Chronic estradiol exposure – harmful effects on behavior, cardiovascular and reproductive functions

Sheba M J MohanKumar¹, Priya Balasubramanian², Madhan Subramanian³ and P S MohanKumar¹

¹Department of Veterinary Biosciences and Diagnostic Imaging, University of Georgia, Athens, Georgia, USA,

²Reynolds Oklahoma Center on Aging, Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA and

³Department of Physiological Sciences, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, Oklahoma, USA

Correspondence should be addressed to S M J MohanKumar; Email: shebamk@uga.edu

Abstract

Estradiol (E2) is a female hormone that is produced largely by the ovaries, but also by the adrenal glands, fat and liver. It is present in the circulation of both males and females. Many studies in the literature have described how E2 is beneficial to the body in terms of preventing bone loss, affording protection in ischemia reperfusion injury, relieving symptoms of menopause, maintaining vaginal health and helping with ovarian failure or hypogonadism. Beneficial effects on the brain have been reported to include protection against memory loss, neuronal degeneration, changes in cognition, mood and behavior. However, the effects of E2 exposure on the neuroendocrine system have not been understood completely. This is because differences in doses, preparation and duration of exposure have produced variable results ranging from beneficial, to no change, or to detrimental. Studies in our lab over the last few years have shown that chronic exposures to low levels of E2 in young rats can produce specific effects on the neuroendocrine system. We have observed that these exposures can induce reproductive senescence, hypertension, anxiety-like behavior and cause degenerative changes in specific neuronal populations leading to hyperprolactinemia. The purpose of the review is to present evidence from the literature for these effects and to discuss the underlying molecular mechanisms.

Reproduction (2018) **156** R169–R186

Introduction

Estrogens are female hormones that are responsible for the onset and progression of female reproductive cycles and the development of secondary sexual characteristics typical of females. There are three kinds of estrogens in circulation: estrone (E1), estradiol (E2) and estriol (E3). Of these, E2 is present at higher levels, fluctuates throughout the life of females, and much of the endogenous estrogenic activity has been attributed to this hormone. E2 plays important roles in the regulation of reproduction and energy metabolism and influences a number of other hormones such as thyroid hormones (Santin & Furlanetto 2011). It is also reported to produce a variety of beneficial effects especially when it is used to restore estrogens in older women or ovariectomized animal models. These effects include preventing bone loss in early postmenopausal women (Cooper *et al.* 1999), preventing age-related changes in the skin (Patriarca *et al.* 2013), improving insulin sensitivity in ovariectomized animals (Shen *et al.* 2014) and in postmenopausal women (Raudaskoski *et al.* 1999), preventing the incidence of type 2 diabetes early in menopause (Pereira *et al.* 2015) and improving cognitive functions after focal ischemia in experimental

animals (Soderstrom *et al.* 2009) and in postmenopausal women with Alzheimer's disease (Wharton *et al.* 2011).

There are numerous studies describing the beneficial effects of E2 exposure on the brain and many of these beneficial effects have been observed after acute E2 exposures (Bagger *et al.* 2005, Whitmer *et al.* 2011, Zhang *et al.* 2018). However, the effects of chronic E2 exposures have not been explored in great detail. These exposures are more relevant since humans are exposed to a variety of estrogenic compounds from endogenous estrogens to phytoestrogens and estrogenic endocrine disruptors in the environment (Adeel *et al.* 2017). Most of these exposures occur at low, persistent levels and their effects on neuroendocrine functions are not understood, recognized or are frequently dismissed. More recently, these effects have received considerable attention from the scientific community and regulatory bodies (Vandenberg *et al.* 2012). This is because low-dose exposures may contribute to many non-communicable diseases such as hypertension, reproductive and metabolic disorders that are widely prevalent today (Gore *et al.* 2015). Therefore, it is important to understand the effects of chronic exposure to low levels of E2 on neuroendocrine functions and its relationship to the development of these disorders.

E2 is capable of influencing a variety of neurotransmitter systems (Barth *et al.* 2015) that in turn, could mediate its effects on neuroendocrine function. For the purpose of this review, we will focus on norepinephrine (NE). This is because the catecholamines, NE and dopamine (DA) have been implicated in the neuroendocrine regulation of reproductive functions for a long time. There are several early studies in the 1970s and 1980s that have used agonists, inhibitors, catecholamine depletors and neurotoxins that suggest that catecholamines play an important role in GnRH and prolactin regulation and alterations in behavior (Mueller & Nistico 1989). NE is believed to play a prominent role especially when ovarian steroids are administered (Mueller & Nistico 1989). Moreover, there is strong evidence to suggest that catecholamines are important for the regulation of cardiovascular function (Blessing & Reis 1982, Blessing *et al.* 1982, Madden & Sved 2003).

Chronic E2 exposure and reproductive functions

E2 in reproductive physiology

Reproduction is a complex process involving coordinated regulation of the brain stem, the hypothalamus, the anterior pituitary and the gonads. When follicles in the ovaries mature, they produce E2. Increase in circulating E2 levels stimulates brainstem noradrenergic neurons (Barraclough 1994) to release NE, which in turn stimulates gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus (MohanKumar *et al.* 1994). E2 can also act directly on GnRH neurons to stimulate them (Herbison & Pape 2001). Upon stimulation, these neurons release GnRH via their terminals located in the median eminence and GnRH enters the portal circulation to reach the anterior pituitary and acts on gonadotrophs (Schally *et al.* 1971). Here, it stimulates the release of luteinizing hormone (LH), which, in turn acts on the ovaries to cause ovulation. This process is common to mammals, including humans (Barraclough & Wise 1982).

Female rats attain puberty when they are about 2 months old and begin to have regular estrous cycles that are 4–5 days long. Each day is a specific stage of the cycle and the rat goes through proestrous, estrous, metestrous and diestrous in one cycle (Long & Evans 1922). Proestrous is marked by a gradual rise in circulating E2 in the afternoon that stimulates noradrenergic neurons in the brain stem (McEwen *et al.* 2012) and increases NE release in the medial preoptic area (MPA) of the hypothalamus (MohanKumar *et al.* 1994), an area intimately involved in GnRH regulation (Herbison 1997). This activates GnRH neurons leading to the preovulatory surge in LH that causes ovulation (Wise *et al.* 1981, Wise 1984, MohanKumar *et al.* 1994). Age-related reduction in NE activity in the MPA plays a role in subduing the LH surge during middle

age (MohanKumar & MohanKumar 2004). This causes irregular cyclicity as the animals reach 11–12 months of age. Soon after that, rats enter a state of acyclicity called ‘constant estrus or persistent estrus’ because there is not enough LH to induce ovulation and they are stuck in the ‘estrus’ state (Steger *et al.* 1980). It is believed that repeated exposure to endogenous E2 peaks during each estrous cycle is the primary cause for loss of LH surges and cessation of estrous cyclicity in rodents (Lu *et al.* 1981, Nelson *et al.* 1981).

