Published in final edited form as:
Pflugers Arch. 2013 May ; 465(5): 595–600. doi:10.1007/s00424-013-1260-x.

Stroke: Understanding the Differences between Males and Females

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Abstract

Stroke is a significant cause of death and long-term disability in the United States. The incidence, mortality and outcomes of stroke are significantly different between men and women. As with many diseases that affect men and women differently, an understanding of the reasons underlying those differences is critical to effective diagnosis and treatment. This review will examine the sex differences in stroke in both humans and animal models of stroke and review what is known about potential mechanisms underlying these differences. It is clear that there is a complex interaction between hormonal, genetic and unknown factors at play in generating the sex differences in stroke.

Keywords

Gender; estrogen; progesterone; testosterone; epigenetics; stroke

INTRODUCTION

Stroke is currently the fourth leading cause of death in the United States following heart disease and cancers [25]. Each year approximately seven hundred thousand people suffer a stroke and it causes approximately one out of nineteen deaths in the United States [25]. Overall, women have a lower risk of stroke compared to men, but this disparity diminishes following menopause when they have more strokes and worse outcomes as compared to men [21, 45, 68]. Retrospective studies have shown, however, that post-menopausal women on hormone replacement therapy appear to have better functional outcomes following a stroke [27]. Data from the Women's Heath Initiative in which either placebo, estrogens or progestins plus progesterone were administered to post-menopausal women in a double-blind trial, however, failed to show a protective effect of current hormone replacement therapies against stroke [76]. This may be due to the timing of hormone replacement as the average age of women in the study was 63, although this has not been conclusively determined [28, 32]. In addition to sex differences observed in humans, there are numerous studies in animal models of ischemic stroke suggesting sex differences in brain damage and recovery [29, 42, 35]. In this manuscript we will discuss some of the most current literature of what may be the source of this sex difference in stroke. A complete understanding of these complex events are not entirely known.

SEX DIFFERENCES IN STROKE IN HUMANS

Someone dies of a stroke in the United States every four minutes. The vast majority of these strokes are ischemic strokes where blood flow to part of the brain is blocked resulting in damage to the brain [25]. The single largest risk factor of stroke is age, however, males are more likely to suffer a stroke as compared to females [59, 74]. Males are more likely to

suffer a pediatric stroke and this trend continues through adolescence [26]. Interestingly, in young adults females are more likely than males to experience stroke [52, 56]. This is likely due to the pro-thrombotic actions of oral contraception [8]. After age thirty, however, male predominance returns [56, 67]. Following menopause, however, the protection in females is lost [30]. This is likely due to the increased number of women who live longer life spans than men, but also due to loss of female hormones after menopause.

When women suffer a stroke they are significantly more likely to have what is classified as a "severe" stroke more likely to result in death [20]. The women who survive are also more likely to experience more severe outcomes with less of a chance of complete recovery [21]. Women are more likely to experience long-term handicaps, a reduced quality of life and are more likely to experience depression [4, 57]. The contributing reasons for this likely have to do with age in that they may not have family caregivers and are older at the time of the stroke, in addition to an underlying sex difference in recovery. Overall there are significant sex differences in the incidence, severity and recovery from stroke.

SEX DIFFERENCES IN ANIMAL MODELS OF STROKE

Animal models have been extensively used to begin to understand the mechanisms, outcomes and sex differences underlying stroke. Methods to transiently or permanent block one or more of the cerebral arteries have been used by many investigators to produce a reproducible infarct in the brain. Females consistently experience less brain injury than males in numerous rodent models. Female gerbils suffered less neuronal pathology compared to males after ischemia was induced by unilateral carotid artery occlusion [31]. Similar results were obtained in rats in studies utilizing several models of transient cerebral ischemia [3, 40, 84] where it was demonstrated that both young and middle-aged females with similar levels of circulating estrogen had significantly less infarct volume when compared to gonadally intact males or to ovariectomized female rats whose estrogen levels are virtually undetectable.

