.

30. Liu L, Liu X. Contributions of Drug Transporters to Blood-Brain Barriers. In: Liu X, Pan G (eds) *Drug Transporters in Drug Disposition, Effects and Toxicity*. Singapore: Springer Singapore, pp. 407–466.

31. Choudhuri S, Klaassen CD. Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. *Int J Toxicol* 2006; 25: 231–259.

32. Löscher W, Potschka H. Blood-Brain Barrier Active Efflux Transporters: ATP-Binding Cassette Gene Family. *NeuroRx* 2005; 2: 86–98.

33. De Bock M, Van Haver V, Vandenbroucke RE, et al. Into rather unexplored terrain-transcellular transport across the blood-brain barrier. *Glia* 2016; 64: 1097–1123.

34. Deng J, Huang Q, Wang F, et al. The Role of Caveolin-1 in Blood–Brain Barrier Disruption Induced by Focused Ultrasound Combined with Microbubbles. *J Mol Neurosci* 2011; 46: 677–687.

35. Ikezu T, Ueda H, Trapp BD, et al. Affinity-purification and characterization of caveolins from the brain: Differential expression of caveolin-1, -2, and -3 in brain endothelial and astroglial cell types. *Brain Res* 1998; 804: 177–192.

36. von Tell D, Armulik A, Betsholtz C. Pericytes and vascular stability. *Exp Cell Res* 2006; 312: 623–629.

37. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res* 2005; 97: 512–523.

38. Sweeney MD, Ayyadurai S, Zlokovic BV. Pericytes of the neurovascular unit: Key functions and signaling pathways. *Nat Neurosci* 2016; 19: 771–783.

39. Peppiatt CM, Howarth C, Mobbs P, et al. Bidirectional control of CNS capillary diameter by pericytes. *Nature* 2006; 443: 700–704.

40. Armulik A, Genové G, Betsholtz C. Pericytes: Developmental, Physiological, and Pathological Perspectives, Problems, and Promises. *Dev Cell* 2011; 21: 193–215.

41. Daneman R, Zhou L, Kebede AA, et al. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* 2010; 468: 562–566.

42. Su X, Huang L, Qu Y, et al. Pericytes in Cerebrovascular Diseases: An Emerging Therapeutic Target. *Front Cell Neurosci* 2019; 13: 519.

43. Engelhardt B, Sorokin L. The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction. *Semin Immunopathol* 2009; 31: 497–511.

44. Hawkins BT. The Blood-Brain Barrier/Neurovascular Unit in Health and Disease. *Pharmacol Rev* 2005; 57: 173–185.

45. Wang CX, Shuaib A. Critical role of microvasculature basal lamina in ischemic brain injury. *Prog Neurobiol* 2007; 83: 140–148.

46. Wolf SA, Boddeke HWGM, Kettenmann H. Microglia in Physiology and Disease. *Annu Rev Physiol* 2017; 79: 619–643.

47. Alekseeva OS, Kirik OV, Gilerovich EG, et al. Microglia of the Brain: Origin, Structure, Functions. *J Evol Biochem Physiol* 2019; 55: 257–268.

48. Davis EJ, Foster TD, Thomas WE. Cellular forms and functions of brain microglia. *Brain Res Bull* 1994; 34: 73–78.

49. Johann S, Beyer C. Neuroprotection by gonadal steroid hormones in acute brain damage requires cooperation with astroglia and microglia. *J Steroid Biochem Mol Biol* 2013; 137: 71–81.

50. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol (Berl)* 2010; 119: 7–35.

51. Cabezas R, Ávila M, Gonzalez J, et al. Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease. *Front Cell Neurosci*; 8. Epub ahead of print 4 August 2014. DOI: 10.3389/fncel.2014.00211.

52. Wong AD, Ye M, Levy AF, et al. The blood-brain barrier: an engineering perspective. *Front Neuroengineering*; 6. Epub ahead of print 2013. DOI: 10.3389/fneng.2013.00007.

53. DeBault LE, Cancilla PA. gamma-Glutamyl transpeptidase in isolated brain endothelial cells: induction by glial cells in vitro. *Science* 1980; 207: 653–655.

54. Zhao Z, Nelson AR, Betsholtz C, et al. Establishment and Dysfunction of the Blood-Brain Barrier. *Cell* 2015; 163: 1064–1078.

55. Prasad S, Sajja RK, Naik P, et al. Diabetes Mellitus and Blood-Brain Barrier Dysfunction: An Overview. *J Pharmacovigil* 2014; 2: 125.

56. Liu R, Pan M-X, Tang J-C, et al. Role of neuroinflammation in ischemic stroke. *Neuroimmunol Neuroinflammation* 2017; 4: 158.

57. Gonzales RJ. Androgens and the cerebrovasculature: modulation of vascular function during normal and pathophysiological conditions. *Pflugers Arch* 2013; 465: 627–642.

58. Vodo S, Bechi N, Petroni A, et al. Testosterone-Induced Effects on Lipids and Inflammation. *Mediators of Inflammation*. Epub ahead of print 2013. DOI: 10.1155/2013/183041.

59. Feldman HA, Longcope C, Derby CA, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *J Clin Endocrinol Metab* 2002; 87: 589–598.

60. Thibaut F. The role of sex and gender in neuropsychiatric disorders. *Dialogues Clin Neurosci* 2016; 18: 351–352.

61. Lynch AM, Murphy KJ, Deighan BF, et al. The Impact of Glial Activation in the Aging Brain. *Aging Dis* 2010; 1: 262–278.

62. Lecce L, Lam YT, Lindsay LA, et al. Aging Impairs VEGF-Mediated, Androgen-Dependent Regulation of Angiogenesis. *Mol Endocrinol* 2014; 28: 1487–1501.

63. Sandoval KE, Witt KA. Age and 17β-estradiol effects on blood-brain barrier tight junction and estrogen receptor proteins in ovariectomized rats. *Microvasc Res* 2011; 81: 198–205.

64. Mahmoud R, Wainwright SR, Galea LAM. Sex hormones and adult hippocampal neurogenesis: Regulation, implications, and potential mechanisms. *Front Neuroendocrinol* 2016; 41: 129–152.

65. Ngun TC, Ghahramani N, Sánchez FJ, et al. The genetics of sex differences in brain and behavior. *Front Neuroendocrinol* 2011; 32: 227–246.

66. Robison LS, Gannon OJ, Salinero AE, et al. Contributions of sex to cerebrovascular function and pathology. *Brain Res* 2019; 1710: 43–60.

