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Stroke Neuroprotection: Oestrogen and Insulin-Like Growth Factor-1 Interactions and the Role of Microglia

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Abstract

Oestrogen has been shown to be neuroprotective for stroke and other neural injury models. Oestrogen promotes a neuroprotective phenotype through myriad actions, including stimulating neurogenesis, promoting neuronal differentiation and survival, suppressing neuroinflammation and maintaining the integrity of the blood–brain barrier. At the molecular level, oestrogen directly modulates genes that are beneficial for repair and regeneration via the canonical oestrogen receptor. Increasingly, evidence indicates that oestrogen acts in concert with growth factors to initiate neuroprotection. Oestrogen and insulin-like growth factor (IGF)-1 act cooperatively to influence cell survival, and combined steroid hormone/growth factor interaction has been well documented in the context of neurones and astrocytes. Here, we summarise the evidence that oestrogen-mediated neuroprotection is critically dependent on IGF-1 signalling, and specifically focus on microglia as the source of IGF-1 and the locus of oestrogen–IGF-1 interactions in stroke neuroprotection.

Keywords

growth factor; oestrogens; IGF-1; microglia; stroke

In experimental models of several neurologic diseases, including stroke, Parkinson's disease and multiple sclerosis, oestrogen treatment has been shown to reduce neuronal damage and improve recovery. In the case of stroke, oestrogen treatment to ovariectomised young female animals is neuroprotective in a transient intraluminal middle cerebral artery occlusion (MCAo-suture) model (1–5) and in transient global ischaemia (6,7). Oestrogen also improves histological signs and clinical outcomes in experimental autoimmune encephalomyelitis, a model of multiple sclerosis (8) and attenuates dopamine loss in the striatum resulting from 6-hydroxydopamine injections, a model for Parkinson's disease (9).

The neuroprotective effects of oestrogen are well studied in the context of stroke, although the precise mechanism of hormone action is not fully understood, and more than one mechanism may be in effect. Some studies have shown that oestrogen-mediated infarct reduction may be related to delayed increases in cerebral blood flow (10,11), although not

all studies support this idea (3,12). Oestrogen may directly affect NMDA receptor activation (13) and modify caspase-12 (14) activation to affect neuroprotection. Oestrogen has been shown to attenuate β -amyloid precursor protein mRNA (15), increase glucose transporter protein type 1 transporter (16), decrease programmed cell death in cortical neurones (17,18) and attenuate c-fos induction (17,18). Oestrogen also regulates heat shock proteins (19), cytochrome P450 (20), the cell death effectors Bcl2 and Bcl-xl (21), the cocaine- and amphetamine regulated transcript (22) and nuclear factor-kB, a key signalling element of the inflammatory pathway (23).

The actions of oestrogen are transduced by oestrogen receptor (ER) α and ER β , and the more recently discovered GPR30 (24). Studies of the ER α knockout mouse (ERKO) suggest that this receptor is crucial for the protective effects of oestrogen in stroke (25,26); however, *in vitro*, some of the neuroprotective effects of oestrogen are not blocked by the receptor antagonist, and may occur as a result of oestrogen's action as a free radical scavenger (27). Furthermore, the ERKO mouse paradoxically has a smaller cortical lesion than the wild-type mouse (28), suggesting that ablation of ER α may also initiate neuroprotective pathways. Oestrogen analogues, which do not bind either oestrogen receptor, have also been shown to be neuroprotective in a stroke model, suggesting that some of the effects of oestrogen may be receptor-independent (29). The selective oestrogen receptor modulator (SERM) tamoxifen also reduces infarct size at higher doses, when given prior to the injury; however, its effects are not mediated through cerebral blood flow (30) or inhibited by the receptor antagonist (31), nor is the SERM LY353381.HCl (32).

It should be noted that oestrogen replacement is not uniformly protective, and these circumstances may illuminate new mechanisms of action of oestrogen. For example, although oestrogen has been shown to reduce infarct in older animals using the MCAo-intraluminal stroke model (33–35), oestrogen failed to protect CA1 neurones in older female gerbils in a bilateral carotid (global) ischaemia model (36). In the vasoconstrictive MCAo-endothelin-1 (ET-1) model, oestrogen treatment was neuroprotective to young adult female rats but highly deleterious for older acyclic females (37,38). Irrespective of oestrogen treatment, older animals sustain a more severe infarction compared to younger animals (39), suggesting that the microenvironment of the ageing brain may not cope adequately with an ischaemic insult. The fact that oestrogen is ineffective in older females where the ischaemic event is more severe raises the possibility that the loss of other neuroprotective molecules in ageing not only renders the older animal more susceptible to stroke-induced cell death, but also affects the ability of oestrogen to render neuroprotection.

Our recent studies suggest that the age-related loss of insulin-like growth factor (IGF-1) is related to oestrogen's impaired protective capacity in older females. Several studies have shown that oestrogen cooperates with IGF-1 to promote cell proliferation and protect against neural injury (40). Furthermore, IGF-1 is dramatically reduced with age. We found that i.c.v. infusions of IGF-1 to oestrogen-treated middle-aged females reversed the toxic effects of oestrogen in this group. Furthermore, i.c.v. infusions of the IGF receptor (IGFR) inhibitor JB-1 abolished oestrogen's neuroprotective effects in young females (37,38), confirming that oestrogen and IGF-1 collaborate to promote neuroprotection, and also supporting the

hypothesis that poor bioavailability of IGF-1 in older females impairs the neuroprotective actions of oestrogen.

IGF-1 bioavailability and neuroprotection

Circulating IGF-1 levels are markedly reduced with ageing in almost all species (41,42), and, although this phenomenon increases longevity (43,44), it may be deleterious for the injured brain. Although the liver is the single largest source, IGF-1 is synthesised by diverse organs, including the brain (45). Brain IGF-1 thus represents a mixed pool of peripherally-derived IGF-1 and locally produced peptide. IGF-1 delivered peripherally enters the brain through saturable transport systems (46) and circulating IGF-1 and growth hormone (GH) levels in turn are considered to elevate brain IGF-1 levels and regulate local transcription (47). IGF-1 is produced by both neurones and glia, although it has distinct actions on each cell type, producing proliferation in astrocytes (48), as well as activating phosphoinositide 3-kinase (PI3K) and Akt signalling in microglia (49), whereas it stimulated pro-survival pathways in neurones (50).

Not unexpectedly, exogenous IGF-1 is shown to reduce ischaemic injury in many species (51–54), stimulate stroke-induced neurogenesis (55) and promote neuronal survival, neuronal myelination and angiogenesis (56,57). Intravenous (58), i.c.v. and intranasal delivery of IGF-1 (59) all improve stroke outcomes. Additionally, treatment with IGF-1, IGF-2 and IGF binding protein (IGFBP) ligand inhibitors, which displace and therefore increase bioactive IGFs from their binding protein, all improved infarct volume in MCAo models (60), as does the delayed peripheral administration of the N-terminal tripeptide of IGF-1 (61). IGF-1 has been shown to increase neurogenesis in the periventricular and parenchymal zones of the hypothalamus (62). In a spinal cord injury model, IGF-1 gene transfer promoted motor recovery, anti-inflammatory responses and reduced apoptotic cell death (63), whereas i.c.v. infusion of IGF-1 increased progenitor cell proliferation in stroke affected animals (64).