Permanent occlusion of the middle cerebral artery (MCAO) is a well-established model of stroke. In this model, there is a 50% reduction in cerebral blood flow to the striatum and overlaying cortex [11]. This decrease in blood supply causes necrotic cell death in the striatum followed by programmed cell death, or apoptosis, in the overlaying cortex. In this model of permanent occlusion, ovariectomized females and intact males had a much larger MCAO-induced injury than animals with higher circulating estradiol concentrations [11, 60, 63, 73]. Pretreatment of these animals with low doses of 17β-estradiol is sufficient to exert dramatic protection in the brains of both female [80, 81] and male rats [51, 73], suggesting that indeed the female sex steroid hormone, estradiol, at least in part contributes to the sex difference in stroke vulnerability.

ROLE OF STEROID HORMONES

Estrogen

Many sex differences are mediated by the presence of the sex-specific steroid hormones; estrogens and progesterone, in females and androgens, including testosterone in males. Studies have shown that even low physiological doses of estradiol replacement are sufficient to exert dramatic protection in the brains of young and middle-aged female [13] and male [73] rats. Treatment with lower physiological doses of estradiol must occur prior to the onset of injury [11] while higher doses of estradiol can still be protective when given immediately before injury or even after injury [44, 73]. There are likely numerous mechanisms by which estrogen can prevent cell death following stroke, some requiring the action of the classical estrogen receptor and others that do not.

Steroid hormone receptors classically mediate the actions of steroid hormones by acting as transcription factors regulating target genes. This estrogen receptor function is necessary for the prevention of secondary apoptosis observed in cortex following focal ischemia [12]. Protection by estradiol is dependent on the presence of estrogen receptor alpha (ERα) specifically in the cortex. In ERα knockout mice, estradiol is not neuroprotective [14]. In the uninjured adult cerebral cortex, ERα is virtually absent as it is only transiently expressed during neonatal development [47, 55]. Twenty-four hours after MCAO, both ERα mRNA and protein are increased in the cortex of rats and mice [12, 15]. In ovariectomized females, this increase in ERα expression occurs in both oil and estradiol-treated groups [15]. One potential mechanism of this re-expression is thought to be due to the release of repressive DNA methylation [77]. DNA methylation of the promoter regions of the ERα gene is inversely correlated with expression in a time course that matches the re-expression following injury.

Estradiol also appears to enhance cellular viability by regulating the expression of anti-apoptotic gene, *bcl-2* [71]. In the hypothalamus, Bcl-2 mRNA was elevated in ovariectomized, estradiol-treated rats [22]. Additionally, estradiol prevented the injury-induced decrease in Bcl-2 gene expression in the cerebral cortex after ischemic injury [12]. Similarly, in hippocampal cells, estradiol increased expression of Bcl-xL, a Bcl-2 family member that interacts with Bcl-2 and Bcl-xL to prevent the pro-apoptotic actions of Bax, inhibit free radical production and suppress the activation of cysteine proteases [53]. Therefore, the regulation of this family of genes by estradiol may have multiple downstream effects that together suppress apoptosis and favor cell survival. In an *in vitro* model of ischemia it was demonstrated that pharmacologically blocking estradiol action via its receptors did indeed, reduce cell death and specifically, apoptosis [78, 79].

While the inflammatory response following ischemia is very complex, in general estradiol is believed to have an anti-inflammatory effect in the brain [58]. Estradiol can prevent the inflammatory response in cerebral blood vessels in young rats, however, this effect appears to be lost in older animals [69]. Additionally, estradiol has also been shown to regulate plasma levels of IL-6, TNF-α, granulocyte-macrophage colony-stimulating factor, IL-4 and IL-5 leading to a reduction in cell death in females following MCAO [70]. Together, these studies suggest that estradiol has the potential to regulate multiple aspects of inflammation that could result in less neuronal cell death following a stroke.

Estradiol may also exert trophic and protective effects by acting via classical receptor-mediated mechanisms on a variety of genes including the neurotrophins and their receptors [66]. Ovariectomy reduces brain derived neurotrophic factor (BDNF) mRNA levels in the cortex and hippocampus of female rats. In addition, estradiol may influence neurotrophin receptors, (trk A, trkB, trkC) and/or the pan-neurotrophin receptor, p75NTR [48]. During development estradiol receptor mRNA colocalizes with NGF, BDNF and NT3 in subsets of cells in the cortex and hippocampus and with the receptors for these neurotropins, p75NTR, trkA and trkB in the basal forebrain [48, 66]. The colocalization of estradiol receptors, neurotrophins, and their cognate receptors suggests potential complex autocrine and paracrine interactions between estradiol and the neurotrophins [65]. Estradiol may exert trophic and protective effects by influencing the expression of genes that encode for survival factors in the brain.