67. Atallah A, Mhaouty-Kodja S, Grange-Messent V. Chronic depletion of gonadal testosterone leads to blood–brain barrier dysfunction and inflammation in male mice. *J Cereb Blood Flow Metab* 2017; 37: 3161–3175.

68. Bielecki B, Mattern C, Ghoumari AM, et al. Unexpected central role of the androgen receptor in the spontaneous regeneration of myelin. *Proc Natl Acad Sci* 2016; 113: 14829–14834.

69. Witt KA, Sandoval KE. Steroids and the Blood–Brain Barrier. In: *Advances in Pharmacology*. Elsevier, pp. 361–390.

70. Honda H, Unemoto T, Kogo H. Different mechanisms for testosterone-induced relaxation of aorta between normotensive and spontaneously hypertensive rats. *Hypertens Dallas Tex 1979* 1999; 34: 1232–1236.

71. Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. *Am J Physiol Regul Integr Comp Physiol* 2004; 286: R233-249.

72. Ceballos G, Figueroa L, Rubio I, et al. Acute and Nongenomic Effects of Testosterone on Isolated and Perfused Rat Heart. *J Cardiovasc Pharmacol* 1999; 33: 691.

73. Masuda A, Mathur R, Halushka PV. Testosterone increases thromboxane A2 receptors in cultured rat aortic smooth muscle cells. *Circ Res* 1991; 69: 638–643.

74. Costarella CE, Stallone JN, Rutecki GW, et al. Testosterone causes direct relaxation of rat thoracic aorta. *J Pharmacol Exp Ther* 1996; 277: 34–39.

75. Yue P, Chatterjee K, Beale C, et al. Testosterone relaxes rabbit coronary arteries and aorta. *Circulation* 1995; 91: 1154–1160.

76. Lorigo M, Mariana M, Lemos MC, et al. Vascular mechanisms of testosterone: The non-genomic point of view. *J Steroid Biochem Mol Biol* 2020; 196: 105496.

77. Bennett NC, Gardiner RA, Hooper JD, et al. Molecular cell biology of androgen receptor signalling. *Int J Biochem Cell Biol* 2010; 42: 813–827.

78. Melcangi RC, Poletti A, Cavarretta I, et al. The 5α-reductase in the central nervous system: expression and modes of control11Proceedings of the 13th International Symposium of the Journal of Steroid Biochemistry & Molecular Biology “Recent Advances in Steroid Biochemistry & Molecular Biology” Monaco 25–28 May 1997. *J Steroid Biochem Mol Biol* 1998; 65: 295–299.

79. Patchev VK, Schroeder J, Goetz F, et al. Neurotropic action of androgens: principles, mechanisms and novel targets. *Exp Gerontol* 2004; 39: 1651–1660.

80. Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor-α and -β mRNA in the rat central nervous system. *J Comp Neurol* 1997; 388: 507–525.

81. Simerly RB, Chang C, Muramatsu M, et al. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *J Comp Neurol* 1990; 294: 76–95.

82. Lauber AH, Mobbs CV, Muramatsu M, et al. Estrogen receptor messenger RNA expression in rat hypothalamus as a function of genetic sex and estrogen dose. *Endocrinology* 1991; 129: 3180–3186.

83. Ohtsuki S, Tomi M, Hata T, et al. Dominant expression of androgen receptors and their functional regulation of organic anion transporter 3 in rat brain capillary endothelial cells; comparison of gene expression between the blood-brain and -retinal barriers. *J Cell Physiol* 2005; 204: 896–900.

84. MacLusky NJ, Hajszan T, Prange-Kiel J, et al. Androgen modulation of hippocampal synaptic plasticity. *Neuroscience* 2006; 138: 957–965.

85. Hussain R, Ghoumari AM, Bielecki B, et al. The neural androgen receptor: a therapeutic target for myelin repair in chronic demyelination. *Brain* 2013; 136: 132–146.

86. Forner S, Baglietto-Vargas D, Martini AC, et al. Synaptic Impairment in Alzheimer's Disease: A Dysregulated Symphony. *Trends Neurosci* 2017; 40: 347–357.

87. Frye CA, Edinger KL, Seliga AM, et al. 5alpha-reduced androgens may have actions in the hippocampus to enhance cognitive performance of male rats. *Psychoneuroendocrinology* 2004; 29: 1019–1027.

88. Jia J, Cui C, Yan X, et al. Effects of testosterone on synaptic plasticity mediated by androgen receptors in male SAMP8 mice. *J Toxicol Environ Health A* 2016; 79: 849–855.

89. Brown TJ, Sharma M, Heisler LE, et al. In vitro labeling of gonadal steroid hormone receptors in brain tissue sections. *Steroids* 1995; 60: 726–737.

90. Tabori NE, Stewart LS, Znamensky V, et al. Ultrastructural evidence that androgen receptors are located at extranuclear sites in the rat hippocampal formation. *Neuroscience* 2005; 130: 151–163.

91. Leranth C, Petnehazy O, MacLusky NJ. Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *J Neurosci Off J Soc Neurosci* 2003; 23: 1588–1592.

92. Hatanaka Y, Hojo Y, Mukai H, et al. Rapid increase of spines by dihydrotestosterone and testosterone in hippocampal neurons: Dependence on synaptic androgen receptor and kinase networks. *Brain Res* 2015; 1621: 121–132.

93. Xiao L, Jordan CL. Sex Differences, Laterality, and Hormonal Regulation of Androgen Receptor Immunoreactivity in Rat Hippocampus. *Horm Behav* 2002; 42: 327–336.

94. Cohen LS, Soares CN, Vitonis AF, et al. Risk for New Onset of Depression During the Menopausal Transition: The Harvard Study of Moods and Cycles. *Arch Gen Psychiatry* 2006; 63: 385.

95. Galea L a. M, Wainwright SR, Roes MM, et al. Sex, hormones and neurogenesis in the hippocampus: hormonal modulation of neurogenesis and potential functional implications. *J Neuroendocrinol* 2013; 25: 1039–1061.

96. McEwen BS. Stress and hippocampal plasticity. *Annu Rev Neurosci* 1999; 22: 105–122.

97. Salvador E, Shityakov S, Förster C. Glucocorticoids and endothelial cell barrier function. *Cell Tissue Res* 2014; 355: 597–605.

98. Handa RJ, Nunley KM, Lorens SA, et al. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. *Physiol Behav* 1994; 55: 117–124.