More recent studies reveal that IGF-1 elevations are seen routinely after brain injury and are considered to confer endogenous neuroprotection. For example, IGF-1 expression is rapidly up-regulated after traumatic brain injury in cells located around the cortical contusion site (65). Human bone marrow stromal cells, which are shown to enhance neurological recovery after stroke, increased the number of newborn, migrating neurones that express IGF-1 (66). Exercise also increases brain uptake of IGF-1 and concomitantly reduces MCAo-induced infarct volume and improves motor performance. This effect can be abolished by infusion of an IGFR inhibitor (67), implicating this growth factor and receptor in neural recovery. In the case of mesenchymal stem cells (MSCs), transplantation suppresses MCAO induced inflammation, and these MSCs are shown to produce cytokines including growth factors such as IGF-1, vascular endothelial growth factor and glial-derived neurotrophic factor (GDNF) (68). Similarly, interferon (IFN)- γ mediated improvement in locomotor activity in a spinal cord injury model is also accompanied by elevation of IGF-1 and GDNF (69). Thus, elevating local IGF-1 levels appear to act as a common path for stimulating neuroprotection in several injury models.

IGF-1/oestrogen interactions

It is quite well-accepted that IGF-1 and oestrogen act synergistically in experimental models of neurodegenerative diseases (40,70). Oestrogen and IGF-1 receptors often co-localise to the same cell, promote the survival and differentiation of the same groups of neurones and stimulate adult neurogenesis (71). Oestrogen and IGF-1 also have overlapping neuroprotective actions in the forebrain. Both prevent excitotoxin-induced cell death in hilar neurones (72), and the actions of one agent are inhibited by the other's receptor antagonist. Similarly, 6-hydroxydopamine lesions targeting the nigrostriatal dopaminergic pathway are attenuated by either oestrogen or IGF-1, whereas the IGF-1 inhibitor JB-1 can inhibit the protective actions of both oestrogen and IGF-1 (73). Indeed, IGF-1 can activate oestrogen receptors in the absence of oestradiol (74). Both *in vivo* (75) and *in vitro* studies show that oestrogen can regulate the IGF-1 receptor (76). *In vivo*, oestrogen transiently activates the IGF-1 receptor through tyrosine phosphorylation, leading to an interaction of ER α with IGFR-1 and a greater interaction with components of the PI3K pathway (77). In a medial forebrain bundle injury that models Parkinson's disease, IGF-1 attenuates lesion effects and also mediates the neuroprotective effects of oestrogen (73). IGF-1 and oestrogen both signal through the mitogen-activated protein kinase (MAPK) and Akt pathways and Akt inhibitors blocked the survival effects of both oestrogen and IGF-1, although the MAPK inhibitor had no effect (78), implicating the Akt survival pathway in oestrogen and IGF-1 mediated neuroprotection. Furthermore, oestrogen and IGF-1 suppress ERK activation in the damaged substantia nigra (78) and in the ischaemic cortex (37,38).

An interesting aspect of oestrogen and IGF-1 mediated neuroprotection is whether oestrogen and IGF-1 are independently protective or whether the actions of oestrogen are completely mediated by IGF-1. Although not widely studied, the evidence indicates that the effects of oestrogen appear to be transduced via IGF receptor systems. In a Parkinson's disease model, inhibiting IGFR abrogated the protective response to oestrogen, suggesting that steroid-mediated neuroprotection involves the IGF-1 signalling pathway (73). In the endothelin-1 stroke model, JB-1 induced blockade of the IGF receptor in young females completely abolished the protective effect of oestrogen (37,38). *In vitro* studies have also confirmed that oestrogen mediated neuroprotection is mediated by IGFR, such that the addition of JB-1 to the media of human foetal neuroblast cells abolished the protective effects of oestrogen on peroxide and amyloid-induced toxicity (79).

Our data obtained from older animals further suggest that the converse is also true, namely that IGF-1 is not neuroprotective in the absence of oestrogen. In ovariectomised older females, IGF-1 infusions did not improve (or worsen) infarct volume. However, IGF-1 infusions to animals replaced with oestrogen resulted in a significant reduction of infarct volume. Interestingly, in a different stroke model (bilateral carotid artery occlusion), loss of hippocampal cells in middle-aged females was reduced by either oestrogen or IGF-1, with no additional benefit from combining the two hormones (80). In the case of the sodium calcium exchanger in cortical neurones, however, combining the two compounds enhanced the independent action of oestrogen and IGF-1. Furthermore, this action was independent of the classical receptors because neither oestrogen, nor IGF-1 receptor antagonists could prevent these effects (81). Similarly, both oestrogen and IGF-1 enhance neurogenesis in the

dentate gyrus of the hippocampus and the hormones appear to have a synergistic effect. The ER antagonist, ICI 182,780 blocked the actions of IGF-1 in the presence or absence of oestrogen, implicating the oestrogen receptor in IGF-1 mediated effects (82). Conversely, in a kainate acid injury model, where both oestrogen and IGF-1 protected hippocampal hilar cells, an antagonist to either the oestrogen receptor or the IGF receptor blocked the effects of oestrogen. Reciprocally, the protective actions of IGF-1 were inhibited by the ICI 182,780 compound, suggesting that IGF-1 acts through the oestrogen receptor (72).

Microglia and the convergence of IGF-1/oestrogen action

Although several brain cells express oestrogen and IGF-1 receptor, cross-talk between these two systems has been particularly well studied in neurones and astrocytes (83). Growing evidence shows that microglia may also be an important source of IGF-1, and may provide another cellular substrate for oestrogen-IGF-1 interactions.

Microglia are the main effectors of the innate response in the ischaemic brain. However, their role in post-stroke pathogenesis is widely debated. On the one hand, activated microglia are responsible for clearing debris, phagocytosis of nonfunctional cells and the synthesis and release of cytokines resulting in further cell death. On the other hand, microglia also produce growth factors that are neuroprotective, and can elevate growth factor synthesis in neighbouring cells (84). For example, MSCs cultured in microglial media exhibit elevated level of several growth factors including IGF-1 (85). Exogenous application of microglia reduces ischaemic injury (86), whereas selective ablation of proliferating microglia exacerbates ischaemic injury and reduces IGF-1 levels in the ischaemic hemisphere (87).

Although still controversial, at least two activation states have been proposed for microglia. The classical response is referred to as M1 and is induced by microbial agents or T helper cell type 1 secretions and results in the production of pro-inflammatory cytokines [interleukin (IL)-1 β , tumour necrosis factor (TNF)- α , IL-6, and IL-12], which are cytotoxic. The alternative form, called M2 activation, is induced by Th2 cytokines (IL-4, IL-13), and is characterised by anti-inflammatory responses and tissue repair. This latter form of activation is also associated with increased IGF-1 transcript (88). Both age (88) and mitochondrial dysfunction (89) impair IL-4-mediated alternate activation and fail to elevate IGF-1 levels.

Stroke up-regulates IGF-1 in the ischaemic hemisphere, and many cell types, including microglia, display IGF-1 expression. In the ischaemic hippocampus, there is a rapid increase in neuronal IGF-1, followed a few days later by astroglial and microglial IGF-1 expression (90). A similar time frame was noted in a rat hypoxic/ischaemic injury model, where IGF-1 mRNA was elevated in microglia and astrocytes adjacent to surviving neurones at 3 days post-ischaemia, and IGFBP-3 and -5 were increased in reactive microglia (91), as well as a cytotoxic injury to dentate granule cells, where microglial IGF-1 levels were elevated 4 days post-lesion (92). Among haemopoietic cells, it is mainly cells of myeloid origin (macrophages and microglia) that show IGF-1 mRNA and synthesis (93). Although microglia synthesise IGF-1 as well as several IGF binding proteins, activation of microglia appears to elevate ligand but reduces IGFBP-4 and -6, at the same time as maintaining levels

of IGFBP-2 and -3 (94,95). In a new study, lipopolysaccharide (LPS) or IFN- γ activation of human microglia reduces IGF-1 in these cells (96). Microglial proliferation is enhanced in ageing and can be reduced by wheel running activity, which not only reduces the number of new microglia, but also increases the proportion of IGF-1-expressing microglial cells (97), thus promoting a proneurogenic environment. In a cuprizone model of demyelination, IGF-1 levels were elevated in the microglial fraction during the peak period of demyelination (98). Using green fluorescent protein-labelled donors, bone marrow-derived macrophage (BMDM) recruitment was found to outnumber resident microglia at the lesion site in a spinal ischaemic injury, and BMDM in the most severely injured animals were the ones found to express IGF-1 (99). Thus, several injury models show that microglial mobilisation involves IGF-1 expression. However, an important issue is whether microglial-derived IGF-1 is neuroprotective.