In addition to the receptor-mediated genomic actions of estradiol, several studies have suggested receptor-independent actions of estradiol as well. In female rats, pharmacological doses of estradiol can protect against the injury induced by transient cerebral ischemia [63]. Pretreatment or acute treatment with either estrogen isomer, 17β-estradiol or the receptor inactive 17α-estradiol, achieved equivalent neuroprotection, suggesting a mechanism of

protection independent of transcriptional activation [44]. This may be due to the antioxidative properties of the estrogen molecule or effects at the mitochondria that prevent cytochrome C release following injury [41, 64]. Additionally, acute treatment of male rats with pharmacological levels that are thousands of times higher than physiological levels and pretreatment with physiological levels of estrogen protect against injury induced by transient cerebral ischemia [73]. Together, these findings suggest that the sex-related differences in the extent of brain injury and the protective effects of estrogen replacement may be mediated via multiple cellular and molecular mechanisms.

Progesterone

Much less is known about how the other female sex steroid hormone, progesterone, acts as a potential neuroprotective agent in the brain. Several studies have suggested it can have a neuroprotective role in stroke [43]. Administration of progesterone can reduce cortical infarct size in middle-aged, reproductively senescent females and this protection is not related to the ability of progesterone to regulated cerebral blood flow [6]. Also administration of progesterone during the reperfusion in a model of transient ischemia in ovariectomized young females is neuroprotective [50]. Progesterone administration after a stroke has also been shown to reduce infarct size and enhance functional recovery in males [24]. While the pontential protective mechanisms are not as well known as those of estradiol, progesterone may act as an antioxidant and has been shown to modulate gamma-aminobutryic acid (GABA) receptor channel activity expression in neurons [16]. It can also on stimulate PI3 kinase activity leading to an increase in vascular endothelial growth factor and BDNF [36]. The overall effect of progesterone in stroke, however, is not entirely clear as some studies have demonstrated that progesterone administration can have either no effect or actually increase infarct size in certain models of stroke [10, 49]. Finally, the estrogen plus progesterone arm of the Women's Health Initiative suggests that combined hormone therapy may slightly increase the incidence of stroke [46].

Testosterone

The male sex is a known risk factor for stroke in humans. There are relatively few studies using this animal model testing the effects of androgens on ischemia and of those there are contradictory findings. For example in male rats, castration and the resulting low testosterone has been shown to either have no effect or decrease infarct size following MCAO [33, 73]. Testosterone administration to intact male rats has also been shown to increase infarct size, potentially due to the ability of testosterone to exacerbate glutamate excitotoxicity [83]. On the other hand, in humans, testosterone levels are inversely associated with stroke severity, infarct size and improved functional recovery [37]. Additionally, several *in vitro* studies suggest that testosterone in protective against oxidative stress, beta-amyloid toxicity and serum deprivation [1, 2, 54]. Testosterone and its metabolite, dihydrotesosterone, can protect against ischemia in adult male rats when administered in the low, physiological range and this protection was mediated through androgen receptors [19], however these effects are dose-specific. Higher levels of androgen replacement resulted in increased infarct volumes [75]. It is possible that these divergent effects can also be due to the activation of membrane-associated versus intracellular androgen receptors as the membrane-associated receptor activation can enhance cell death in an *in vitro* model while activation of the traditional intracellular receptor leads to neuroprotection [23]. These data suggest that there is a complex effect of testosterone on neuronal survival after stroke, where dose, timing and the type of androgen all influence its neuronal effects. Clearly, more needs to be done to decipher the overall effects and potential mechanisms of action of androgens.