In our studies, we mimicked this phenomenon by exposing intact young (2–3 month old) cycling rats to low levels of exogenous E2 (slow release pellets implanted subcutaneously, capable of releasing 20 ng estradiol-17 β /day) for 30, 60 or 90 days and evaluated NE activity in the MPA, serum LH, serum estradiol and estrous cyclicity. A majority (about 80%) of the animals that were exposed to E2 for 30 days exhibited regular estrous cycles and had circulating E2 levels of about 35 pg/mL. When they were exposed to E2 for 60 days, their E2 levels increased to 70 pg/mL and 70–80% of them were in persistent estrus; however, a majority of them returned to normal cyclicity at the end of E2 exposure (about 70%). More than 80% of the animals that were exposed to E2 for 90 days were in persistent estrus and did not regain normal cycles at the end of E2 exposure. These animals had circulating E2 levels of 95 pg/mL. In these animals, there was a significant reduction in NE in the MPA and serum LH correlating well with the loss of cyclicity (Kasturi *et al.* 2009). The serum levels of E2 in these animals were in the range of 45–80 pg/mL, which corresponds to the levels observed during the afternoon of proestrus in regularly cycling animals (Lu *et al.* 1979). In other words, rats implanted with E2 pellets were continuously exposed to proestrous levels of E2. If we consider the normal aging process in rats, they experience roughly 7 estrous cycles (and therefore 7 days of proestrous) a month. They enter the constant estrous state after 8 months of regular cycling (or after 56 days of proestrous). In our studies, the dose of E2 used made animals enter constant estrous after 60 days of exposure (Kasturi *et al.* 2009, Gilbreath *et al.* 2014). These results suggest that chronic exposure to 20 ng of E2/day accelerates reproductive aging.

Other studies in ovariectomized rats have found that E2 implants can suppress GnRH activation and block the steroid-induced LH surge (Tsai & Legan 2001, 2002). These implants were capable of generating circulating E2 levels of 75 pg/mL and were kept in place for a maximum of 4 weeks. The steroid-induced LH surges began to decrease in amplitude by 2 weeks and were completely absent after 4 weeks of E2 exposure (Tsai & Legan 2002). These investigators also reported that the reduction in LH levels after E2 exposure did not involve changes in estrogen receptor (ER) expression on GnRH neurons (Legan & Tsai 2003). Further, using the ovariectomized rat model, they demonstrated that acute

E2 exposure was capable of suppressing NE release in the hypothalamus (Legan & Callahan 1999).

In an effort to understand the underlying mechanism by which chronic E2 exposure decreases NE levels, we have focused on the biosynthetic pathway for NE. Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of NE and dopamine. One means by which noradrenergic activity can be reduced is by nitration of tyrosine residues on TH (Huie & Padmaja 1993, Ischiropoulos *et al.* 1995). Peroxynitrite, an oxidant that arises from the reaction of nitric oxide and superoxide anion, gives rise to nitrating species that can nitrate tyrosine moieties on proteins (Radi 2013). This can lead to inactivation of protein function, particularly of proteins like TH, which contain several tyrosine residues in their structure (Ara *et al.* 1998). The tyrosine moieties in TH are clustered around the active center of the enzyme (Imam *et al.* 2001) and nitration of the tyrosine residues causes steric hindrance, which, in turn inhibits the activity of this enzyme (Imam *et al.* 2001). Thus, measurement of nitration of TH can be used as an indicator of degeneration of noradrenergic neurons. In fact, we have shown that there is a gradual increase in nitration of TH in the MPA with increasing duration of E2 exposure that parallels the reduction in NE levels (Kasturi *et al.* 2013). Therefore, it is possible that E2-induced increase in nitric oxide metabolism in the hypothalamus could cause nitration of TH and lead to a loss of noradrenergic neuronal function.

Brain-derived neurotrophic factor (BDNF) is another likely candidate to be involved in the neuroendocrine effects of chronic E2 exposure. BDNF is widely distributed throughout the brain including cerebral and cerebellar cortex, hypothalamus, midbrain, hindbrain and the brain stem and is considered to be a protective factor (Hofer *et al.* 1990, Wetmore *et al.* 1990, Merlio *et al.* 1992, Masana *et al.* 1993, Viant *et al.* 2000). E2 is a potent regulator of BDNF (Singh *et al.* 1995, Sohrabji *et al.* 1995, Wang & Zheng 1998, Kenny *et al.* 2000, West *et al.* 2001, Wu *et al.* 2004, Yan *et al.* 2004) since the BDNF gene promoter region has an estrogen-responsive element (Amantea *et al.* 2005). Moreover, ERs have been identified in BDNF-synthesizing neurons (Miranda *et al.* 1993). E2 and BDNF have overlapping actions in the forebrain and act through common second messengers such as ERK1 and ERK 2 (Singh *et al.* 1999). BDNF and NE can be co-localized in neurons (Hwang *et al.* 2013) and can influence each others' expression (Juric *et al.* 2008, Sakata & Duke 2014). Therefore, there is a possibility that changes in BDNF expression can affect NE levels and vice versa. In the chronic E2 exposure model, we have found that BDNF levels decrease in specific brain regions such as the amygdala (Balasubramanian *et al.* 2014). However, there are no studies exploring the involvement of BDNF in the neuroendocrine regulation of reproductive functions. Further studies are needed to explore the role of BDNF

in the regulation of noradrenergic activity in the context of GnRH regulation after chronic E2 exposure.

Other possible mechanisms of decreasing NE levels in the MPA involve increasing GABA release locally (Demling *et al.* 1985, Sirivelu *et al.* 2009a) or by increasing cytokine levels, which can in turn act on brainstem NE neurons (Sirivelu *et al.* 2012) and inhibit NE synthesis. E2 can directly stimulate cytokine production from a variety of cells including peritoneal macrophages (Calippe *et al.* 2008). In our model, E2 exposure elevated IL-1 β levels in specific brain regions such as the MPA (unpublished) and the arcuate nucleus (MohanKumar *et al.* 2011). The source of proinflammatory cytokines in our model is not clear. It is likely that E2 acts through its receptors to increase cytokines. These receptors have been identified in specific cell types such as ependymal, endothelial cells, neurons and glial cells in several areas of the hypothalamus and the brain (Langub & Watson 1992, Paech *et al.* 1997, Azcoitia *et al.* 1999, Cardona-Gomez *et al.* 2000, Donahue *et al.* 2000, Gundlach *et al.* 2000, Hosli *et al.* 2000, Su *et al.* 2001, Garcia-Ovejero *et al.* 2002). Cytokines such as IL-1 β are known to cross the blood-brain barrier and inhibit NE synthesis by acting on brain stem noradrenergic neurons (Sirivelu *et al.* 2009b) or suppress NE release by increasing GABA levels locally in the hypothalamus (Sirivelu *et al.* 2009a). Therefore, the cytokines generated locally in response to chronic E2 exposure could act similarly.