99. Quillinan N, Deng G, Grewal H, et al. Androgens and stroke: good, bad or indifferent? *Exp Neurol* 2014; 259: 10–15.

100. Cheng J, Alkayed NJ, Hurn PD. Deleterious effects of dihydrotestosterone on cerebral ischemic injury. *J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab* 2007; 27: 1553–1562.

101. Nakano T, Hurn PD, Herson PS, et al. Testosterone exacerbates neuronal damage following cardiac arrest and cardiopulmonary resuscitation in mouse. *Brain Res* 2010; 1357: 124–130.

102. Cheng J, Uchida M, Zhang W, et al. Role of salt-induced kinase 1 in androgen neuroprotection against cerebral ischemia. *J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab* 2011; 31: 339–350.

103. Ishihara Y, Fujitani N, Sakurai H, et al. Effects of sex steroid hormones and their metabolites on neuronal injury caused by oxygen-glucose deprivation/reoxygenation in organotypic hippocampal slice cultures. *Steroids* 2016; 113: 71–77.

104. Stephenson J, Nutma E, van der Valk P, et al. Inflammation in CNS neurodegenerative diseases. *Immunology* 2018; 154: 204–219.

105. Daneman R. The blood-brain barrier in health and disease. *Ann Neurol* 2012; 72: 648–672.

106. Hampton DW, Serio A, Pryce G, et al. Neurodegeneration progresses despite complete elimination of clinical relapses in a mouse model of multiple sclerosis. *Acta Neuropathol Commun* 2013; 1: 84.

107. Liu W, Tang Y, Feng J. Cross talk between activation of microglia and astrocytes in pathological conditions in the central nervous system. *Life Sci* 2011; 89: 141–146.

108. Malkin CJ, Pugh PJ, Jones RD, et al. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J Clin Endocrinol Metab* 2004; 89: 3313–3318.

109. Norata GD, Tibolla G, Seccomandi PM, et al. Dihydrotestosterone decreases tumor necrosis factor-alpha and lipopolysaccharide-induced inflammatory response in human endothelial cells. *J Clin Endocrinol Metab* 2006; 91: 546–554.

110. Barreto G, Veiga S, Azcoitia I, et al. Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihydrotestosterone. *Eur J Neurosci* 2007; 25: 3039–3046.

111. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. *Nat Med* 2013; 19: 1584–1596.

112. Campelo AE, Cutini PH, Massheimer VL. Testosterone modulates platelet aggregation and endothelial cell growth through nitric oxide pathway. *J Endocrinol* 2012; 213: 77–87.

113. Lenz KM, McCarthy MM. Organized for sex – steroid hormones and the developing hypothalamus. *Eur J Neurosci* 2010; 32: 2096–2104.

114. Sieveking DP, Chow RW, Ng MK. Androgens, angiogenesis and cardiovascular regeneration: *Curr Opin Endocrinol Diabetes Obes* 2010; 17: 277–283.

115. Liao W, Huang W, Guo Y, et al. Testosterone promotes vascular endothelial cell migration via upregulation of ROCK-2/moesin cascade. *Mol Biol Rep* 2013; 40: 6729–6735.

116. Liu P, Li X, Song F, et al. Testosterone promotes tube formation of endothelial cells isolated from veins via activation of Smad1 protein. *Mol Cell Endocrinol* 2017; 446: 21–31.

117. Sun Y, Jin K, Xie L, et al. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J Clin Invest* 2003; 111: 1843–1851.

118. Chen Z, Ye R, Goldman SA. Testosterone modulation of angiogenesis and neurogenesis in the adult songbird brain. *Neuroscience* 2013; 239: 139–148.

119. Dittrich F, Ramenda C, Grillitsch D, et al. Regulatory mechanisms of testosterone-stimulated song in the sensorimotor nucleus HVC of female songbirds. *BMC Neurosci*; 15. Epub ahead of print 2 December 2014. DOI: 10.1186/s12868-014-0128-0.

120. Hall ZJ, MacDougall-Shackleton SA. Influence of Testosterone Metabolites on Song-Control System Neuroplasticity during Photostimulation in Adult European Starlings (Sturnus vulgaris). *PLOS ONE* 2012; 7: e40060.

121. Ohtsuki S, Asaba H, Takanaga H, et al. Role of blood–brain barrier organic anion transporter 3 (OAT3) in the efflux of indoxyl sulfate, a uremic toxin: its involvement in neurotransmitter metabolite clearance from the brain. *J Neurochem* 2002; 83: 57–66.

122. Kobayashi Y, Hirokawa N, Ohshiro N, et al. Differential gene expression of organic anion transporters in male and female rats. *Biochem Biophys Res Commun* 2002; 290: 482–487.

123. Giuliano F, Allard J. Dopamine and male sexual function. *Eur Urol* 2001; 40: 601–608.

124. Patching SG. Glucose Transporters at the Blood-Brain Barrier: Function, Regulation and Gateways for Drug Delivery. *Mol Neurobiol* 2017; 54: 1046–1077.

125. Banks WA. Brain Meets Body: The Blood-Brain Barrier as an Endocrine Interface. *Endocrinology* 2012; 153: 4111–4119.

126. Videbech P. PET measurements of brain glucose metabolism and blood flow in major depressive disorder: a critical review. *Acta Psychiatr Scand* 2000; 101: 11–20.

127. Desai BS, Monahan AJ, Carvey PM, et al. Blood-brain barrier pathology in Alzheimer's and Parkinson's disease: implications for drug therapy. *Cell Transplant* 2007; 16: 285–299.

128. Patching SG. Glucose Transporters at the Blood-Brain Barrier: Function, Regulation and Gateways for Drug Delivery. *Mol Neurobiol* 2017; 54: 1046–1077.

129. Shi J, Simpkins JW. 17 beta-Estradiol modulation of glucose transporter 1 expression in blood-brain barrier. *Am J Physiol* 1997; 272: E1016-1022.

130. Pan W, Stone KP, Hsuchou H, et al. Cytokine Signaling Modulates Blood-Brain Barrier Function. *Curr Pharm Des* 2011; 17: 3729–3740.

131. Salkeni MA, Lynch JL, Otamis-Price T, et al. Lipopolysaccharide impairs blood-brain barrier P-glycoprotein function in mice through prostaglandin- and nitric oxide-independent pathways. *J Neuroimmune Pharmacol Off J Soc NeuroImmune Pharmacol* 2009; 4: 276–282.