SEX HORMONE-INDEPENDENT MECHANISMS

In addition to the hormone-regulated events described above, several aspects of the sex differences observed following stroke appear to be independent of direct hormone action. There are clear differences in body size and vascular anatomy that are associated with an increased risk of stroke in males [72]. Increasingly the contribution of the sex chromosomal makeup of the organism is being recognized as a potential source of the sex difference. Cultured neurons from either male or female rats are differentially susceptible to an *in vitro* model of ischemia where male neurons are more vulnerable to cell death than female neurons cultured under identical conditions [18]. It was subsequently demonstrated that, neurons from males expressed higher levels of soluble epoxide hydrolase that contributes to neurotoxicity. Additionally it is possible that X or Y chromosome-linked genes may contribute to the inherent sex difference in response to stroke. Genes on the Y-chromosome are involved in regulating hypertension, which is a risk factor associated with the increased stroke risk in men [9]. Furthermore, polymorphisms of this gene are associated with increased hypertension. In females, an X-chromosome linked gene, XIAP, has been identified that inhibits apoptosis [34]. XIAP has recently been shown to be specifically regulated by a microRNA in females following stroke [62]. Disruption of this expression in females resulted in ischemic cell death comparable to that in males.

Another hormone-independent mechanism that appears to be in play in the brain is the epigenetic regulation of genes involved in neuroprotection or apoptosis. Epigenetic regulation involves post-replication and post-translational modification of DNA and histones that lead to lasting changes in chromatin structure and thus changes in gene transcription (for review see [82] and [39]). DNA methylation is central to epigenetic control of gene expression. Methylation typically occurs at repeats of cytosine-phosphate-guanine sites primarily in the promoters of genes. DNA methyltransferase (DNMT) transfers a methyl group to the cytosine resulting in 5-methylcytosine [61]. The methylated region of DNA is then bound by DNA methyl binding proteins which can then prevent transcription machinery from binding, resulting in gene silencing in many cases [7, 39]. Increased DNA methyltransferase activity is associated with stroke and inhibiting DNA methyltransferase activity confers neuroprotection following MCAO [17]. In the hippocampus, expression of the methyl DNA binding proteins is altered in a spatial and temporal fashion following a transient ischemia [38]. We have demonstrated that the promoter of the ERα gene is progressively methylated during development and is essentially turned off in the adult rodent cortex [55]. Following MCAO the ERα gene is demethylated and subsequently re-expressed [15, 77]. Interestingly, this demethylation occurs only in female rats and is independent of estradiol treatment in the females[77]. MCAO also regulates the expression of the DNA methyltransferase that regulates the maintenance of DNA methylation, DNMT 1, in a similar sex-specific manner. Sex differences in DNMT gene expression have also been observed in the developing hypothalamus [5]. Together this data suggest that epigenetic modification of genes may play a role in regulating genes involved in neuronal survival and cell death following brain injury in a sex specific manner.

CONCLUSIONS AND FUTURE DIRECTIONS

Many factors impact the incidence and outcomes of individuals that experience an ischemic stroke. It is clear that age, sex and hormone exposure all contribute to the responses to stroke. We have reviewed several potential mechanisms underlying these contributions. Further investigation is needed to come to a more complete understanding of this complicated relationship. It is also increasingly clear that the age and sex of the animal models needs to be taken into consideration very carefully when drawing conclusions about causes and mechanisms of stroke. A deeper understanding of these mechanisms will

eventually result in the design of better prevention, treatment and therapeutic paradigms when particularly applied to aging men and women.