Is microglial IGF-1 neuroprotective?

Several new studies directly implicate microglial-derived IGF-1 in neuroprotection. Furthermore, microglial cells may also mediate the neuroprotective effects of exogenously provided IGF-1. In a mouse model of retinitis pigmentosa, IGF-1-induced cell survival is concomitant with an increase in the number of microglial cells in the outer nuclear layer. Depletion of microglia by clodronate in this model reduced the neuroprotective effect of IGF-1 (100). Interestingly, depleting microglia in the absence of IGF-1 was also neuroprotective, suggesting that IGF-1 may change the phenotypic role of this immune cell. A cuprizone model of demyelination also highlights the dual role played by these cells. Microglia increase phagocytosis and phagocytic markers such as TREM2b and the inflammatory cytokine TNF- α . However, both IGF-1 and fibroblast growth factor-2 were also up-regulated, suggesting that these cells may also promote a regenerative environment (101). In a neonatal hypoxia-induced periventricular white matter disease, IGF-1 and IGF-2 were up-regulated primarily in amoeboid microglial cells (102). However, subsequent action of glutamate resulted in an increase in microglial cytokines, suggesting that IGF-1 may be an early protective response by these cells. Activated microglia are also shown to decrease T cell apoptosis and stimulate T cell expansion via an O $\times$ 40-mediated mechanism that also elevated IGF-1 synthesis (103).

In an adult model of hypoxia/ischaemia, injured regions of the brain display cells positive for IGF-1 and F4/80, a marker for microglia/macrophages. Indeed, these cells were also positive for proliferating cell nuclear antigen (PCNA) and *ex vivo* experiments confirmed that IGF-1 treatment to microglia was mitogenic for microglia (104). In a sheep model of demyelination, a low dose of IGF was proliferative for oligodendroglia as well as astrocytes and microglia (105).

Microglia are also assumed to promote a neurogenic environment after stroke. Microglia expressing IGF-1 are seen in the subventricular zone of the ischaemic hemisphere as rapidly as 2 weeks after stroke and persist as late as 16 weeks. At 16 weeks, the only cells in the subventricular zone that express IGF-1 are microglia (106). Microglial activation may also increase the therapeutic window of IGF-1 in a model of neonatal hypoxia. Specifically,

IGF-1 was protective even 6 h after hypoxia-ischaemia if rat pups were subject to hyperthermia, a condition that resulted in activation of microglia and macrophages (100).

Direct evidence of the protective role of microglia was shown in a study where microglia were selectively ablated by ganciclovir treatment to mice carrying the herpes simplex virus driven by the CD11b promoter (87). Specifically, microglial ablation increased infarct volume and neuronal cell death and significantly reduced IGF-1. Conversely, stimulating microglial cells with macrophage-colony stimulating factor caused an increase in both microglial cells and IGF-1 levels. Thus, microglia may reduce infarction by providing a source for IGF-1 post-stroke. Ablation of the lectin galectin-3 in microglia further reduced microglial proliferation and increased the volume of infarction following stroke. Indeed, galectin-3 ablated microglia also failed to proliferate in response to IGF-1 (107). In an amyotrophic lateral sclerosis model, a decrease in IGF-1 levels was associated with reduced motoneuronal cell viability and reduced microglial recruitment in the mSOD1 transgenic mouse (108).

Are microglial cells responsive to oestrogen and IGF-1?

Although IGF-1 exerts proliferative effects on microglia, very little is known about the expression of the IGF receptor system in this cell type. The IGF receptor system consists of the IGFR1, a receptor tyrosine kinase, and IGFR2, a transmembrane glycoprotein also known as the cation-independent mannose 6-phosphate, as well as IGFBP-1–7. The tyrosine kinase receptor IGFR1 initiates intracellular signalling pathways, whereas the IGFR2 is not known to transduce signals. The IGFBP, similarly, do not initiate cellular signalling cascades but can either enhance or reduce the presentation of IGF-1 to the IGFR at the level of the extracellular matrix or cell membrane. In a multiple sclerosis model, microglia were shown to express IGFR1 and IGFBP-2 and 3 (109), whereas microglia cells from the human brain samples were found to not express IGF receptor at the resting state but expressed IGFBP-2 when activated (94,95). The proliferative actions of IGF-1 on microglia are likely dependent on the IGFR1. In a hypoxic/ischaemic injury, IGF-1 was seen mainly in microglia and co-localised PCNA. Moreover, IGF-1 enhanced DNA synthesis *in vitro* in microglia (104), consistent with data indicating that IGF-1 is mitogenic for peripheral macrophages (110,111). This also suggested that, besides providing trophic support to injured neurones, microglial IGF-1 may be important for initiating autocrine/paracrine actions.

Much more is known of the effects of oestrogen and oestrogen receptor systems in microglia. The effect of sex steroids on microglial cells was recognised more than a decade ago (112), and microglia secretions were shown to influence the hypothalamic, pituitary gonadal axis (113). Ovariectomy elevates microglial genes, which can be attenuated by exogenous oestradiol (114,115) and oestrogen treatment *in vivo* suppresses microglial expression in the penumbra region of an MCAo stroke model (116). Both ER α and ER β , have been localised to microglial cells (112,117), as are steroid-converting enzymes that are involved in the metabolism of oestrogens and androgens (118). In primary rat microglial cultures, 17 β -oestradiol or DPN (ER β agonist) and PPT (ER α agonist) all suppressed LPS-induced cytokine expression and reduced cell death, whereas pretreatment with the ER antagonist fulvestrant abrogated these effects (119). LPS treatment was able to induce ER α

and ER β in these cells (119), although other reports indicate that only ER β transcripts are increased (120). In *ex vivo* studies of adult-derived microglia, however, 17 β -oestradiol did not suppress microglial activation (117,121), although the ER β agonist DPN was able to suppress LPS-induced cytokine expression in these cells (122). ER β agonists have also been shown to reduce microglial activation and improve mortality in an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (123). 5-Androsten-3 β -17 β -diol, which functions as a selective modulator of ER β , represses microglial activation and inflammatory genes in response to LPS (124) and oestradiol is immunosuppressive in the BV-2 microglial cell line, which exclusively expresses ER β (125). It should be noted that ER α is also implicated in microglial suppression, as supported by studies using the ER α null mouse (126) and the ER α expressing macrophage line RAW 264.7 (127). Additionally, the effects of oestrogen effects can be transduced by the G-protein coupled receptor GPR30, and activation of this receptor through its ligand G-1 ameliorates the severity of disease in an EAE model (128). The SERMS tamoxifen and raloxifene also suppress microglial activation after LPS injections in male and female brains (129). Interestingly, in a conditional ER α mutant model where ER α was deleted in either neurones or microglia, loss of neuronal ER α caused more severe damage in an MCAo stroke model, whereas ER α deletion in microglia did not affect the outcome (130). Collectively, these studies highlight the importance of oestrogenic regulation of microglial cells and suggest that nonclassical oestrogen receptors, ER β and GPR30, may be crucial for suppressing the activation of microglial and peripheral immune cells. Furthermore, the action of SERMs on microglial activation may not engage the classical receptors but act instead via interaction with pro-inflammatory signalling cascades (131).