132. Miller DS, Bauer B, Hartz AMS. Modulation of P-Glycoprotein at the Blood-Brain Barrier: Opportunities to Improve Central Nervous System Pharmacotherapy. *Pharmacol Rev* 2008; 60: 196–209.

133. Poller B, Drewe J, Krähenbühl S, et al. Regulation of BCRP (ABCG2) and P-Glycoprotein (ABCB1) by Cytokines in a Model of the Human Blood–Brain Barrier. *Cell Mol Neurobiol* 2010; 30: 63–70.

134. Virgintino D, Robertson D, Errede M, et al. Expression of caveolin-1 in human brain microvessels. *Neuroscience* 2002; 115: 145–152.

135. Badaut J, Ajao DO, Sorensen DW, et al. Caveolin expression changes in the neurovascular unit after juvenile traumatic brain injury: signs of blood-brain barrier healing? *Neuroscience* 2015; 285: 215–226.

136. Jasmin J-F, Malhotra S, Singh Dhallu M, et al. Caveolin-1 deficiency increases cerebral ischemic injury. *Circ Res* 2007; 100: 721–729.

137. Jasmin J-F, Malhotra S, Singh Dhallu M, et al. Caveolin-1 deficiency increases cerebral ischemic injury. *Circ Res* 2007; 100: 721–729.

138. Huang Q, Zhong W, Hu Z, et al. A review of the role of cav-1 in neuropathology and neural recovery after ischemic stroke. *J Neuroinflammation*; 15. Epub ahead of print 20 December 2018. DOI: 10.1186/s12974-018-1387-y.

139. Nag S, Venugopalan R, Stewart DJ. Increased caveolin-1 expression precedes decreased expression of occludin and claudin-5 during blood-brain barrier breakdown. *Acta Neuropathol (Berl)* 2007; 114: 459–469.

140. Stamatovic SM, Keep RF, Wang MM, et al. Caveolae-mediated internalization of occludin and claudin-5 during CCL2-induced tight junction remodeling in brain endothelial cells. *J Biol Chem* 2009; 284: 19053–19066.

141. Chen H, Chen X, Li W, et al. Targeting RNS/caveolin-1/MMP signaling cascades to protect against cerebral ischemia-reperfusion injuries: potential application for drug discovery. *Acta Pharmacol Sin* 2018; 39: 669–682.

142. Bennett N, Hooper JD, Lee CS, et al. Androgen receptor and caveolin-1 in prostate cancer. *IUBMB Life* 2009; 61: 961–970.

143. Lu ML, Schneider MC, Zheng Y, et al. Caveolin-1 Interacts with Androgen Receptor: A POSITIVE MODULATOR OF ANDROGEN RECEPTOR MEDIATED TRANSACTIVATION. *J Biol Chem* 2001; 276: 13442–13451.

144. Baeten KM, Akassoglou K. Extracellular matrix and matrix receptors in blood-brain barrier formation and stroke. *Dev Neurobiol* 2011; 71: 1018–1039.

145. Fujimura M, Gasche Y, Morita-Fujimura Y, et al. Early appearance of activated matrix metalloproteinase-9 and blood–brain barrier disruption in mice after focal cerebral ischemia and reperfusion. *Brain Res* 1999; 842: 92–100.

146. Ridnour LA, Windhausen AN, Isenberg JS, et al. Nitric oxide regulates matrix metalloproteinase-9 activity by guanylyl-cyclase-dependent and -independent pathways. *Proc Natl Acad Sci* 2007; 104: 16898–16903.

147. pubmeddev, al S-AY et. Matrix metalloproteinase (MMP) system in brain: identification and characterization of brain-specific MMP highly expressed in cerebellum. - PubMed - NCBI, https://www.ncbi.nlm.nih.gov/pubmed/11264666 (accessed 6 September 2019).

148. Lakhan SE, Kirchgessner A, Tepper D, et al. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Front Neurol* 2013; 4: 32.

149. Candelario-Jalil Eduardo, Thompson Jeffrey, Taheri Saeid, et al. Matrix Metalloproteinases Are Associated With Increased Blood–Brain Barrier Opening in Vascular Cognitive Impairment. *Stroke* 2011; 42: 1345–1350.

150. Rempe RG, Hartz AM, Bauer B. Matrix metalloproteinases in the brain and blood–brain barrier: Versatile breakers and makers. *J Cereb Blood Flow Metab* 2016; 36: 1481–1507.

151. Yang F, Zhao K, Zhang X, et al. ATP Induces Disruption of Tight Junction Proteins via IL-1 Beta-Dependent MMP-9 Activation of Human Blood-Brain Barrier In Vitro. *Neural Plast* 2016; 2016: 8928530.

152. Dhanda S, Sandhir R. Blood-Brain Barrier Permeability Is Exacerbated in Experimental Model of Hepatic Encephalopathy via MMP-9 Activation and Downregulation of Tight Junction Proteins. *Mol Neurobiol* 2018; 55: 3642–3659.

153. Gu Y, Zheng G, Xu M, et al. Caveolin-1 regulates nitric oxide-mediated matrix metalloproteinases activity and blood-brain barrier permeability in focal cerebral ischemia and reperfusion injury. *J Neurochem* 2012; 120: 147–156.

154. Berthiaume A-A, Hartmann DA, Majesky MW, et al. Pericyte Structural Remodeling in Cerebrovascular Health and Homeostasis. *Front Aging Neurosci*; 10. Epub ahead of print 17 July 2018. DOI: 10.3389/fnagi.2018.00210.

155. Krause DN, Duckles SP, Pelligrino DA. Influence of sex steroid hormones on cerebrovascular function. *J Appl Physiol Bethesda Md 1985* 2006; 101: 1252–1261.

156. Willems A, Batlouni SR, Esnal A, et al. Selective Ablation of the Androgen Receptor in Mouse Sertoli Cells Affects Sertoli Cell Maturation, Barrier Formation and Cytoskeletal Development. *PLOS ONE* 2010; 5: e14168.

157. Sumi N, Nishioku T, Takata F, et al. Lipopolysaccharide-activated microglia induce dysfunction of the blood-brain barrier in rat microvascular endothelial cells co-cultured with microglia. *Cell Mol Neurobiol* 2010; 30: 247–253.

158. Ronaldson PT, Davis TP. Blood-brain barrier integrity and glial support: mechanisms that can be targeted for novel therapeutic approaches in stroke. *Curr Pharm Des* 2012; 18: 3624–3644.