Kisspeptin, neurokinin B and dynorphin could also be potential mediators in chronic E2-induced suppression of reproductive functions. KNDy neurons that secrete these mediators are believed to relay the negative feedback effects of E2 on GnRH secretion (Rance 2009, Mittelman-Smith *et al.* 2012). Chronic exposure to E2 was found to decrease KNDy neuron size and spine density and reduce the expression of kisspeptin and neurokinin B in these neurons. These changes could potentially contribute to the reduction in GnRH levels after chronic E2 exposure (Cholanian *et al.* 2015). It should be noted that these KNDy neurons are distinct from subpopulations of kisspeptin-positive neurons in the hypothalamus that are involved in stimulating GnRH secretion (Herbison 2008).

Chronic E2 exposure and effects on TIDA neurons

Tuberoinfundibular dopaminergic (TIDA) neurons are responsible for secreting dopamine that enters the portal circulation to inhibit prolactin secretion by the anterior pituitary (Fuxe 1964). The number of dopamine receptors in the anterior pituitary increases sharply during the afternoon of proestrous, coinciding with the occurrence of the surge in plasma prolactin (Heiman & Ben-Jonathan 1982a). During this time frame, dopamine concentrations in the portal circulation and dopamine turnover in the median eminence drop (Ben-Jonathan *et al.* 1977, Rance *et al.* 1981), suggesting that the

reduction in dopamine levels contribute to the increase in prolactin secretion. E2 is believed to be one of the contributing factors for the increase in prolactin, either by decreasing dopaminergic activity in TIDA neurons or possibly through a direct effect on the anterior pituitary (Raymond *et al.* 1978, Heiman & Ben-Jonathan 1982a, Demarest *et al.* 1984, Kohama *et al.* 1992, Lawson *et al.* 1993, Maeda *et al.* 1996, Csakvari *et al.* 2008, Morel *et al.* 2009) (Table 1). E2 treatment for up to 10 days produced a dramatic increase in prolactin mRNA and serum prolactin levels (Lawson *et al.* 1993). E2 also decreases the number of dopamine-binding sites in the anterior pituitary (Heiman & Ben-Jonathan 1982b). These effects are probably mediated through ERs located on lactotrophs (Keefer *et al.* 1976). Although these studies were acute in nature, they provided clues to what might happen during the natural aging process in rats.

Female rats enter the constant estrous state when they are 11–12 months of age and they remain in this state for about 6 months. Prolactin levels in the early stages of constant estrous are about 40 ng/mL and increase to around 200 ng/mL toward the end of constant estrous (Goya *et al.* 1991). The reason for this several fold increase has been attributed to a reduction in dopamine levels in the median eminence (Sarkar *et al.* 1984). Initially, it was believed that the persistent increase in E2 levels in the constant estrous state continuously stimulated prolactin secretion. This, in turn increased dopamine production in TIDA neurons to turn down prolactin production (Simpkins 1983, Sarkar *et al.* 1984). However, with time, the continuous stimulation by prolactin causes TIDA neurons to become exhausted ultimately leading to lower dopamine production (Simpkins 1983, Sarkar *et al.* 1984). There are other physiological changes that occur with aging in the hypothalamus leading to the notion that other mechanisms could also be in play. Gliosis is a phenomenon that is commonly observed with aging. E2 exposure is known to increase astrocyte activity specifically in the arcuate nucleus of the hypothalamus where TIDA neuronal cell bodies are located (Kohama *et al.* 1995, Stone *et al.* 1998). Moreover, glial fibrillary acidic protein (GFAP) that is produced by activated astrocytes has an estrogen responsive element in its promoter region (Stone *et al.* 1998). We have found an increase in GFAP expression in the chronic E2 exposure model after 60 and 90 days of exposure that was comparable to what is seen in constant estrous rats (MohanKumar *et al.* 2011). Glial cells are highly dynamic cells that are responsible for maintaining homeostasis in the brain (Araque 2006). Although astrocytes are the predominant glial cells in the CNS, microglia also contribute significantly to the pathophysiology of the CNS. Microglia when activated, are capable of releasing a variety of proinflammatory cytokines, including IL-1 β (Lynch *et al.* 2010). In the chronic E2 exposure model, we found significant elevations in IL-1 β levels in the arcuate nucleus after 60 and 90 days of exposure

and paralleled IL-1 β levels in old constant estrous rats (MohanKumar *et al.* 2011). E2 stimulates microglia most probably through a toll-like receptor (Young *et al.* 2014). Besides cytokines, microglia have been implicated in the generation of a variety of other molecules: superoxide and other oxidants through the NADPH oxidase pathway and nitric oxide and related molecules through the inducible nitric oxide synthase (iNOS) pathway (Block & Hong 2007). They can also decrease the supply of protective factors, such as BDNF and insulin-like growth factor-1 (IGF-1) (Brown & Vilalta 2015). In fact, chronic E2 exposure increases nitric oxide levels in the arcuate nucleus and the levels are similar to what is seen in constant estrous rats (MohanKumar *et al.* 2011). Elevations in nitric oxide-related free radicals can cause nitration of TH as explained previously. Peroxynitrite, a powerful oxidant that is formed by the reaction between nitric oxide and superoxide anion, reacts with the metal center of TH and nitrates tyrosine residue 423. This results in a loss of enzyme activity (Blanchard-Fillion *et al.* 2001). Loss of TH function, not only impairs the synthesis of NE, but also drastically affects dopamine production since it is a synthesizing enzyme required for both NE and dopamine (Nakashima *et al.* 2009). We have been able to block this effect in iNOS-knockout mice, but not IL-1 receptor-knockout mice (our unpublished observations), suggesting that iNOS plays a key role in this phenomenon. Besides nitration of TH, dopamine itself is capable of being nitrated. In fact, there is experimental evidence that indicates that significant amounts of dopamine can get nitrated at pH 7 and nitrated dopamine fails to bind to D1 dopamine receptor (Daveu *et al.* 1997). These studies indicate that nitric oxide-related free radical generation can have a significant impact on aging processes and processes that mimic aging.