An interesting hypothesis that has not been directly tested is whether oestrogen and IGF-1 act coordinately on microglia to mediate both an increase in IGF-1 synthesis, as well as microglia proliferation and M2 activation. In view of the neuroprotective effects of these two hormones, and their cooperative actions on neurones and astrocytes, oestrogen either alone or in combination with IGF-1 may act to suppress pro-inflammatory microglial function at the same time as enhancing its protective roles. Although direct evidence is lacking, indirect support for oestrogen/IGF-1 action on microglia comes from our recent study on IGF-1 associated microRNA. In the ischaemic hemisphere, the microRNA Let7f is largely seen in microglia, and antagomirs to Let7f promote stroke neuroprotection and elevate microglial IGF-1 (37,38). However, antagomir treatment is only effective in young females, and not in oestrogen deficient middle-aged females or ovariectomised young females, suggesting that the ovarian endocrine environment is critical for microglial function. Coupled with the evidence that the microglial inhibitor minocycline is only neuroprotective for males but not females (132), these studies highlight the need for direct evidence of coordinated oestrogen and IGF-1 interaction in microglia, as has been successfully shown for neurones and astrocytes. Such studies will further our understanding of ischaemic pathology and promote the development of new therapeutic targets for stroke.

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References

1. Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finkelstein SP, Rau SW, Wise PM. Estradiol protects against ischemic injury. *J Cereb Blood Flow Metab.* 1998; 18:1253–1258. [PubMed: 9809515]
2. Hurn PD, Littleton-Kearney MT, Kirsch JR, Dharmarajan AM, Traystman RJ. Postischemic cerebral blood flow recovery in the female: effect of 17beta-estradiol. *J Cereb Blood Flow Metab.* 1995; 15:666–672. [PubMed: 7790416]
3. Rusa R, Alkayed NJ, Crain BJ, Traystman RJ, Kimes AS, London ED, Klaus JA, Hurn PD. 17beta-estradiol reduces stroke injury in estrogen-deficient female animals. *Stroke.* 1999; 30:1665–1670. [PubMed: 10436119]
4. Sawada M, Alkayed NJ, Goto S, Crain BJ, Traystman RJ, Shaivitz A, Nelson RJ, Hurn PD. Estrogen receptor antagonist ICI182,780 exacerbates ischemic injury in female mouse. *J Cereb Blood Flow Metab.* 2000; 20:112–118. [PubMed: 10616799]
5. Simpkins JW, Rajakumar G, Zhang YQ, Simpkins CE, Greenwald D, Yu CJ, Bodor N, Day AL. Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. *J Neurosurg.* 1997; 87:724–730. [PubMed: 9347981]
6. Jover T, Tanaka H, Calderone A, Oguro K, Bennett MV, Etgen AM, Zukin RS. Estrogen protects against global ischemia-induced neuronal death and prevents activation of apoptotic signaling cascades in the hippocampal CA1. *J Neurosci.* 2002; 22:2115–2124. [PubMed: 11896151]
7. Zhang QG, Raz L, Wang R, Han D, De Sevilla L, Yang F, Vadlamudi RK, Brann DW. Estrogen attenuates ischemic oxidative damage via an estrogen receptor alpha-mediated inhibition of NADPH oxidase activation. *J Neurosci.* 2009; 29:13823–13836. [PubMed: 19889994]
8. Offner H, Polanczyk M. A potential role for estrogen in experimental autoimmune encephalomyelitis and multiple sclerosis. *Ann N Y Acad Sci.* 2006; 1089:343–372. [PubMed: 17261780]
9. Dluzen D. Estrogen decreases corpus striatal neurotoxicity in response to 6-hydroxydopamine. *Brain Res.* 1997; 767:340–344. [PubMed: 9367266]
10. Roof RL, Hall ED. Estrogen-related gender difference in survival rate and cortical blood flow after impact-acceleration head injury in rats. *J Neurotrauma.* 2000; 17:1155–1169. [PubMed: 11186229]
11. Yang SH, Shi J, Day AL, Simpkins JW. Estradiol exerts neuroprotective effects when administered after ischemic insult. *Stroke.* 2000; 31:745–749. [PubMed: 10700514]
12. Carswell HV, Anderson NH, Morton JJ, McCulloch J, Dominiczak AF, Macrae IM. Investigation of estrogen status and increased stroke sensitivity on cerebral blood flow after a focal ischemic insult. *J Cereb Blood Flow Metab.* 2000; 20:931–936. [PubMed: 10894176]
13. Connell BJ, Crosby KM, Richard MJ, Mayne MB, Saleh TM. Estrogen-mediated neuroprotection in the cortex may require NMDA receptor activation. *Neuroscience.* 2007; 146:160–169. [PubMed: 17317017]
14. Crosby KM, Connell BJ, Saleh TM. Estrogen limits ischemic cell death by modulating caspase-12-mediated apoptotic pathways following middle cerebral artery occlusion. *Neuroscience.* 2007; 146:1524–1535. [PubMed: 17433554]
15. Shi J, Panickar KS, Yang SH, Rabbani O, Day AL, Simpkins JW. Estrogen attenuates over-expression of beta-amyloid precursor protein messenger RNA in an animal model of focal ischemia. *Brain Res.* 1998; 810:87–92. [PubMed: 9813255]
16. Shi J, Zhang YQ, Simpkins JW. Effects of 17beta-estradiol on glucose transporter 1 expression and endothelial cell survival following focal ischemia in the rats. *Exp Brain Res.* 1997; 117:200–206. [PubMed: 9419067]