159. Sasaki Y, Ohsawa K, Kanazawa H, et al. Iba1 Is an Actin-Cross-Linking Protein in Macrophages/Microglia. *Biochem Biophys Res Commun* 2001; 286: 292–297.

160. Ito D, Imai Y, Ohsawa K, et al. Microglia-specific localisation of a novel calcium binding protein, Iba1. *Brain Res Mol Brain Res* 1998; 57: 1–9.

161. Raivich G, Bohatschek M, Kloss CU, et al. Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function. *Brain Res Brain Res Rev* 1999; 30: 77–105.

162. Parkhurst CN, Gan W-B. Microglia dynamics and function in the CNS. *Curr Opin Neurobiol* 2010; 20: 595–600.

163. Lenz KM, Nelson LH. Microglia and Beyond: Innate Immune Cells As Regulators of Brain Development and Behavioral Function. *Front Immunol*; 9. Epub ahead of print 13 April 2018. DOI: 10.3389/fimmu.2018.00698.

164. Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 2017; 541: 481–487.

165. Hol EM, Pekny M. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. *Curr Opin Cell Biol* 2015; 32: 121–130.

166. Wilhelmsson U, Li L, Pekna M, et al. Absence of glial fibrillary acidic protein and vimentin prevents hypertrophy of astrocytic processes and improves post-traumatic regeneration. *J Neurosci Off J Soc Neurosci* 2004; 24: 5016–5021.

167. Sherman MP, Griscavage JM, Ignarro LJ. Nitric oxide-mediated neuronal injury in multiple sclerosis. *Med Hypotheses* 1992; 39: 143–146.

168. Askalan R, Deveber G, Ho M, et al. Astrocytic-inducible nitric oxide synthase in the ischemic developing human brain. *Pediatr Res* 2006; 60: 687–692.

169. Liddelow SA, Barres BA. Reactive Astrocytes: Production, Function, and Therapeutic Potential. *Immunity* 2017; 46: 957–967.

170. Hanisch U-K, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci* 2007; 10: 1387–1394.

171. Cragnolini AB, Lampitella G, Virtuoso A, et al. Regional brain susceptibility to neurodegeneration: what is the role of glial cells? *Neural Regen Res* 2020; 15: 838–842.

172. Wood PL. *Neuroinflammation: Mechanisms and Managemen*t. Springer Science & Business Media, 2002.

173. Acaz-Fonseca E, Avila-Rodriguez M, Garcia-Segura LM, et al. Regulation of astroglia by gonadal steroid hormones under physiological and pathological conditions. *Prog Neurobiol* 2016; 144: 5–26.

174. Garcia-Segura LM, Melcangi RC. Steroids and glial cell function. *Glia* 2006; 54: 485–498.

175. Chapouly C, Tadesse Argaw A, Horng S, et al. Astrocytic TYMP and VEGFA drive blood–brain barrier opening in inflammatory central nervous system lesions. *Brain* 2015; 138: 1548–1567.

176. da Fonseca ACC, Matias D, Garcia C, et al. The impact of microglial activation on blood-brain barrier in brain diseases. *Front Cell Neurosci*; 8. Epub ahead of print 3 November 2014. DOI: 10.3389/fncel.2014.00362.

177. Oldendorf WH, Cornford ME, Brown WJ. The large apparent work capability of the blood-brain barrier: a study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. *Ann Neurol* 1977; 1: 409–417.

178. Di Meo S, Reed TT, Venditti P, et al. Role of ROS and RNS Sources in Physiological and Pathological Conditions. *Oxid Med Cell Longev*; 2016. Epub ahead of print 2016. DOI: 10.1155/2016/1245049.

179. Phaniendra A, Jestadi DB, Periyasamy L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. *Indian J Clin Biochem* 2015; 30: 11–26.

180. Doll DN, Hu H, Sun J, et al. Mitochondrial Crisis in Cerebrovascular Endothelial Cells Opens the Blood-Brain Barrier. *Stroke J Cereb Circ* 2015; 46: 1681–1689.

181. Taylor JM, Main BS, Crack PJ. Neuroinflammation and oxidative stress: Co-conspirators in the pathology of Parkinson's disease. *Neurochem Int* 2013; 62: 803–819.

182. Rovira-Llopis S, Bañuls C, de Marañon AM, et al. Low testosterone levels are related to oxidative stress, mitochondrial dysfunction and altered subclinical atherosclerotic markers in type 2 diabetic male patients. *Free Radic Biol Med* 2017; 108: 155–162.

183. Ota H, Akishita M, Akiyoshi T, et al. Testosterone Deficiency Accelerates Neuronal and Vascular Aging of SAMP8 Mice: Protective Role of eNOS and SIRT1. *PLoS ONE*; 7. Epub ahead of print 4 January 2012. DOI: 10.1371/journal.pone.0029598.

184. Liguori I, Russo G, Curcio F, et al. Oxidative stress, aging, and diseases. *Clin Interv Aging* 2018; 13: 757–772.

185. Pintana H, Pongkan W, Pratchayasakul W, et al. Testosterone replacement attenuates cognitive decline in testosterone-deprived lean rats, but not in obese rats, by mitigating brain oxidative stress. *Age Dordr Neth* 2015; 37: 84.

186. Haring R, Baumeister SE, Volzke H, et al. Prospective Inverse Associations of Sex Hormone Concentrations in Men With Biomarkers of Inflammation and Oxidative Stress. *J Androl* 2012; 33: 944–950.

187. Toro-Urrego N, Garcia-Segura LM, Echeverria V, et al. Testosterone Protects Mitochondrial Function and Regulates Neuroglobin Expression in Astrocytic Cells Exposed to Glucose Deprivation. *Front Aging Neurosci*; 8. Epub ahead of print 27 June 2016. DOI: 10.3389/fnagi.2016.00152.

188. Grimm A, Mensah-Nyagan AG, Eckert A. Alzheimer, mitochondria and gender. *Neurosci Biobehav Rev* 2016; 67: 89–101.

189. Túnez I, Feijóo M, Collado JA, et al. Effect of testosterone on oxidative stress and cell damage induced by 3-nitropropionic acid in striatum of ovariectomized rats. *Life Sci* 2007; 80: 1221–1227.

190. Cunningham RL, Singh M, O'Bryant SE, et al. Oxidative stress, testosterone, and cognition among Caucasian and Mexican American men with and without Alzheimer's disease. *J Alzheimers Dis JAD* 2014; 40: 563–573.