Chronic E2 exposure and cardiovascular function

It is well established that the prevalence of hypertension increases with age. In the United States, roughly 1 in 3 people are affected by high blood pressure and only half of them maintain good control over their blood pressures using medication or lifestyle changes (Booth *et al.* 2017). While roughly 25% of men and 19% of women develop hypertension when they are 35 years of age, the prevalence is 66% in men and 78% in women when they are 75 years or older (Hayward & Kelly 1997). The reason for the dramatic change in older women is attributed to loss of estrogen due to menopause and related changes in arterial walls (Lima *et al.* 2012). This led to the increasing use of hormonal replacement therapy (HRT) in postmenopausal women as a way to replace the loss of endogenous estrogen. Use of HRT in postmenopausal women became controversial after the results from two large randomized clinical trials, namely the Women's Health Initiative (WHI) (Manson

Table 1 Effects of chronic administration of different estrogenic preparations on TIDA activity in animal models.

Species	Type of E2	Conadectomy	Route	Dose	Duration	Age at exposure	Effects on TIDA neurons and prolactin	Reference
Female Holtzman rats	17 β -Estradiol or diethylstilbesterol	Yes	s.c. infusion via silastic capsules		4 h to 10 days	Adulthood	Prolactin mRNA in the anterior pituitary doubled on the 10th day of treatment. Pituitary prolactin levels paralleled mRNA levels until day 7 then decreased significantly at day 10. Serum prolactin levels also significantly increased at day 10 with fluctuations during earlier timepoints	Lawson <i>et al.</i> (1993)
Female rats	Estradiol benzoate	Yes	s.c. infusion via silastic capsules		6, 12 and 18 days	Adulthood	Serum prolactin concentrations were markedly increased at all timepoints whereas the rate of DOPA accumulation was increased at 6 days but not at 12 days, and was decreased at 18 days. The concentration of DA in the median eminence was reduced at all timepoints	Demarest <i>et al.</i> (1984)
Female Sprague–Dawley rats	17 β -Estradiol	Yes	s.c. infusion via silastic capsules and intramuscular injections	40 μ g – once a week	7 weeks	4 months	Subcutaneous or i.m. administration of estrogen for 7 weeks induced a marked hyperprolactinemia with no detectable changes in THmRNA in the arcuate nucleus. However, 30 weeks after estrogen withdrawal, TH neuron numbers were back to normal and serum prolactin returned to basal levels	Morel <i>et al.</i> (2009)
Female Wistar rats	17 β -Estradiol	Yes	ICV infusion	1 μ m using Alzet osmotic minipumps	1, 3 and 7 days	Adulthood	While serum prolactin and prolactin mRNA increased initially at 3 days, there was no change at 7 days of E2 treatment. Similarly, DOPAC/DA ratio was significantly higher at day 1, but no longer different at 3 and 7 days of treatment	Maeda <i>et al.</i> (1996)
Female Sprague–Dawley rats	17 β -Estradiol	No	s.c. slow release pellets	20 ng/day	30, 60 or 90 days	4–5 months old	Chronic estrogen treatment increased GFAP, IL-1 β and nitrate levels in the arcuate nucleus and increased nitration of TH in the median eminence. This was associated with increase in serum prolactin levels	Mohankumar <i>et al.</i> (2011)
Female C57BL/6J mice	17 β -Estradiol	No	Through drinking water	850 μ g/kg/day	3 months	4 and 9 months old	Loss of cyclicity due to chronic E2 treatment was not associated with changes in the number of TIDA neurons in the arcuate nucleus	Kohama <i>et al.</i> (1992)
Male and female CFY albino rats	17 β -Estradiol	Yes	Single dose by s.c. injection	45 μ g/kg/day	24 h	3 months	In females, E2 treatment decreased the numerical density of GABAergic synapses on TH immunoreactive neurons, but not on nondopaminergic neurons, whereas in males, E2I treatment increased inhibitory synapses onto nondopaminergic neurons but did not affect the number of inhibitory terminals onto TH-IR neurons	Csakvari <i>et al.</i> (2008)

et al. 2003) and the Heart and Estrogen/progestin Replacement Study (HERS I and II) (Hulley *et al.* 2002, 1998), showed increased incidence of cardiovascular diseases like stroke and myocardial infarction in women on HRT. Table 2 provides a list of studies using various estrogenic preparations and their effects on cardiovascular function.

In light of these findings, it was important to understand the effects of chronic E2 exposure on the cardiovascular system. In fact, the dose of E2 used in the chronic exposure model mimicked oral contraceptive use in young women and HRT in older women. Chronic E2 exposure increased systolic and diastolic blood pressure (BP) without affecting heart rate in female rats (Subramanian *et al.* 2011). In fact, systolic BP begins to rise 3 weeks after the beginning of E2 exposure (Subramanian *et al.* 2017). A number of studies (both basic and clinical) have reported increases in BP in experimental animals and women after varying doses and duration of E2 exposure (Lim *et al.* 1987, Woods 1988, Byrne *et al.* 1994, Chasan-Taber *et al.* 1996, Olatunji & Soladoye 2006, 2008). In contrast to these findings, shorter durations of exposure (3 or 8 weeks) to E2 (1.5 mg, s.c.) have shown beneficial effects on the cardiovascular system both in ovariectomized and experimental hypertensive animals (Brosnihan *et al.* 1997, Gimenez *et al.* 2006). In these animals, there was a reduction in mean arterial pressure due to reduced synthesis of and response to angiotensin II (Brosnihan *et al.* 1997) and an improvement in microvascular density (Gimenez *et al.* 2006). Collectively, these findings support the idea that whether E2 exposure is detrimental or beneficial to the cardiovascular system depends largely on the dose, duration of E2 exposure and the age of the animal.

There are several central mechanisms by which chronic E2 exposure could increase BP. Some of the central regions involved in BP regulation include the rostral ventrolateral medulla (RVLM), the nucleus tractus solitarius and area postrema of the brainstem, periventricular regions, such as the subfornical organ and the organum vasculosum lamina terminalis, and hypothalamic nuclei, such as the paraventricular nucleus (PVN), supraoptic nucleus (SON), the lateral hypothalamus and medial septal area (Menani *et al.* 2014). Chronic E2 exposure could affect one or several of these areas to produce a concerted effect on BP. Activation of neurons in the PVN and RVLM could also result in activation of the sympathetic nervous system through the intermediolateral cell column of the spinal cord (Westerhaus & Loewy 1999, Guyenet 2006).