References

1. Ahlbom E, Grandison L, Bonfoco E, Zhivotovsky B, Ceccatelli S. Androgen treatment of neonatal rats decreases susceptibility of cerebellar granule neurons to oxidative stress in vitro. The European journal of neuroscience. 1999; 11:1285–1291. [PubMed: 10103123]
2. Ahlbom E, Prins GS, Ceccatelli S. Testosterone protects cerebellar granule cells from oxidative stress-induced cell death through a receptor mediated mechanism. Brain research. 2001; 892:255–262. [PubMed: 11172772]
3. Alkayed NJ, Harukuni I, Kimes AS, London ED, Traystman RJ, Hurn PD. Gender-linked brain injury in experimental stroke. Stroke. 1998; 29:159–165. discussion 166. [PubMed: 9445346]
4. Appelros P, Stegmayr B, Terent A. A review on sex differences in stroke treatment and outcome. Acta Neurol Scand. 2010; 121:359–369. [PubMed: 20002005]
5. Auger AP, Jessen HM, Edelmann MN. Epigenetic organization of brain sex differences and juvenile social play behavior. Horm Behav. 2011; 59:358–363. [PubMed: 20619265]
6. Azcoitia I, Sierra A, Veiga S, Garcia-Segura LM. Aromatase expression by reactive astroglia is neuroprotective. Annals of the New York Academy of Sciences. 2003; 1007:298–305. [PubMed: 14993062]
7. Bird AP, Wolffe AP. Methylation-induced repression--belts, braces, and chromatin. Cell. 1999; 99:451–454. [PubMed: 10589672]
8. Bousser MG. Estrogens, migraine, and stroke. Stroke. 2004; 35:2652–2656. [PubMed: 15459439]
9. Charchar FJ, Tomaszewski M, Padmanabhan S, Lacka B, Upton MN, Inglis GC, Anderson NH, McConnachie A, Zukowska-Szczechowska E, Grzeszczak W, Connell JM, Watt GC, Dominiczak AF. The Y chromosome effect on blood pressure in two European populations. Hypertension. 2002; 39:353–356. [PubMed: 11882572]
10. Coomber B, Gibson CL. Sustained levels of progesterone prior to the onset of cerebral ischemia are not beneficial to female mice. Brain research. 2010; 1361:124–132. [PubMed: 20850417]
11. Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM. Estradiol protects against ischemic injury. J Cereb Blood Flow Metab. 1998; 18:1253–1258. [PubMed: 9809515]
12. Dubal DB, Shughrue PJ, Wilson ME, Merchenthaler I, Wise PM. Estradiol modulates bcl-2 in cerebral ischemia: a potential role for estrogen receptors. J Neurosci. 1999; 19:6385–6393. [PubMed: 10414967]
13. Dubal DB, Wise PM. Neuroprotective effects of estradiol in middle-aged female rats. Endocrinology. 2001; 142:43–48. [PubMed: 11145565]
14. 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 U S A. 2001; 98:1952–1957. [PubMed: 11172057]
15. Dubal DB, Rau SW, Shughrue PJ, Zhu H, Yu J, Cashion AB, Suzuki S, Gerhold LM, Bottner MB, Dubal SB, Merchanthaler 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]
16. El-Ashry D, Chrysogelos SA, Lippman ME, Kern FG. Estrogen induction of TGF-alpha is mediated by an estrogen response element composed of two imperfect palindromes. The Journal of steroid biochemistry and molecular biology. 1996; 59:261–269. [PubMed: 9010318]
17. Endres M, Meisel A, Biniszkiewicz D, Namura S, Prass K, Ruscher K, Lipski A, Jaenisch R, Moskowitz MA, Dirnagl U. DNA methyltransferase contributes to delayed ischemic brain injury. J Neurosci. 2000; 20:3175–3181. [PubMed: 10777781]
18. Fairbanks SL, Young JM, Nelson JW, Davis CM, Koerner IP, Alkayed NJ. Mechanism of the sex difference in neuronal ischemic cell death. Neuroscience. 2012; 219:183–191. [PubMed: 22641086]