17. Rau SW, Dubal DB, Bottner M, Wise PM. Estradiol differentially regulates c-Fos after focal cerebral ischemia. *J Neurosci.* 2003; 23:10487–10494. [PubMed: 14627632]
18. Rau SW, Dubal DB, Bottner M, Gerhold LM, Wise PM. Estradiol attenuates programmed cell death after stroke-like injury. *J Neurosci.* 2003; 23:11420–11426. [PubMed: 14673006]
19. Lu A, Rau RQ, Clark J, Reilly M, Nee A, Sharp FR. 17-beta-estradiol induces heat shock proteins in brain arteries and potentiates ischemic heat shock protein induction in glia and neurons. *J Cereb Blood Flow Metab.* 2002; 22:183–195. [PubMed: 11823716]
20. McCullough LD, Blizzard K, Simpson ER, Oz OK, Hurn PD. Aromatase cytochrome P450 and extragonadal estrogen play a role in ischemic neuroprotection. *J Neurosci.* 2003; 23:8701–8705. [PubMed: 14507969]
21. Won CK, Kim MO, Koh PO. Estrogen modulates Bcl-2 family proteins in ischemic brain injury. *J Vet Med Sci.* 2006; 68:277–280. [PubMed: 16598173]
22. Xu Y, Zhang W, Klaus J, Young J, Koerner I, Sheldahl LC, Hurn PD, Martinez-Murillo F, Alkayed NJ. Role of cocaine-and amphetamine-regulated transcript in estradiol-mediated neuroprotection. *Proc Natl Acad Sci USA.* 2006; 103:14489–14494. [PubMed: 16971488]
23. Wen Y, Yang S, Liu R, Perez E, Yi KD, Koulen P, Simpkins JW. Estrogen attenuates nuclear factor-kappa B activation induced by transient cerebral ischemia. *Brain Res.* 2004; 1008:147–154. [PubMed: 15145751]
24. Schreihöfer DA, Ma Y. Estrogen receptors and ischemic neuroprotection: who, what, where and when? *Brain Res.* 2013; 1514:107–122. [PubMed: 23500634]
25. Dubal DB, Rau SW, Shughrue PJ, Zhu H, Yu J, Cashion AB, Suzuki S, Gerhold LM, Bottner MB, Dubal SB, Merchenthaler I, Kindy MS, Wise PM. Differential modulation of estrogen receptors (ERs) in ischemic brain injury: a role for ERalpha in estradiol-mediated protection against delayed cell death. *Endocrinology.* 2006; 147:3076–3084. [PubMed: 16527848]
26. Dubal DB, Zhu H, Yu J, Rau SW, Shughrue PJ, Merchenthaler I, Kindy MS, Wise PM. Estrogen receptor alpha, not beta, is a critical link in estradiol-mediated protection against brain injury. *Proc Natl Acad Sci USA.* 2001; 98:1952–1957. [PubMed: 11172057]
27. Culmsee C, Vedder H, Ravati A, Junker V, Otto D, Ahlemeyer B, Krieg JC, Kriegstein J. Neuroprotection by estrogens in a mouse model of focal cerebral ischemia and in cultured neurons: evidence for a receptor-independent antioxidative mechanism. *J Cereb Blood Flow Metab.* 1999; 19:1263–1269. [PubMed: 10566973]
28. Sampei K, Goto S, Alkayed NJ, Crain BJ, Korach KS, Traystman RJ, Demas GE, Nelson RJ, Hurn PD. Stroke in estrogen receptor-alpha-deficient mice. *Stroke.* 2000; 31:738–743. [PubMed: 10700513]
29. Liu R, Yang SH, Perez E, Yi KD, Wu SS, Eberst K, Prokai L, Prokai-Tatrai K, Cai ZY, Covey DF, Day AL, Simpkins JW. Neuroprotective effects of a novel non-receptor-binding estrogen analogue: *in vitro* and *in vivo* analysis. *Stroke.* 2002; 33:2485–2491. [PubMed: 12364742]
30. Mehta SH, Dhandapani KM, De Sevilla LM, Webb RC, Mahesh VB, Brann DW. Tamoxifen, a selective estrogen receptor modulator, reduces ischemic damage caused by middle cerebral artery occlusion in the ovariectomized female rat. *Neuroendocrinology.* 2003; 77:44–50. [PubMed: 12624540]
31. Zhang Y, Milatovic D, Aschner M, Feustel PJ, Kimelberg HK. Neuroprotection by tamoxifen in focal cerebral ischemia is not mediated by an agonist action at estrogen receptors but is associated with antioxidant activity. *Exp Neurol.* 2007; 204:819–827. [PubMed: 17321521]
32. Rossberg MI, Murphy SJ, Traystman RJ, Hurn PD. LY353381.HCl, a selective estrogen receptor modulator, and experimental stroke. *Stroke.* 2000; 31:3041–3046. [PubMed: 11108769]
33. Alkayed NJ, Murphy SJ, Traystman RJ, Hurn PD, Miller VM. Neuroprotective effects of female gonadal steroids in reproductively senescent female rats. *Stroke.* 2000; 31:161–168. [PubMed: 10625733]
34. Dubal DB, Wise PM. Neuroprotective effects of estradiol in middle-aged female rats. *Endocrinology.* 2001; 142:43–48. [PubMed: 11145565]
35. Toung TJ, Chen TY, Littleton-Kearney MT, Hurn PD, Murphy SJ. Effects of combined estrogen and progesterone on brain infarction in reproductively senescent female rats. *J Cereb Blood Flow Metab.* 2004; 24:1160–1166. [PubMed: 15529016]

36. De Butte-Smith M, Nguyen AP, Zukin RS, Etgen AM, Colbourne F. Failure of estradiol to ameliorate global ischemia-induced CA1 sector injury in middle-aged female gerbils. *Brain Res.* 2007; 1153:214–220. [PubMed: 17462607]
37. Selvamani A, Sohrabji F. The neurotoxic effects of estrogen on ischemic stroke in older female rats is associated with age-dependent loss of insulin-like growth factor-1. *J Neurosci.* 2010; 30:6852–6861. [PubMed: 20484627]
38. Selvamani A, Sohrabji F. Reproductive age modulates the impact of focal ischemia on the forebrain as well as the effects of estrogen treatment in female rats. *Neurobiol Aging.* 2010; 31:1618–1628. [PubMed: 18829137]
39. Liu F, Yuan R, Benashski SE, McCullough LD. Changes in experimental stroke outcome across the life span. *J Cereb Blood Flow Metab.* 2009; 29:792–802. [PubMed: 19223913]
40. Garcia-Segura LM, Sanz A, Mendez P. Cross-talk between IGF-I and estradiol in the brain: focus on neuroprotection. *Neuroendocrinology.* 2006; 84:275–279. [PubMed: 17124377]
41. Denti L, Annoni V, Cattadori E, Salvagnini MA, Visioli S, Merli MF, Corradi F, Ceresini G, Valenti G, Hoffman AR, Ceda GP. Insulin-like growth factor I as a predictor of ischemic stroke outcome in the elderly. *Am J Med.* 2004; 117:312–317. [PubMed: 15336580]
42. Waters DL, Yau CL, Montoya GD, Baumgartner RN. Serum sex hormones, IGF-1, and IGFBP3 exert a sexually dimorphic effect on lean body mass in aging. *J Gerontol A Biol Sci Med Sci.* 2003; 58:648–652. [PubMed: 12865482]
43. Bartke A, Chandrashekar V, Dominici F, Turyn D, Kinney B, Steger R, Kopchick JJ. Insulin-like growth factor-I (IGF-1) and aging: controversies and new insights. *Biogerontology.* 2003; 4:1–8.
44. Brown-Borg HM. Hormonal regulation of longevity in mammals. *Ageing Res Rev.* 2007; 6:28–45. [PubMed: 17360245]
45. Baskin DG, Wilcox BJ, Figlewicz DP, Dorsa DM. Insulin and insulin-like growth factors in the CNS. *Trends Neurosci.* 1988; 11:107–111. [PubMed: 2465607]
46. Pan W, Kastin AJ. Interactions of IGF-1 with the blood-brain barrier *in vivo* and *in situ*. *Neuroendocrinology.* 2000; 72:171–178. [PubMed: 11025411]
47. Yan H, Mitschelen M, Bixler GV, Brucklacher RM, Farley JA, Han S, Freeman WM, Sonntag WE. Circulating IGF1 regulates hippocampal IGF1 levels and brain gene expression during adolescence. *J Endocrinol.* 2011; 211:27–37. [PubMed: 21750148]
48. Torres-Aleman I, Barriors V, Berciano J. The peripheral insulin-like growth factor system in amyotrophic lateral sclerosis and in multiple sclerosis. *Neurology.* 1998; 50:772–776. [PubMed: 9521273]
49. Strle K, Zhou JH, Broussard SR, Venters HD, Johnson RW, Freund GG, Dantzer R, Kelley KW. IL-10 promotes survival of microglia without activating Akt. *J Neuroimmunol.* 2002; 122:9–19. [PubMed: 11777539]
50. Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME. Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science.* 1997; 275:661–665. [PubMed: 9005851]
51. Gluckman P, Klempt N, Guan J, Mallard C, Sirimanne E, Dragunow M, Klempt M, Singh K, Williams C, Nikolics K. A role for IGF-1 in the rescue of CNS neurons following hypoxic-ischemic injury. *Biochem Biophys Res Commun.* 1992; 182:593–599. [PubMed: 1370886]
52. Guan J, Bennet L, George S, Wu D, Waldvogel HJ, Gluckman PD, Faull RL, Crosier PS, Gunn AJ. Insulin-like growth factor-I reduces postischemic white matter injury in fetal sheep. *J Cereb Blood Flow Metab.* 2001; 21:493–502. [PubMed: 11333359]
53. Johnston BM, Mallard EC, Williams CE, Gluckman PD. Insulin-like growth factor-1 is a potent neuronal rescue agent after hypoxic-ischemic injury in fetal lambs. *J Clin Invest.* 1996; 97:300–308. [PubMed: 8567948]
54. Lee WH, Bondy C. Insulin-like growth factors and cerebral ischemia. *Ann N Y Acad Sci.* 1993; 679:418–422. [PubMed: 7685573]
55. Yan YP, Sailor KA, Vemuganti R, Dempsey RJ. Insulin-like growth factor-I is an endogenous mediator of focal ischemia-induced neural progenitor proliferation. *Eur J Neurosci.* 2006; 24:45–54. [PubMed: 16882007]