Since chronic low-dose E2 exposure is capable of inducing oxidative stress in specific hypothalamic areas, it is likely that this could be a possible molecular mechanism that contributes to increased BP in this model. In fact, oxidative stress has been implicated in the pathogenesis of hypertension in other experimental

animal models of obesity, angiotensin II- or salt-induced hypertension (Kishi *et al.* 2004, Chan *et al.* 2005, Stocker *et al.* 2007, Hirooka 2008, Oliveira-Sales *et al.* 2009, Hilzendeger *et al.* 2012, Konno *et al.* 2012). In the chronic low-dose E2 exposure model, the levels of superoxide, a reactive oxygen species, were elevated after 90 days of exposure and both superoxide and BP parameters were markedly reduced when rats were treated with resveratrol, a natural phenol and antioxidant (Subramanian *et al.* 2011). The source of the superoxide anion is not clear. As explained previously, microglia could be a potential source of this molecule. Microglia express ER alpha (Sierra *et al.* 2008) and they also contain NADPH oxidase, which gives them the ability to generate superoxide and produce neurodegenerative changes in chronic conditions (Surace & Block 2012). In fact, NADPH oxidase gene expression is increased in the RVLM after chronic E2 exposure (Subramanian *et al.* 2015). This agrees well with published evidence that NADPH oxidase is activated in the RVLM of neurogenic hypertensive rats contributing to oxidative stress in these models (Hirooka 2008, 2011, Bai *et al.* 2009, Kishi & Hirooka 2012). There is some evidence to suggest that oxidative stress in the RVLM could activate the sympathetic nervous system leading to hypertension (Agarwal *et al.* 2011, Winklewski *et al.* 2015).

Angiotensin II (Ang II), a vasoconstrictor peptide, has also been reported to increase oxidative stress leading to hypertension (Young & Davisson 2015). To our surprise, both Ang II levels and gene expression in the RVLM were unaffected in the chronic E2 exposure model. However, a vasoconstrictor peptide, endothelin-1 (ET-1), was dramatically upregulated in the RVLM of E2-treated animals after 90 days of exposure (Subramanian *et al.* 2017). ET-1 has been shown to activate the sympathetic nervous system (Bruno *et al.* 2011). It is also capable of increasing superoxide production through activation of NADPH oxidase to cause vasoconstriction in the aorta of rats (Loomis *et al.* 2005, Dammanahalli & Sun 2008). Therefore, it is very likely that ET-1 increases NADPH oxidase in the RVLM as well. This effect is most probably produced through the ETA receptor, since the gene expression of this receptor was upregulated in the chronic E2 exposure model, both in the RVLM and the PVN (Subramanian *et al.* 2017).

Nitration of proteins is present at a basal level in normal tissues and is important for certain signaling pathways. However, when there is serious imbalance in the generation of nitrating species, there is abnormal level of nitration that leads to altered cardiovascular function (Turko & Murad 2002). Nitration of free radical scavenging enzymes such as manganese superoxide dismutase (MnSOD) could also result in the accumulation of free radicals. MnSOD is capable of undergoing peroxynitrite-dependent nitration of the tyrosine residue (Tyr34) that is located very close to the active site of the enzyme. This results in loss of enzyme activity leading

Table 2 Chronic administration of various estrogenic preparations and their impact on cardiovascular functions.

Species	Type of E2	Gonadectomy	Route	Dose	Duration	Age at exposure	Effects on cardiovascular function	Reference
Female SD rats	Ethinyl estradiol plus norgestrel		Oral gavage	0.1 µg/day and 1.0 µg/day (low EEN) and 10.0 µg/day respectively (high EEN)	6 weeks	12 weeks	Elevates SBP and endothelial dysfunction	Olatunji <i>et al.</i> (2016)
	Ethinyl estradiol		s.c. injection	0.2 µg/day	14 weeks	10–12 weeks	Elevates SBP	Geraghty <i>et al.</i> (1990)
	17β-Estradiol		s.c. implant	0.05 mg and 0.1 mg	21 days	180 and 250	E2 replacement prevents cardiac hypertrophy and interstitial fibrosis induced by DOCA-salt administration	Shenoy <i>et al.</i> (2009)
Chimeric mice	17β-Estradiol	Yes	s.c. implant	5.6 µg/day	10 weeks	12 weeks	17β-Estradiol is atheroprotective	Shelton <i>et al.</i> (2013)
ApoE(–/–) mice	17β-Estradiol	Yes	Oral	6 µg/day	12 weeks	11 weeks of age and E2 exposure at 12 weeks	E2 treatment reduces ROS levels and atherosclerosis progression in apoE(–/–) mice	Wing <i>et al.</i> (2009)
ApoE(–/–) mice	17β-Estradiol	Yes	Western diet	1.1 and 6 µg/day	90 days	Ovariectomized at 28 days and E2 exposure at 42 days of age	Low dose increased and high dose decreased atherosclerotic plaque	Freudenberger <i>et al.</i> (2010)
Cynomolgus macaques	Estradiol	Yes	Oral	1 mg/day micronized E2	8 months	1 month or 54 months	E2 treatment 1 month after ovx inhibits macrophage accumulation in carotid artery but not in the 54 month group	Sophonsritsuk <i>et al.</i> (2013)
Female mice	17β-Estradiol	Yes	s.c. implant	150–200 pg/mL	5, 14, 28, 49 days	9–11 weeks	E2 is able to suppress reactive gliosis	McAsey <i>et al.</i> (2006)
C57BL/6 mice	17β-Estradiol	No	s.c. implant	0.21 mg/day	3 months and 1 month	17 months – every month until 20 months of age; 17 months – implanted once at 20 months of age	Chronic E2 improved infarct outcomes in female mice but acute treatment did not	Liu <i>et al.</i> (2012)

to a gradual change in mitochondrial redox homeostasis that further aggravates the oxidative insult (Radi 2013). Besides nitration of specific enzymes, myofibrillar proteins in the heart and aorta can be nitrated especially in hypertensive animals (Cabassi *et al.* 2001). Further studies are needed to determine if similar changes are obvious in the chronic E2 exposure model.

Unlike other neurogenic hypertensive models such as spontaneously hypertensive rats or Ang II/salt-induced hypertension, chronic E2 exposure only results in a moderate increase in mean arterial pressure. This represents the current scenario in society where women are exposed to a very small dose of estrogenic compounds on a day-to-day basis in the form of oral contraceptives, cosmetics or hormone replacement therapy. It is possible that this constant exposure could move a woman from the pre-hypertensive state to a hypertensive state over a period of time. Hence, it is of utmost clinical importance to understand the mechanisms behind chronic E2-related hypertension.