19. Frye CA, McCormick CM. Androgens are neuroprotective in the dentate gyrus of adrenalectomized female rats. Stress. 2000; 3:185–194. [PubMed: 10938579]
20. Gall SL, Donnan G, Dewey HM, Macdonell R, Sturm J, Gilligan A, Srikanth V, Thrift AG. Sex differences in presentation, severity, and management of stroke in a population-based study. Neurology. 2010; 74:975–981. [PubMed: 20181922]
21. Gall SL, Tran PL, Martin K, Blizzard L, Srikanth V. Sex differences in long-term outcomes after stroke: functional outcomes, handicap, and quality of life. Stroke. 2012; 43:1982–1987. [PubMed: 22569940]
22. Garcia-Segura LM, Cardona-Gomez P, Naftolin F, Chowen JA. Estradiol upregulates Bcl-2 expression in adult brain neurons. Neuroreport. 1998; 9:593–597. [PubMed: 9559922]
23. Gatson JW, Singh M. Activation of a membrane-associated androgen receptor promotes cell death in primary cortical astrocytes. Endocrinology. 2007; 148:2458–2464. [PubMed: 17303658]
24. Gibson CL, Murphy SP. Progesterone enhances functional recovery after middle cerebral artery occlusion in male mice. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 2004; 24:805–813. [PubMed: 15241189]
25. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Executive Summary: Heart Disease and Stroke Statistics--2013 Update: A Report From the American Heart Association. Circulation. 2013; 127:143–152. [PubMed: 23283859]
26. Golomb MR, Fullerton HJ, Nowak-Gottl U, Deveber G. Male predominance in childhood ischemic stroke: findings from the international pediatric stroke study. Stroke. 2009; 40:52–57. [PubMed: 18787197]
27. Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, Ernster VL, Cummings SR. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med. 1992; 117:1016–1037. [PubMed: 1443971]
28. Grodstein F, Manson JE, Stampfer MJ, Rexrode K. Postmenopausal hormone therapy and stroke: role of time since menopause and age at initiation of hormone therapy. Arch Intern Med. 2008; 168:861–866. [PubMed: 18443262]
29. Haast RA, Gustafson DR, Kiliaan AJ. Sex differences in stroke. J Cereb Blood Flow Metab. 2012; 32:2100–2107. [PubMed: 23032484]
30. Haberman S, Capildeo R, Rose FC. Sex differences in the incidence of cerebrovascular disease. J Epidemiol Community Health. 1981; 35:45–50. [PubMed: 7264532]
31. Hall ED, Pazara KE, Linseman KL. Sex differences in postischemic neuronal necrosis in gerbils. J Cereb Blood Flow Metab. 1991; 11:292–298. [PubMed: 1997500]
32. Harman SM, Naftolin F, Brinton EA, Judelson DR. Is the estrogen controversy over? Deconstructing the Women's Health Initiative study: a critical evaluation of the evidence. Ann N Y Acad Sci. 2005; 1052:43–56. [PubMed: 16024750]
33. Hawk T, Zhang YQ, Rajakumar G, Day AL, Simpkins JW. Testosterone increases and estradiol decreases middle cerebral artery occlusion lesion size in male rats. Brain research. 1998; 796:296–298. [PubMed: 9689481]
34. Holcik M, Korneluk RG. XIAP, the guardian angel. Nature reviews Molecular cell biology. 2001; 2:550–556.
35. Hurn PD, Macrae IM. Estrogen as a neuroprotectant in stroke. J Cereb Blood Flow Metab. 2000; 20:631–652. [PubMed: 10779008]
36. Ishrat T, Sayeed I, Atif F, Hua F, Stein DG. Progesterone is neuroprotective against ischemic brain injury through its effects on the phosphoinositide 3-kinase/protein kinase B signaling pathway. Neuroscience. 2012; 210:442–450. [PubMed: 22450229]
37. Jeppesen LL, Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS, Winther K. Decreased serum testosterone in men with acute ischemic stroke. Arteriosclerosis, thrombosis, and vascular biology. 1996; 16:749–754.