56. Smith A, Chan SJ, Gutierrez J. Autoradiographic and immunohistochemical localization of insulin-like growth factor-I receptor binding sites in brain of the brown trout, *Salmo trutta*. *Gen Comp Endocrinol*. 2005; 141:203–213. [PubMed: 15804507]
57. Wang JM, Hayashi T, Zhang WR, Sakai K, Shiro Y, Abe K. Reduction of ischemic brain injury by topical application of insulin-like growth factor-I after transient middle cerebral artery occlusion in rats. *Brain Res*. 2000; 859:381–385. [PubMed: 10719091]
58. Rizk NN, Myatt-Jones J, Rafols J, Dunbar JC. Insulin like growth factor-1 (IGF-1) decreases ischemia-reperfusion induced apoptosis and necrosis in diabetic rats. *Endocrine*. 2007; 31:66–71. [PubMed: 17709900]
59. Lin S, Fan LW, Rhodes PG, Cai Z. Intranasal administration of IGF-1 attenuates hypoxic-ischemic brain injury in neonatal rats. *Exp Neurol*. 2009; 217:361–370. [PubMed: 19332057]
60. Mackay KB, Loddick SA, Naeve GS, Vana AM, Verge GM, Foster AC. Neuroprotective effects of insulin-like growth factor-binding protein ligand inhibitors *in vitro* and *in vivo*. *J Cereb Blood Flow Metab*. 2003; 23:1160–1167. [PubMed: 14526226]
61. Shapira S, Mathai S, Zhang R, Guan J. Delayed peripheral administration of the N-terminal tripeptide of IGF-1 (GPE) reduces brain damage following microsphere induced embolic damage in young adult and aged rats. *Neurosci Lett*. 2009; 454:53–57. [PubMed: 19429053]
62. Perez-Martin M, Cifuentes M, Grondona JM, Lopez-Avalos MD, Gomez-Pinedo U, Garcia-Verdugo JM, Fernandez-Llebrez P. IGF-1 stimulates neurogenesis in the hypothalamus of adult rats. *Eur J Neurosci*. 2010; 31:1533–1548. [PubMed: 20525067]
63. Hung KS, Tsai SH, Lee TC, Lin JW, Chang CK, Chiu WT. Gene transfer of insulin-like growth factor-I providing neuroprotection after spinal cord injury in rats. *J Neurosurg Spine*. 2007; 6:35–46. [PubMed: 17233289]
64. Dempsey RJ, Sailor KA, Bowen KK, Tureyen K, Vemuganti R. Stroke-induced progenitor cell proliferation in adult spontaneously hypertensive rat brain: effect of exogenous IGF-1 and GDNF. *J Neurochem*. 2003; 87:586–597. [PubMed: 14535942]
65. Madathil SK, Evans HN, Saatman KE. Temporal and regional changes in IGF-1/IGF-1R signaling in the mouse brain after traumatic brain injury. *J Neurotrauma*. 2010; 27:95–107. [PubMed: 19751099]
66. Zhang J, Li Y, Chen J, Yang M, Katakowski M, Lu M, Chopp M. Expression of insulin-like growth factor 1 and receptor in ischemic rats treated with human marrow stromal cells. *Brain Res*. 2004; 1030:19–27. [PubMed: 15567334]
67. Chang HC, Yang YR, Wang PS, Kuo CH, Wang RY. Insulin-like growth factor I signaling for brain recovery and exercise ability in brain ischemic rats. *Med Sci Sports Exerc*. 2011; 43:2274–2280. [PubMed: 21606872]
68. Sheikh AM, Nagai A, Wakabayashi K, Narantuya D, Kobayashi S, Yamaguchi S, Kim SU. Mesenchymal stem cell transplantation modulates neuroinflammation in focal cerebral ischemia: contribution of interleukin-1 and IL-5. *Neurobiol Dis*. 2011; 41:717–724. [PubMed: 21168500]
69. Fujiyoshi T, Kubo T, Chan CC, Koda M, Okawa A, Takahashi K, Yamazaki M. Interferon- γ decreases chondroitin sulfate proteoglycan expression and enhances hindlimb function after spinal cord injury in mice. *J Neurotrauma*. 2010; 27:2283–2294. [PubMed: 20925481]
70. Garcia-Segura LM, Cardona-Gomez GP, Chowen JA, Azcoitia I. Insulin-like growth factor-1 receptors and estrogen receptors interact in the promotion of neuronal survival and neuroprotection. *J Neurocytol*. 2000; 29:425–437. [PubMed: 11424959]
71. Mendez P, Azcoitia I, Garcia-Segura LM. Interdependence of oestrogen and insulin-like growth factor-1 in the brain: potential for analyzing neuroprotective mechanisms. *J Endocrinol*. 2005; 185:11–17. [PubMed: 15817823]
72. Azcoitia I, Sierra A, Garcia-Segura LM. Neuroprotective effects of estradiol in the adult rat hippocampus: interaction with insulin-like growth factor-I signalling. *J Neurosci Res*. 1999; 58:815–822. [PubMed: 10583912]
73. Quesada A, Micevych PE. Estrogen interacts with the IGF-1 system to protect nigrostriatal dopamine and maintain motoric behavior after 6-hydroxydopamine lesions. *J Neurosci Res*. 2004; 75:107–116. [PubMed: 14689453]