Chronic E2 exposure and behavior

In the previous sections, we described the effects of low-dose chronic E2 exposures on neuroendocrine functions related to reproduction and prolactin secretion and its impact on the development of moderate hypertension. Besides these effects, E2 is also capable of affecting behavior. The most common association is between E2 and mood swings in women. Although mood disorders are common in women of all reproductive ages, the incidence is higher during the luteal phase of menstrual cycle (premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD) and during the perimenopausal period (anxiety and depression)). Sex hormones, particularly E2, are strongly implicated in the pathogenesis of these behavioral disorders as their incidence coincides with periods of drastic fluctuations in circulating levels of E2 in women. A recent study has found a significant association between oral contraceptive use in adolescents and subsequent use of anti-depressants, suggesting chronic exposure to E2 may impact mental health (Skovlund *et al.* 2016). Further, clinical studies in postmenopausal women who are exposed to E2 in the form of HRT (conjugated equine estrogen (CEE) alone/plus medroxyprogesterone acetate) have also reported minimal or modest adverse effects on cognition and mood associated with its long-term use (Espeland *et al.* 2004, 2017). Some of the early studies, such as Women's Health Initiative Memory Study (Espeland *et al.* 2004, Shumaker *et al.* 2004) and Heart and Estrogen/progestin Replacement Study (HERS) (Grady *et al.* 2002), found an increased risk for dementia and decreased cognitive profile in older postmenopausal women treated with E2 alone or E2 plus progestin pills. As these studies involved women who initiated HRT later in their life, concerns were raised as to the timing

of HRT initiation in postmenopausal women. This led to the proposal of 'critical window hypothesis', which states that the outcomes of HRT depend on the timing of initiation of treatment with respect to age and/or the onset of menopause, with benefits limited to early initiation of treatment (Resnick & Henderson 2002). According to this theory, there is a critical window around perimenopause when hormonal withdrawal increases the susceptibility of the brain to pathologic processes and initiation of HRT around that time might provide the maximal benefits (Marder & Sano 2000). Supporting this idea, clinical studies like Research into Memory, Brain function and Estrogen Replacement (REMEMBER) pilot study, Cache county study and Cognitive and Affective Study (KEEPS-Cog) of the Kronos Early Estrogen Prevention Study (KEEPS) showed that early initiation of HRT around the perimenopausal period was associated with better cognitive outcomes, reduced risk of Alzheimer's disease, anxiety and depression (Zandi *et al.* 2002, MacLennan *et al.* 2006, Gleason *et al.* 2015). Neuroimaging studies also found that women who started using HRT around perimenopause had larger hippocampal volume and better memory performance than the never users (Erickson *et al.* 2010) (Maki *et al.* 2011). One study that investigated the effects of a combination of estrogen and progestogen on verbal memory in women younger than 65 years of age and did not find a significant association with short and long delay-free recall (Maki *et al.* 2007). On the other hand, another study found improvements in verbal memory (Linzmayr *et al.* 2001). Based on these studies, a clear consensus does not exist on whether timing of HRT affects its outcomes. Also, some of these studies involve estrogen plus progestin pills; hence, the extent to which progesterone contributes to these outcomes needs to be clarified. While the concept of hormonal therapy for menopause is centered around the fact that stabilizing fluctuating estrogen levels will attenuate the adverse outcomes of menopausal transition, perimenopause is also characterized by periods of increased E2 levels. Whether this period of exposure to elevated E2 levels programs the brain for increased susceptibility to anxiety and depression as observed in postmenopausal women needs to be addressed.

Other studies have observed variable effects of estrogen on behavior. Factors such as dose and duration of estrogen exposure, presence or absence of ovaries and age of the animals at the time of exposure might be responsible for the differences observed in terms of anxiogenic vs anxiolytic effects of estrogen treatment (Table 3). For example, in other rodent studies, the beneficial effects of chronic estrogen treatment (3 weeks) in improving depression was observed only at low doses (0.3–3 µg/day) and not at high doses (10 µg/day), which produces supra-physiological estradiol levels (Okada *et al.* 1997). Further, ovariectomized rats displayed increased activity in the open field test only when treated with estrogen for 5 days and not 35 days

Table 3 Effects of various estrogenic preparations administered on a chronic basis on behavioral indices.

Species	Type of E2	Gonadectomy	Route	Dose	Duration	Age at exposure	Effects on cardiovascular function	Citation
Sprague-Dawley female rats	17 β -E2	No	s.c. implantation of slow release pellets	1.8 μ g pellets which release 20 ng per day	90 days	4–5 months	Increases anxiety-like behavior in OFT. No change in EPM test	Balasubramanian <i>et al.</i> (2014)
Sprague-Dawley male and female rats	17 β -E2, PPT, DPT	Yes	Daily s.c. injections	17 β -Estradiol-0.25 mg/kg, PPT and DPT-1 mg/kg	4 days	60–90 days	DPN and E2-treated females reduced anxiety behavior in EPM and OFT test. PPT increased, anxiogenic behaviors such as the number of fecal boli and time spent grooming in females. DPN produced similar anxiolytic effects in male rats too	Lund <i>et al.</i> (2005)
Sprague-Dawley female rats	Estradiol benzoate	Yes	s.c. injection	10 μ g/kg	3 h	Adult	Anxiogenic effects in EPM	Mora <i>et al.</i> (1996)
C57BL/6 N female mice	17 β -E2, PPT, ER β agonist DPT or G-1	Yes	s.c. injection	E2-0.025 and 0.25 mg/kg, other drugs 1 mg/kg BW	2 h	3 months and 3 weeks	E2 produced dose dependent changes. Low-dose-Anxiogenic effects in OFT. High dose-Anxiolytic effects in EPM GPR30 agonist-anxiogenic in EPM and OFT. No changes with PPT or DPT treatment	Kastenberger <i>et al.</i> (2012)
ICR albino female mice	Estradiol benzoate	Yes	s.c. injection	0.3, 1.5, 3, 15 and 30 μ g/kg/day	14 days	9 weeks	Anti-depressive effect at 15 and 30 μ g dose-shortened the duration of immobility in forced swimming test. Increase in uterine weight	Bekku & Yoshimura (2005)
Congenic C57BL/6 background female mice	17 β -E2	No	s.c. injection	10 μ g	1 h	24 months	Decreased anxiety and depression behavior in open field, mirror chamber, light-dark transition and forced swim test. No changes in motor activity and coordination	Walf & Frye (2010a)
Long-Evans female rats	17 β -E2	Yes	s.c. injection	0.09 mg/kg once weekly	14 weeks	8 weeks	Anti-anxiety and depressive effect-More time on open arms in elevated maze test and less immobile time in forced swim test	Walf & Frye (2010b)
Long-Evans female rats*	17 β -E2	Yes	Exp 1: Chronic s.c. infusion via silastic capsules. Exp 2: Weekly s.c. injections	0.03 and 0.09 mg/kg	Exp 1: 2 and 6 months. Exp 2: 3.5 months	4–8 months	Exp 1: Increase in incidence of mammary tumors and uterine weight. Exp 2: Increased sexual behavior and enhanced sexual receptivity (i.e., lordosis in response to mounting by a male rat)	Walf & Frye (2009)