191. Barron AM, Fuller SJ, Verdile G, et al. Reproductive Hormones Modulate Oxidative Stress in Alzheimer's Disease. *Antioxid Redox Signal* 2006; 8: 2047–2059.

192. Alonso-Alvarez C, Bertrand S, Faivre B, et al. Testosterone and oxidative stress: the oxidation handicap hypothesis. *Proc R Soc B Biol Sci* 2007; 274: 819–825.

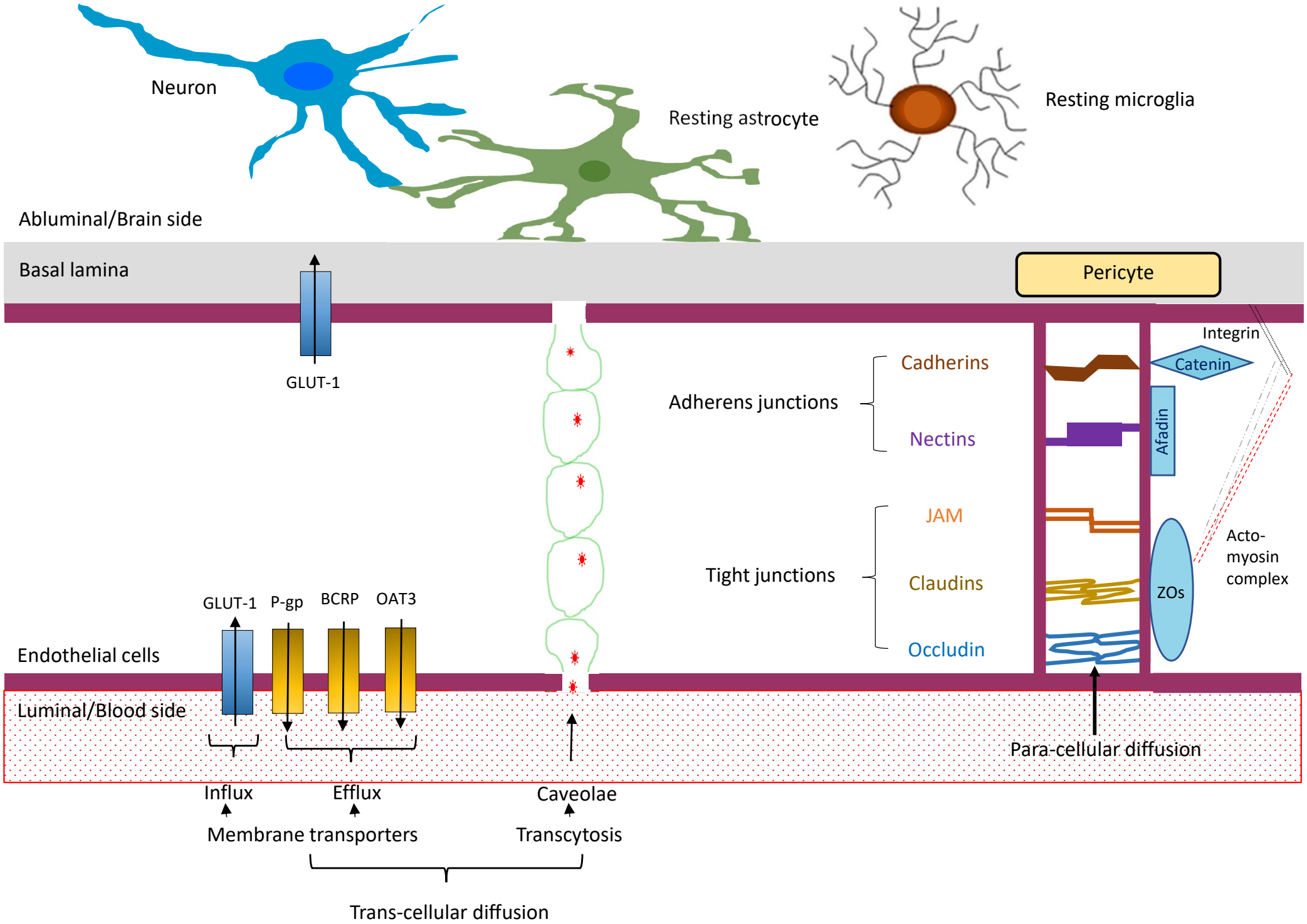
193. Choudhary V, Kaddour-Djebbar I, Lakshmikanthan V, et al. Novel Role of Androgens in Mitochondrial Fission and Apoptosis. *Mol Cancer Res* 2011; 9: 1067–1077.