74. Ma ZQ, Santagati S, Patrone C, Pollio G, Vegeto E, Maggi A. Insulin-like growth factors activate estrogen receptor to control the growth and differentiation of the human neuroblastoma cell line SK-ER3. *Mol Endocrinol*. 1994; 8:910–918. [PubMed: 7984152]
75. El-Bakri NK, Islam A, Suliman I, Lindgren U, Winblad B, Adem A. Ovariectomy and gonadal hormone treatment: effects on insulin-like growth factor-1 receptors in the rat brain. *Growth Horm IGF Res*. 2004; 14:38–93.
76. Cardona-Gomez GP, Mendez P, DonCarlos LL, Azcoitia I, Garcia-Segura LM. Interactions of estrogen and insulin-like growth factor-I in the brain: molecular mechanisms and functional implications. *J Steroid Biochem Mol Biol*. 2002; 83:211–217. [PubMed: 12650718]
77. Mendez P, Azcoitia I, Garcia-Segura LM. Estrogen receptor alpha forms estrogen-dependent multimolecular complexes with insulin-like growth factor receptor and phosphatidylinositol 3-kinase in the adult rat brain. *Brain Res Mol Brain Res*. 2003; 112:170–176. [PubMed: 12670715]
78. Quesada A, Lee BY, Micevych PE. PI3 kinase/Akt activation mediates estrogen and IGF-1 nigral DA neuronal neuroprotection against a unilateral rat model of Parkinson's disease. *Dev Neurobiol*. 2008; 68:632–644. [PubMed: 18278798]
79. Luciani P, Deledda C, Benvenuti S, Callai I, Modi G, Fibbi B, Danza G, Vannelli GB, Peri A. Relationship between the neuroprotective effects of insulin-like growth factor-1 and 17β -oestradiol in human neuroblasts. *J Neuroendocrinol*. 2012; 24:1304–1310. [PubMed: 22621285]
80. Traub ML, De Butte-Smith M, Zukin RS, Etgen AM. Oestradiol and insulin-like growth factor-1 reduce cell loss after global ischaemia in middle-aged female rats. *J Neuroendocrinol*. 2009; 21:1038–1044. [PubMed: 19840235]
81. Sanchez JC, Lopez-Zapata DF, Francis L, De Los RL. Effects of estradiol and IGF-1 on the sodium calcium exchanger in rat cultured cortical neurons. *Cell Mol Neurobiol*. 2011; 31:619–627. [PubMed: 21311966]
82. Perez-Martin M, Azcoitia I, Trejo JL, Sierra A, Garcia-Segura LM. An antagonist of estrogen receptors blocks the induction of adult neurogenesis by insulin-like growth factor-I in the dentate gyrus of adult female rat. *Eur J Neurosci*. 2003; 18:923–930. [PubMed: 12925018]
83. Mendez P, Wandosell F, Garcia-Segura LM. Cross-talk between estrogen receptors and insulin-like growth factor-I receptor in the brain: cellular and molecular mechanisms. *Front Neuroendocrinol*. 2006; 27:391–403. [PubMed: 17049974]
84. Yenari MA, Kauppinen TM, Swanson RA. Microglial activation in stroke: therapeutic targets. *Neurotherapeutics*. 2010; 7:378–391. [PubMed: 20880502]
85. Zhou C, Zhang C, Chi S, Xu Y, Teng J, Wang H, Song Y, Zhao R. Effects of human marrow stromal cells on activation of microglial cells and production of inflammatory factors induced by lipopolysaccharide. *Brain Res*. 2009; 1269:23–30. [PubMed: 19269277]
86. Kitamura Y, Takata K, Inden M, Tsuchiya D, Yanagisawa D, Nakata J, Taniguchi T. Intracerebroventricular injection of microglia protects against focal brain ischemia. *J Pharmacol Sci*. 2004; 94:203–206. [PubMed: 14978360]
87. Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J. Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. *J Neurosci*. 2007; 27:2596–2605. [PubMed: 17344397]
88. Lee DC, Ruiz CR, Lebson L, Selenica ML, Rizer J, Hunt JB Jr, Rojiani R, Reid P, Kammath S, Nash K, Dickey CA, Gordon M, Morgan D. Aging enhances classical activation but mitigates alternative activation in the central nervous system. *Neurobiol Aging*. 2013; 34:1610–1620. [PubMed: 23481567]
89. Ferger AI, Campanelli L, Reimer V, Muth KN, Merdian I, Ludolph AC, Witting A. Effects of mitochondrial dysfunction on the immunological properties of microglia. *J Neuroinflammation*. 2010; 7:45. [PubMed: 20701773]
90. Hwang IK, Yoo KY, Park SK, An SJ, Lee JY, Choi SY, Kang JH, Kwon YG, Kang TC, Won MH. Expression and changes of endogenous insulin-like growth factor-1 in neurons and glia in the gerbil hippocampus and dentate gyrus after ischemic insult. *Neurochem Int*. 2004; 45:149–156. [PubMed: 15082232]
91. Beilharz EJ, Russo VC, Butler G, Baker NL, Connor B, Sirimanne ES, Dragunow M, Werther GA, Gluckman PD, Williams CE, Scheepens A. Co-ordinated and cellular specific induction of the

- components of the IGF-IGFBP axis in the rat brain following hypoxic-ischemic injury. *Brain Res Mol Brain Res.* 1998; 59:119–134. [PubMed: 9729323]
92. Breese CR, D'Costa A, Rollins YD, Adams C, Booze RM, Sonntag WE, Leonard S. Expression of insulin-like growth factor-1 (IGF-1) and IGF-binding protein 2 (IGF-BP2) in the hippocampus following cytotoxic lesion of the dentate gyrus. *J Comp Neurol.* 1996; 369:388–404. [PubMed: 8743420]
 93. Arkins S, Rebeiz N, Biragyn A, Reese DL, Kelley KW. Murine macrophages express abundant insulin-like growth factor-1 class I Ea and Eb transcripts. *Endocrinology.* 1993; 133:2334–2343. [PubMed: 8404686]
 94. Chesik D, De Keyser J, Wilczak N. Involvement of insulin-like growth factor binding protein-2 in activated microglia as assessed in post mortem human brain. *Neurosci Lett.* 2004; 362:14–16. [PubMed: 15147770]
 95. Chesik D, Glazenburg K, Wilczak N, Geeraedts F, De Keyser J. Insulin-like growth factor binding protein-1–6 expression in activated microglia. *NeuroReport.* 2004; 15:1033–1037. [PubMed: 15076729]
 96. Suh HS, Zhao ML, Derico L, Choi N, Lee SC. Insulin-like growth factor 1 and 2 (IGF1, IGF2) expression in human microglia: differential regulation by inflammatory mediators. *J Neuroinflammation.* 2013; 10:37. [PubMed: 23497056]
 97. Kohman RA, DeYoung EK, Bhattacharya TK, Peterson LN, Rhodes JS. Wheel running attenuates microglia proliferation and increases expression of a proneurogenic phenotype in the hippocampus of aged mice. *Brain Behav Immun.* 2012; 26:803–810. [PubMed: 22056294]
 98. Gudi V, Skuljec J, Yildiz O, Frichert K, Skripuletz T, Moharreh-Khiabani D, Voss E, Wissel K, Wolter S, Stangel M. Spatial and temporal profiles of growth factor expression during CNS demyelination reveal the dynamics of repair priming. *PLoS ONE.* 2011; 6:e22623. [PubMed: 21818353]
 99. Nakata T, Kawachi K, Nagashima M, Yasugi T, Izutani H, Ryugo M, Okamura T, Shikata F, Imagawa H, Yano H, Takahashi H, Tanaka J. Transient ischemia-induced paresis and complete paraplegia displayed distinct reactions of microglia and macrophages. *Brain Res.* 2011; 1420:114–124. [PubMed: 21959174]
 100. Lin S, Rhodes PG, Cai Z. Whole body hypothermia broadens the therapeutic window of intranasally administered IGF-1 in a neonatal rat model of cerebral hypoxia-ischemia. *Brain Res.* 2011; 1385:246–256. [PubMed: 21316352]
 101. Voss EV, Skuljec J, Gudi V, Skripuletz T, Pul R, Trebst C, Stangel M. Characterisation of microglia during de- and remyelination: can they create a repair promoting environment? *Neurobiol Dis.* 2012; 45:519–528. [PubMed: 21971527]
 102. Sivakumar V, Ling EA, Lu J, Kaur C. Role of glutamate and its receptors and insulin-like growth factors in hypoxia induced periventricular white matter injury. *Glia.* 2010; 58:507–523. [PubMed: 19795501]
 103. Wang Y, Li M, Song M, Xu X, Xiong J, Yang X, Tan J, Bai Y. Expression of OX40 ligand in microglia activated by IFN-gamma sustains a protective CD4+ T-cell response *in vitro*. *Cell Immunol.* 2008; 251:86–92. [PubMed: 18485335]
 104. O'Donnell SL, Frederick TJ, Krady JK, Vannucci SJ, Wood TL. IGF-1 and microglia/macrophage proliferation in the ischemic mouse brain. *Glia.* 2002; 39:85–97. [PubMed: 12112378]
 105. Cao Y, Gunn AJ, Bennet L, Wu D, George S, Gluckman PD, Shao XM, Guan J. Insulin-like growth factor (IGF)-1 suppresses oligodendrocyte caspase-3 activation and increases glial proliferation after ischemia in near-term fetal sheep. *J Cereb Blood Flow Metab.* 2003; 23:739–747. [PubMed: 12796722]
 106. Thored P, Heldmann U, Gomes-Leal W, Gisler R, Darsalia V, Taneera J, Nygren JM, Jacobsen SE, Ekdahl CT, Kokaia Z, Lindvall O. Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia.* 2009; 57:835–849. [PubMed: 19053043]
 107. Lalancette-Hebert M, Swarup V, Beaulieu JM, Bohacek I, Abdelhamid E, Weng YC, Sato S, Kriz J. Galectin-3 is required for resident microglia activation and proliferation in response to ischemic injury. *J Neurosci.* 2012; 32:10383–10395. [PubMed: 22836271]