(Continued)

Table 3 (Continued)

Species	Type of E2	Gonadectomy	Route	Dose	Duration	Age at exposure	Effects on cardiovascular function	Citation
C57BL/6 female mice	E2, PPT; or DPT	Yes	s.c. injection	10 µg/kg or 30 µg/kg/day	4 weeks	Adult	E2 (both doses) and ERβ agonist (high dose only) reversed depression-like behavior in sucrose preference test. Similarly, E2 and ERβ agonist at high dose reduced anxiety-like behavior in EPM and OFT. ERα agonist had no effect on behavior	Xu et al. (2016)
C57BL/6 female mice	Estradiol benzoate	Yes	Chronic s.c. infusion via silastic capsules	25, 50 or 75 µg in 0.03 mL sesame oil	10 days	8–9 weeks	Increase in fear and anxiety in EPM, OFT, dark and light transition test and conditioned fear learning test. Dose dependent increase in uterine weight	Morgan & Pfaff (2001)
Sprague–Dawley female rats	17β-E2	Yes	s.c. injection (4 weeks after OVX)	30 µg/day	7 days	Adult	Reversed OVX-induced depressive behavior in forced swimming test. Increased number of crossings and rearings in OFT	Li et al. (2014)
Female Wistar rats	17β-E2	Yes	Chronic s.c. infusion via silastic capsules	2 capsules with 400 µg/8 µL in each capsule	30 days	Young (Y) – 3 months old; adult (AD) – 7–8 months old; and middle-aged (MA) – 12–13 months	Improved spatial reference memory of adult and middle-aged rats and anti-depressive behavior in young and adult rats in forced swimming test	Kiss et al. (2012)
HERS involving 2763 women with coronary disease	Conjugated estrogen (0.625 mg) plus medroxyprogesterone acetate (2.5 mg)	ND	Oral	One tablet daily	Mean (±s.d.): 4.2 ± 0.4 years	Mean age of 71 ± 6 years	Cognitive function test-women assigned to hormone replacement scored worse on the Verbal Fluency test compared to placebo group	Grady et al. (2002)
Women's Health Initiative Memory Study	CEE	Yes-prior hysterectomy	Oral	One tablet daily (0.625 mg of CEE)	Mean follow-up: 5.4 years	65–79 years	Global cognitive function test-women assigned to CEE scored less in 3MMSE than the placebo group. The effect was more pronounced among women with lower cognitive function at baseline	Espeland et al. (2004)
Women's Health Initiative Memory Study	CEE alone or conjugated estrogen (0.625 mg) plus medroxyprogesterone acetate (2.5 mg)	ND	Oral	One tablet daily	Mean follow-up of 5.21 years	65–79 years	Estrogen alone and estrogen plus progestin resulted in increased risks for dementia	Shumaker et al. (2004)

REMEMBER pilot study	Estrogen or estrogen plus progesterin (self reported hormone therapy use)	Yes-prior hysterectomy	Oral or transdermal form	Once daily	Atleast 1 year	Early – before 56 years for intact women or within 5 years of hysterectomy and oophorectomy Late -later than the above mentioned times	Early initiators scored significantly higher than late initiators on the MMSE. Early initiators were significantly faster than never users on the TMTA	MacLennan <i>et al.</i> (2006)
ELITE	17 β -E2	Both intact and women with oophorectomy were involved	Oral	Once daily (1 mg)	2–5 years + additional 2.5 years	Early (<6 years after menopause) and late (10+ year after menopause) groups	No difference between early and late groups on verbal memory, executive functions, or global cognition	Henderson <i>et al.</i> (2013)
Cognitive follow-up study of women enrolled in the Melbourne Women's Midlife Health Project	Hormone therapy				~11 years	56–67 years	fMRI-perimenopausal HT users showed increased activation in the left hippocampus and decreased activation in the parahippocampal gyrus bilaterally compared with never users in the verbal recognition task. Each of these patterns of activation was associated with better memory performance on the imaging memory task	Maki <i>et al.</i> (2011)
KEEPS-Cog of KEEPS	17 β -E2 in the transdermal product and estrone in oral CEE	ND	Oral and transdermal	Oral CEE –0.45 mg/day plus 200mg/day m-P for first 12 days of each month t-E2-50 μ g/d) plus 200 mg/d m-P for the first 12 day of each month	Upto 4 years	Recent postmenopausal women, within 3 years of their last menstrual period, mean age -52.6 years	No effect on cognitive outcomes, women treated with o-CEE showed improvements in depression and anxiety symptoms. No effect of transdermal E2 on mood	Gleason <i>et al.</i> (2015)
WHIMSY	CEE with or without medroxyprogesterone acetate	ND	Oral	0.625 mg CEE with or without 2.5 mg medroxyprogesterone acetate	Mean: 7 years	Postmenopausal women aged 50–55 years	No overall sustained benefit or risk to cognitive function in women on CEE based therapies	Espeland <i>et al.</i> (2013)

PPT, propyl pyrazole triol; DPT, diarylpropionitrile; E2, estradiol; G-1, GPR30 agonist; HERS, Heart and Estrogen/progestin Replacement Study; CEE, conjugated equine estrogen; REMEMBER, Research into Memory, Brain function and Estrogen Replacement; ELITE, Early versus Late Intervention Trial; KEEPS, Kronos Early Estrogen Prevention Study; KEEPS-Cog, Cognitive and Affective Study of KEEPS; WHIMSY, Women's health Initiative Memory Study of Younger Women; s.c., subcutaneous; OFT, open field test; EPM, elevated plus maze test; 3MMSE, Modified Mini-Mental State Examination; TMTA, Attention and Concentration Tasks.* not clearly mentioned, but this group used Long-Evans rats in their previous studies.