38. Jung BP, Zhang G, Ho W, Francis J, Eubanks JH. Transient forebrain ischemia alters the mRNA expression of methyl DNA-binding factors in the adult rat hippocampus. Neuroscience. 2002; 115:515–524. [PubMed: 12421618]
39. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci. 2006; 31:89–97. [PubMed: 16403636]
40. Li K, Futrell N, Tovar S, Wang LC, Wang DZ, Schultz LR. Gender influences the magnitude of the inflammatory response within embolic cerebral infarcts in young rats. Stroke. 1996; 27:498–503. [PubMed: 8610320]
41. Liang Y, Belford S, Tang F, Prokai L, Simpkins JW, Hughes JA. Membrane fluidity effects of estratrienes. Brain Res Bull. 2001; 54:661–668. [PubMed: 11403993]
42. Liu F, McCullough LD. Interactions between age, sex, and hormones in experimental ischemic stroke. Neurochem Int. 2012; 61:1255–1265. [PubMed: 23068990]
43. Liu M, Dziennis S, Hurn PD, Alkayed NJ. Mechanisms of gender-linked ischemic brain injury. Restorative neurology and neuroscience. 2009; 27:163–179. [PubMed: 19531872]
44. Liu R, Wang X, Liu Q, Yang SH, Simpkins JW. Dose dependence and therapeutic window for the neuroprotective effects of 17beta-estradiol when administered after cerebral ischemia. Neurosci Lett. 2007; 415:237–241. [PubMed: 17331646]
45. Manolio TA, Kronmal RA, Burke GL, O'Leary DH, Price TR. Short-term predictors of incident stroke in older adults. The Cardiovascular Health Study. Stroke. 1996; 27:1479–1486. [PubMed: 8784116]
46. Marjoribanks J, Farquhar C, Roberts H, Lethaby A. Long term hormone therapy for perimenopausal and postmenopausal women. Cochrane database of systematic reviews. 2012; 7:CD004143.
47. Miranda RC, Toran-Allerand CD. Developmental expression of estrogen receptor mRNA in the rat cerebral cortex: a nonisotopic in situ hybridization histochemistry study. Cereb Cortex. 1992; 2:1–15. [PubMed: 1633405]
48. Miranda RC, Sohrabji F, Toran-Allerand CD. Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. Proc Natl Acad Sci U S A. 1993; 90:6439–6443. [PubMed: 8341652]
49. Murphy SJ, Traystman RJ, Hurn PD, Duckles SP. Progesterone exacerbates striatal stroke injury in progesterone-deficient female animals. Stroke; a journal of cerebral circulation. 2000; 31:1173–1178.
50. Murphy SJ, Littleton-Kearney MT, Hurn PD. Progesterone administration during reperfusion, but not preischemia alone, reduces injury in ovariectomized rats. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 2002; 22:1181–1188. [PubMed: 12368656]
51. Murphy SJ, McCullough LD, Smith JM. Stroke in the female: role of biological sex and estrogen. ILAR J. 2004; 45:147–159. [PubMed: 15111734]
52. Ogeng'o JA, Olabu BO. Cortical stroke in Kenya. Int J Stroke. 2010; 5:517–518. [PubMed: 21050415]
53. Pike CJ. Estrogen modulates neuronal Bcl-xL expression and beta-amyloid-induced apoptosis: relevance to Alzheimer's disease. J Neurochem. 1999; 72:1552–1563. [PubMed: 10098861]
54. Pike CJ. Testosterone attenuates beta-amyloid toxicity in cultured hippocampal neurons. Brain research. 2001; 919:160–165. [PubMed: 11689174]
55. Prewitt AKW, ME. Changes in estrogen receptor-alpha mRNA in the mouse cortex during development. Brain Res. 2007; 1134:62–69. [PubMed: 17207781]
56. Putaala J, Metso AJ, Metso TM, Konkola N, Kraemer Y, Haapaniemi E, Kaste M, Tatlisumak T. Analysis of 1008 consecutive patients aged 15 to 49 with first-ever ischemic stroke: the Helsinki young stroke registry. Stroke. 2009; 40:1195–1203. [PubMed: 19246709]
57. Reeves MJ, Bushnell CD, Howard G, Gargano JW, Duncan PW, Lynch G, Khatiwoda A, Lisabeth L. Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. Lancet Neurol. 2008; 7:915–926. [PubMed: 18722812]