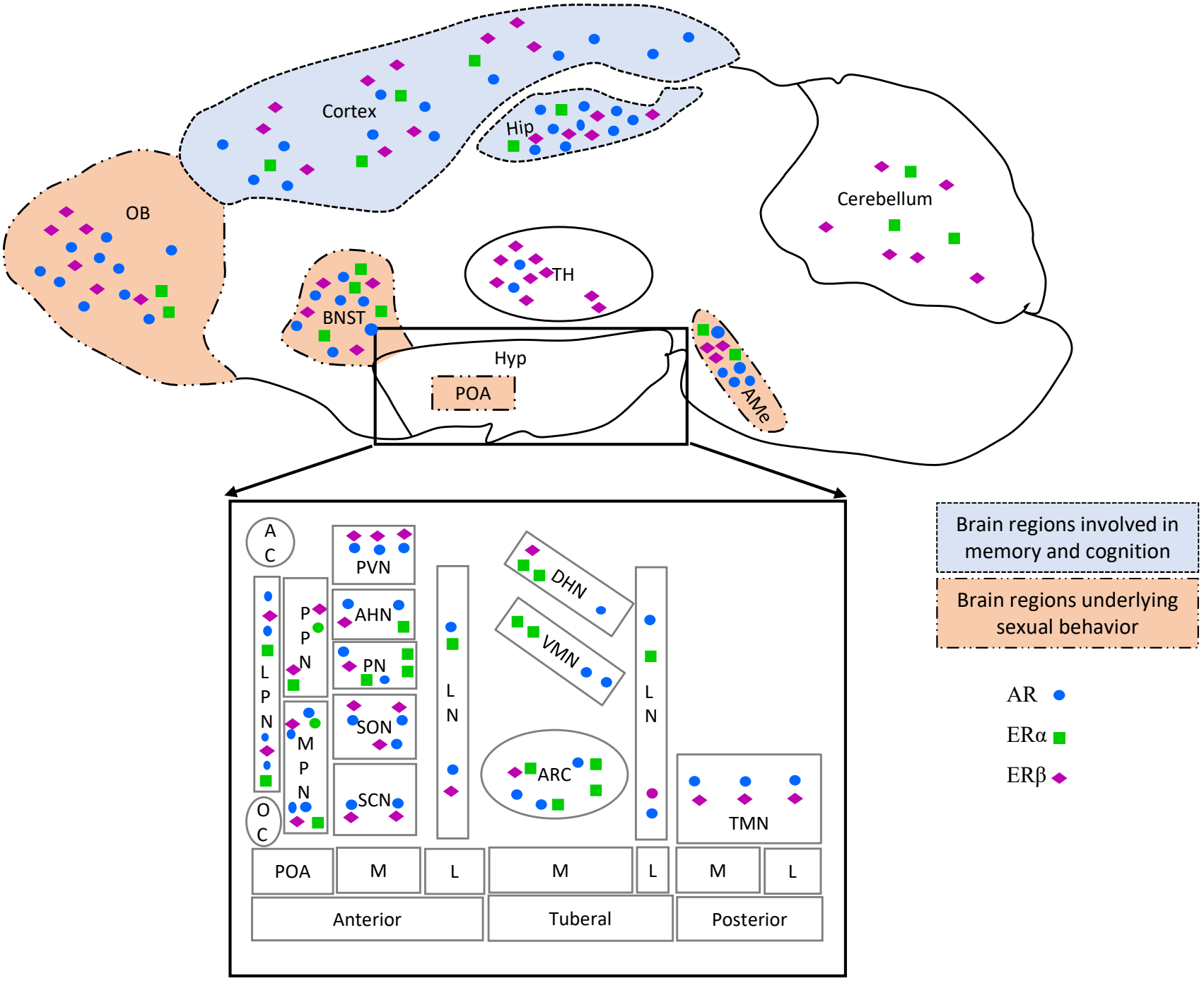
194. Tostes RC, Carneiro FS, Carvalho MHC, et al. Reactive oxygen species: players in the cardiovascular effects of testosterone. *Am J Physiol Regul Integr Comp Physiol* 2016; 310: R1-14.

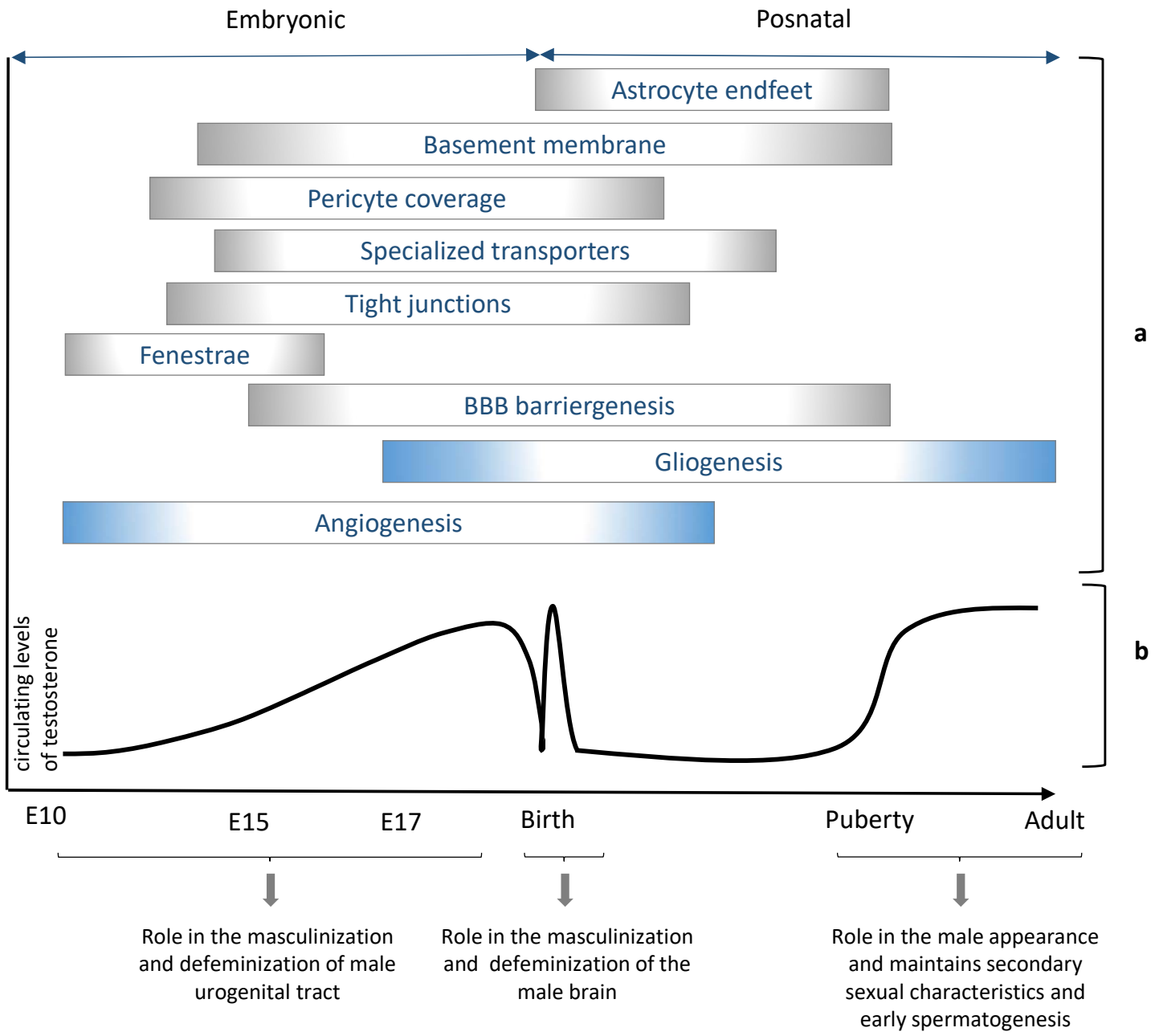
195. Kang J, Jia Z, Ping Y, et al. Testosterone alleviates mitochondrial ROS accumulation and mitochondria-mediated apoptosis in the gastric mucosa of orchiectomized rats. *Arch Biochem Biophys* 2018; 649: 53–59.