108. Kawamura MF, Yamasaki R, Kawamura N, Tateishi T, Nagara Y, Matsushita T, Ohyagi Y, Kira J. Impaired recruitment of neuroprotective microglia and T cells during acute neuronal injury coincides with increased neuronal vulnerability in an amyotrophic lateral sclerosis model. *Exp Neurol*. 2012; 234:437–445. [PubMed: 22293437]
109. Gveric D, Cuzner ML, Newcombe J. Insulin-like growth factors and binding proteins in multiple sclerosis plaques. *Neuropathol Appl Neurobiol*. 1999; 25:215–225. [PubMed: 10417663]
110. Mueller RV, Hunt TK, Tokunaga A, Spencer EM. The effect of insulinlike growth factor I on wound healing variables and macrophages in rats. *Arch Surg*. 1994; 129:262–265. [PubMed: 8129600]
111. Scheven BA, Hamilton NJ. Stimulation of macrophage growth and multinucleated cell formation in rat bone marrow cultures by insulin-like growth factor I. *Biochem Biophys Res Commun*. 1991; 174:647–653. [PubMed: 1993062]
112. Mor G, Nilsen J, Horvath T, Bechmann I, Brown S, Garcia-Segura LM, Naftolin F. Estrogen and microglia: a regulatory system that affects the brain. *J Neurobiol*. 1999; 40:484–496. [PubMed: 10453051]
113. Mor G, Naftolin F. Oestrogen, menopause and the immune system. *J Br Menopause Soc*. 1998; S1:4–8.
114. Johnson AB, Bake S, Lewis DK, Sohrabji F. Temporal expression of IL-1 β protein and mRNA in the brain after systemic LPS injection is affected by age and estrogen. *J Neuroimmunol*. 2006; 174:82–91. [PubMed: 16530273]
115. Sarvari M, Hrabovszky E, Kallo I, Solymosi N, Liko I, Berchtold N, Cotman C, Liposits Z. Menopause leads to elevated expression of macrophage-associated genes in the aging frontal cortex: rat and human studies identify strikingly similar changes. *J Neuroinflammation*. 2012; 9:264. [PubMed: 23206327]
116. Dang J, Mitkari B, Kipp M, Bever C. Gonadal steroids prevent cell damage and stimulate behavioral recovery after transient middle cerebral artery occlusion in male and female rats. *Brain Behav Immun*. 2011; 25:715–726. [PubMed: 21277368]
117. Johnson AB, Sohrabji F. Estrogen's effects on central and circulating immune cells vary with reproductive age. *Neurobiol Aging*. 2005; 26:1365–1374. [PubMed: 16243607]
118. Gottfried-Blackmore A, Sierra A, Jellinck PH, McEwen BS, Bulloch K. Brain microglia express steroid-converting enzymes in the mouse. *J Steroid Biochem Mol Biol*. 2008; 109:96–107. [PubMed: 18329265]
119. Smith JA, Das A, Butler JT, Ray SK, Banik NL. Estrogen or estrogen receptor agonist inhibits lipopolysaccharide induced microglial activation and death. *Neurochem Res*. 2011; 36:1587–1593. [PubMed: 21127968]
120. Liu X, Fan XL, Zhao Y, Luo GR, Li XP, Li R, Le WD. Estrogen provides neuroprotection against activated microglia-induced dopaminergic neuronal injury through both estrogen receptor- α and estrogen receptor- β in microglia. *J Neurosci Res*. 2005; 81:653–665. [PubMed: 16013043]
121. Sierra A, Gottfried-Blackmore A, Milner TA, McEwan BS, Bulloch K. Steroid hormone receptor expression and function in microglia. *Glia*. 2008; 56:659–674. [PubMed: 18286612]
122. Lewis DK, Johnson AB, Stohlgren S, Harms A, Sohrabji F. Effects of estrogen receptor agonists on regulation of the inflammatory response in astrocytes from young adult and middle-aged female rats. *J Neuroimmunol*. 2008; 195:47–59. [PubMed: 18328572]
123. Wu WF, Tan XJ, Dai YB, Krishnan V, Warner M, Gustafsson JA. Targeting estrogen receptor β in microglia and T cells to treat experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA*. 2013; 110:3543–3548. [PubMed: 23401502]
124. Saijo K, Collier JG, Li AC, Katzenellenbogen JA, Glass CK. An ADIOL-ER β -CtBP transrepression pathway negatively regulates microglia-mediated inflammation. *Cell*. 2011; 145:584–595. [PubMed: 21565615]
125. Baker AE, Brautigam VM, Watters JJ. Estrogen modulates microglial inflammatory mediator production via interactions with estrogen receptor beta. *Endocrinology*. 2004; 145:5021–5032. [PubMed: 15256495]

126. Vegeto E, Belcredito S, Etteri S, Ghisletti S, Brsadelli A, Meda C, Krust A, Dupont S, Ciana P, Chambon P, Maggi A. Estrogen receptor-alpha mediates the brain anti-inflammatory activity of estradiol. *Proc Natl Acad Sci USA*. 2003; 100:9614–9619. [PubMed: 12878732]
127. Vegeto E, Ghisletti S, Meda C, Etteri S, Belcredito S, Maggi A. Regulation of the lipopolysaccharide signal transduction pathway by 17beta-estradiol in macrophage cells. *J Steroid Biochem Mol Biol*. 2004; 91:59–66. [PubMed: 15261308]
128. Blasko E, Haskell CA, Leung S, Gualtieri G, Halks-Miller M, Mahmoudi M, Dennis MK, Prossnitz ER, Karpus WJ, Horuk R. Beneficial role of the GPR30 agonist G-1 in an animal model of multiple sclerosis. *J Neuroimmunol*. 2009; 214:67–77. [PubMed: 19664827]
129. Arevalo MA, Diz-Chaves Y, Santos-Galindo M, Bellini MJ, Garcia-Segura LM. Selective oestrogen receptor modulators decrease the inflammatory response of glial cells. *J Neuroendocrinol*. 2012; 24:183–190. [PubMed: 21564348]
130. Elzer JG, Muhammad S, Wintermantel TM, Regnier-Vigouroux A, Ludwig J, Schutz G, Schwaninger M. Neuronal estrogen receptor-alpha mediates neuroprotection by 17beta-estradiol. *J Cereb Blood Flow Metab*. 2010; 30:935–942. [PubMed: 20010956]
131. Suuronen T, Nuutinen T, Huuskonen J, Ojala J, Thornell A, Salminen A. Anti-inflammatory effect of selective estrogen receptor modulators (SERMs) in microglial cells. *Inflamm Res*. 2005; 54:194–203. [PubMed: 15953991]
132. Li J, McCullough LD. Sex differences in minocycline-induced neuroprotection after experimental stroke. *J Cereb Blood Flow Metab*. 2009; 29:670–674. [PubMed: 19190654]