58. Ritzel RM, Capozzi LA, McCullough LD. Sex, stroke, and inflammation: The potential for estrogen-mediated immunoprotection in stroke. Horm Behav. 2013; 63:238–253. [PubMed: 22561337]
59. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2008; 117:e25–146. [PubMed: 18086926]
60. 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]
61. Sharma RP, Grayson DR, Guidotti A, Costa E. Chromatin, DNA methylation and neuron gene regulation--the purpose of the package. J Psychiatry Neurosci. 2005; 30:257–263. [PubMed: 16049569]
62. Siegel C, Li J, Liu F, Benashski SE, McCullough LD. miR-23a regulation of X-linked inhibitor of apoptosis (XIAP) contributes to sex differences in the response to cerebral ischemia. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:11662–11667. [PubMed: 21709246]
63. 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]
64. Simpkins JW, Yang SH, Sarkar SN, Pearce V. Estrogen actions on mitochondria--physiological and pathological implications. Mol Cell Endocrinol. 2008; 290:51–59. [PubMed: 18571833]
65. Singh M, Setalo G Jr, Guan X, Warren M, Toran-Allerand CD. Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. J Neurosci. 1999; 19:1179–1188. [PubMed: 9952396]
66. Sohrabji F, Greene LA, Miranda RC, Toran-Allerand CD. Reciprocal regulation of estrogen and NGF receptors by their ligands in PC12 cells. J Neurobiol. 1994; 25:974–988. [PubMed: 7525871]
67. Spengos K, Vemmos KN. Female predominance at very young ages and other similarities between Finnish and Greek young ischemic stroke patients. Stroke. 2009; 40:e491. author reply e492. [PubMed: 19498177]
68. Sudlow CL, Warlow CP. Comparable studies of the incidence of stroke and its pathological types: results from an international collaboration. International Stroke Incidence Collaboration. Stroke. 1997; 28:491–499. [PubMed: 9056601]
69. Sunday L, Osuna C, Krause DN, Duckles SP. Age alters cerebrovascular inflammation and effects of estrogen. Am J Physiol Heart Circ Physiol. 2007; 292:H2333–2340. [PubMed: 17208996]
70. Suzuki S, Brown CM, Dela Cruz CD, Yang E, Bridwell DA, Wise PM. Timing of estrogen therapy after ovariectomy dictates the efficacy of its neuroprotective and antiinflammatory actions. Proc Natl Acad Sci U S A. 2007; 104:6013–6018. [PubMed: 17389368]
71. Teixeira C, Reed JC, Pratt MA. Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. Cancer Res. 1995; 55:3902–3907. [PubMed: 7641210]
72. Tian Y, Stamova B, Jickling GC, Liu D, Ander BP, Bushnell C, Zhan X, Davis RR, Verro P, Pevec WC, Hedayati N, Dawson DL, Khoury J, Jauch EC, Pancioli A, Broderick JP, Sharp FR. Effects of gender on gene expression in the blood of ischemic stroke patients. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 2012; 32:780–791. [PubMed: 22167233]
73. Toung TJ, Traystman RJ, Hurn PD. Estrogen-mediated neuroprotection after experimental stroke in male rats. Stroke. 1998; 29:1666–1670. [PubMed: 9707210]
74. Turtzo LC, McCullough LD. Sex-specific responses to stroke. Future Neurol. 2010; 5:47–59. [PubMed: 20190872]
75. Uchida M, Palmateer JM, Herson PS, DeVries AC, Cheng J, Hurn PD. Dose-dependent effects of androgens on outcome after focal cerebral ischemia in adult male mice. Journal of cerebral blood

flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 2009; 29:1454–1462. [PubMed: 19436313]

76. Wassertheil-Smoller S, Hendrix SL, Limacher M, Heiss G, Kooperberg C, Baird A, Kotchen T, Curb JD, Black H, Rossouw JE, Aragaki A, Safford M, Stein E, Laowattana S, Mysiw WJ. Effect of estrogen plus progestin on stroke in postmenopausal women: the Women's Health Initiative: a randomized trial. Jama. 2003; 289:2673–2684. [PubMed: 12771114]
77. Westberry JM, Prewitt AK, Wilson ME. Epigenetic Regulation of the Estrogen Receptor Alpha Promoter in the Cereberal Cortex Following Ischemia in Male and Female Rats. Neuroscience. 2008; 152:982–989. [PubMed: 18353557]
78. Wilson ME, Dubal DB, Wise PM. Estradiol protects against injury-induced cell death in cortical explant cultures: a role for estrogen receptors. Brain Res. 2000; 873:235–242. [PubMed: 10930549]
79. Wilson, ME.; Wise, PM. Society for Neuroscience Abstracts. 2001. Estradiol suppresses pro-apoptotic signals in a cortical explant model of injury.
80. Wise PM, Dubal DB, Wilson ME, Rau SW, Bottner M, Rosewell KL. Estradiol is a protective factor in the adult and aging brain: understanding of mechanisms derived from in vivo and in vitro studies. Brain Res Brain Res Rev. 2001; 37:313–319. [PubMed: 11744096]
81. Wise PM, Dubal DB, Wilson ME, Rau SW, Liu Y. Estrogens: trophic and protective factors in the adult brain. Front Neuroendocrinol. 2001; 22:33–66. [PubMed: 11141318]
82. Wolffe AP, Matzke MA. Epigenetics: regulation through repression. Science. 1999; 286:481–486. [PubMed: 10521337]
83. Yang SH, Perez E, Cutright J, Liu R, He Z, Day AL, Simpkins JW. Testosterone increases neurotoxicity of glutamate in vitro and ischemia-reperfusion injury in an animal model. Journal of applied physiology. 2002; 92:195–201. [PubMed: 11744660]
84. Zhang YQ, Shi J, Rajakumar G, Day AL, Simpkins JW. Effects of gender and estradiol treatment on focal brain ischemia. Brain Res. 1998; 784:321–324. [PubMed: 9518671]