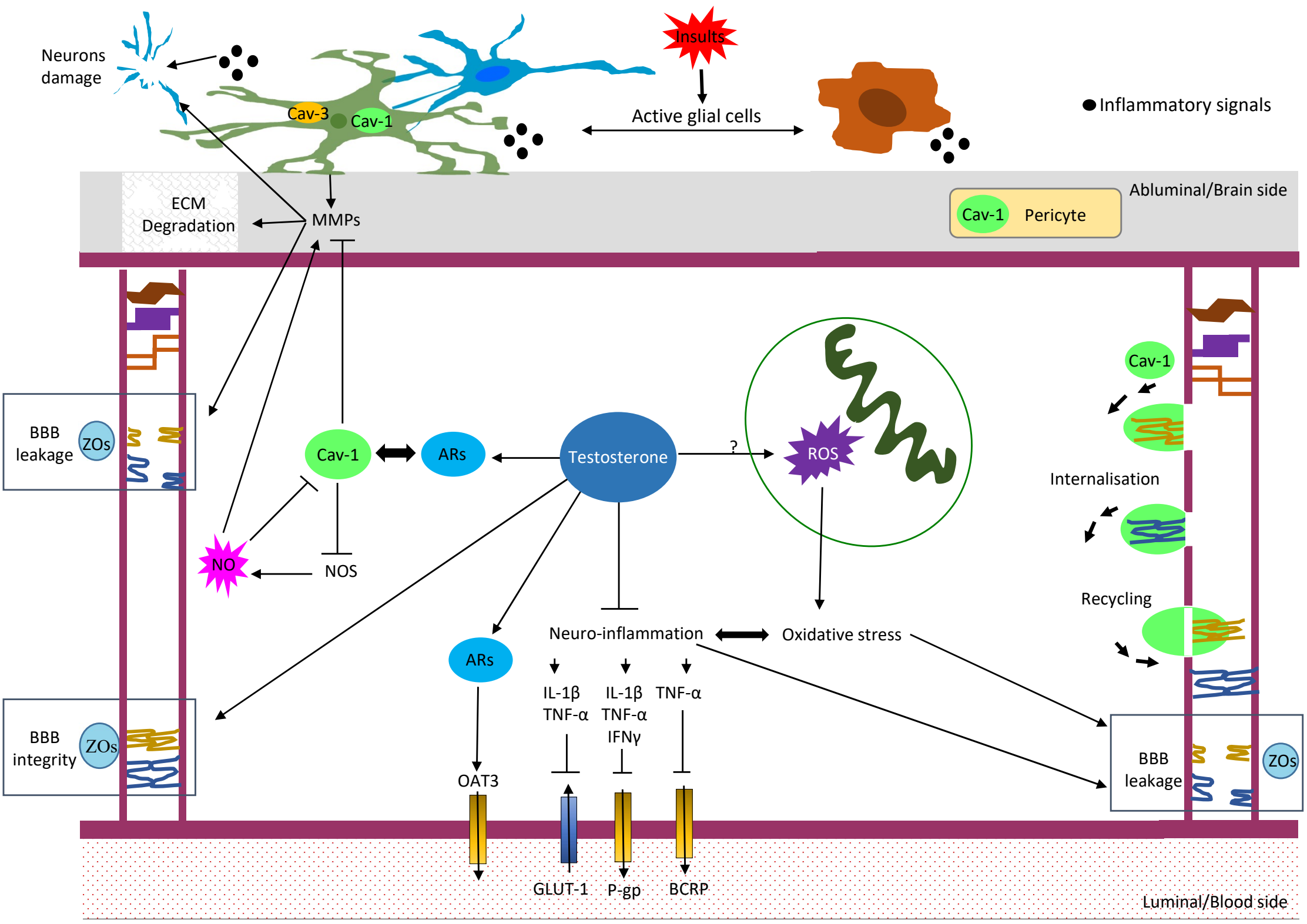
196. Chignalia AZ, Schuldt EZ, Camargo LL, et al. Testosterone induces vascular smooth muscle cell migration by NADPH oxidase and c-Src-dependent pathways. *Hypertens Dallas Tex 1979* 2012; 59: 1263–1271.

197. Singh H, Cheng J, Deng H, et al. Vascular cytochrome P450 4A expression and 20-hydroxyeicosatetraenoic acid synthesis contribute to endothelial dysfunction in androgen-induced hypertension. *Hypertens Dallas Tex 1979* 2007; 50: 123–129.


Neuron
Resting astrocyte
Resting microglia
Abluminal/Brain side
Basal lamina
Pericyte
GLUT-1
Adherens junctions
Cadherins
Nectins
Integrin
Catenin
Afadin
Tight junctions
JAM
Claudins
Occludin
ZOs
Acto-myosin complex
GLUT-1
P-gp
BCRP
OAT3
Endothelial cells
Luminal/Blood side
Para-cellular diffusion
Influx
Efflux
Caveolae
Membrane transporters
Transcytosis
Trans-cellular diffusion


Cortex
Hip
Cerebellum
OB
TH
BNST
Hyp
POA
AMe
A C
PVN
AHN
PN
SON
SCN
P P N
M P N
L P N
O C
L N
DHN
VMN
ARC
L N
TMN
POA
M
L
M
L
M
L
Anterior
Tuberal
Posterior
Brain regions involved in memory and cognition
Brain regions underlying sexual behavior
AR
ERα
ERβ


Embryonic
Posnatal
Astrocyte endfeet
Basement membrane
Pericyte coverage
Specialized transporters
Tight junctions
Fenestrae
BBB barriergenesis
Gliogenesis
Angiogenesis
a
circulating levels of testosterone
b
E10
E15
E17
Birth
Puberty
Adult
Role in the masculinization and defeminization of male urogenital tract
Role in the masculinization and defeminization of the male brain
Role in the male appearance and maintains secondary sexual characteristics and early spermatogenesis


Neurons damage
Cav-3
Cav-1
Insults
Active glial cells
Inflammatory signals
ECM Degradation
MMPs
Abluminal/Brain side
Cav-1
Pericyte
BBB leakage
ZOs
Cav-1
ARs
Testosterone
?
ROS
NO
NOS
Internalisation
Recycling
Neuro-inflammation
Oxidative stress
ARs
IL-1β
TNF-α
IL-1β
TNF-α
IFNγ
TNF-α
BBB integrity
ZOs
OAT3
GLUT-1
P-gp
BCRP
BBB leakage
ZOs
Luminal/Blood side