Figure 1 Schematic above depicts the effects of chronic low-dose E2 exposure on three distinct brain regions: The hypothalamus, brain stem and the limbic system (hippocampus and amygdala). E2-induced glial activation may play a role in both the hypothalamus and the brainstem. Effects on the hypothalamus involve activation of cytokines and oxidative stress-related pathways leading to nitration of tyrosine hydroxylase that results in the reduction in NE and DA levels leading to ovulatory failure and mammary/pituitary tumors respectively. Effects on the brain stem involve increasing ET-1 levels and generation of free radicals that may promote endothelial dysfunction or increased sympathetic nerve activity leading to hypertension. Effects on the hippocampus and amygdala may include reduction in BDNF levels and monoamines leading to anxiety.

(Luine *et al.* 1998). In some cases, even acute estrogen treatment (0.75 µg for 2 days) has been reported to induce depression-like behavior (Galea *et al.* 2002). In comparison with all these studies, the dose of estrogen used in our studies was much lower (20 ng of E2/day) and the duration of exposure was much longer (12 weeks) (Kasturi *et al.* 2009). This resulted in lower BDNF mRNA and protein levels in the amygdala, but not in the hippocampus (Balasubramanian *et al.* 2014). In fact, there was downregulation of one specific BDNF exon (exon VI) (Balasubramanian *et al.* 2014), which has an estrogen-responsive element in its structure (Sohrabji *et al.* 1995). This was associated with a reduction in dopamine (DA) levels in the amygdala that has been linked to anxiety in humans (Kienast *et al.* 2008) and rodents (Perez de la Mora *et al.* 2012). Further studies are needed to determine if chronic E2-induced reduction in BDNF directly influences DA levels or if DA levels are decreased independent of BDNF action as a result of TH nitration in the amygdala.

Conclusions

We need to consider that E2 is a pleiotropic hormone. It could have a number of beneficial effects as numerous studies in the literature indicate. However, it needs to be used in moderation and with caution. Many of these

reported beneficial effects have been observed with higher doses of E2 and for varying durations. According to the studies described here, it appears that chronic exposures to low levels of E2 have widespread effects that are deleterious (Fig. 1). These are important to consider since most of the environmental exposures to estrogenic compounds occur at low levels and for prolonged periods of time. The deleterious effects on the nervous, cardiovascular and reproductive systems described here are based on the generation of oxidative stress and other mechanisms. If the underlying mechanism (oxidative stress) appears as a constant feature for all these effects, it should be possible to correct all of them using antioxidants.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

Work presented here from the MohanKumar laboratory was supported by NIH AG 027697 to P S M. The authors would like to thank Stephanie Pfeiffer, Educational resource center, UGA for preparing the figure and Elyssa Jacob for her editorial assistance.

References

- Adel M, Song X, Wang Y, Francis D & Yang Y 2017 Environmental impact of estrogens on human, animal and plant life: a critical review. *Environment International* **99** 107–119. (<https://doi.org/10.1016/j.envint.2016.12.010>)
- Agarwal D, Welsch MA, Keller JN & Francis J 2011 Chronic exercise modulates RAS components and improves balance between pro- and anti-inflammatory cytokines in the brain of SHR. *Basic Research in Cardiology* **106** 1069–1085. (<https://doi.org/10.1007/s00395-011-0231-7>)
- Amantea D, Russo R, Bagetta G & Corasaniti MT 2005 From clinical evidence to molecular mechanisms underlying neuroprotection afforded by estrogens. *Pharmacological Research* **52** 119–132. (<https://doi.org/10.1016/j.phrs.2005.03.002>)
- Ara J, Przedborski S, Naini AB, Jackson-Lewis V, Trifiletti RR, Horwitz J & Ischiropoulos H 1998 Inactivation of tyrosine hydroxylase by nitration following exposure to peroxynitrite and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *PNAS* **95** 7659–7663. (<https://doi.org/10.1073/pnas.95.13.7659>)
- Araque A 2006 Astrocyte-neuron signaling in the brain – implications for disease. *Current Opinion in Investigational Drugs* **7** 619–624.
- Azcoitia I, Sierra A & Garcia-Segura LM 1999 Localization of estrogen receptor beta-immunoreactivity in astrocytes of the adult rat brain. *Glia* **26** 260–267. ([https://doi.org/10.1002/\(SICI\)1098-1136\(199905\)26:3<260::AID-GLIA7>3.0.CO;2-R](https://doi.org/10.1002/(SICI)1098-1136(199905)26:3<260::AID-GLIA7>3.0.CO;2-R))
- Bagger YZ, Tanko LB, Alexandersen P, Qin G, Christiansen C & Group PS 2005 Early postmenopausal hormone therapy may prevent cognitive impairment later in life. *Menopause* **12** 12–17. (<https://doi.org/10.1097/00042192-200512010-00005>)
- Bai Y, Jabbari B, Ye S, Campese VM & Vaziri ND 2009 Regional expression of NAD(P)H oxidase and superoxide dismutase in the brain of rats with neurogenic hypertension. *American Journal of Nephrology* **29** 483–492. (<https://doi.org/10.1159/000178817>)
- Balasubramanian P, Subramanian M, Nunez JL, Mohankumar SM & Mohankumar PS 2014 Chronic estradiol treatment decreases brain derived neurotrophic factor (BDNF) expression and monoamine levels in the amygdala – implications for behavioral disorders. *Behavioural Brain Research* **261** 127–133. (<https://doi.org/10.1016/j.bbr.2013.12.018>)
- Barracough CA 1994 Neurotransmitter regulation of luteinizing hormone-releasing hormone neuronal function. *Acta Biologica Hungarica* **45** 189–206.
- Barracough CA & Wise PM 1982 The role of catecholamines in the regulation of pituitary luteinizing hormone and follicle-stimulating hormone secretion. *Endocrine Reviews* **3** 91–119. (<https://doi.org/10.1210/edrv-3-1-91>)
- Barth C, Villringer A & Sacher J 2015 Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Frontiers in Neuroscience* **9** 37. (<https://doi.org/10.3389/fnins.2015.00037>)
- Bekku N & Yoshimura H 2005. Animal model of menopausal depressive-like state in female mice: prolongation of immobility time in the forced swimming test following ovariectomy. *Psychopharmacology* **183** 300–307. (<https://doi.org/10.1007/s00213-005-0179-0>)
- Ben-Jonathan N, Oliver C, Weiner HJ, Mical RS & Porter JC 1977 Dopamine in hypophyseal portal plasma of the rat during the estrous cycle and throughout pregnancy. *Endocrinology* **100** 452–458. (<https://doi.org/10.1210/endo-100-2-452>)
- Blanchard-Fillion B, Souza JM, Friel T, Jiang GC, Vrana K, Sharov V, Barron L, Schoneich C, Quijano C, Alvarez B et al. 2001 Nitration and inactivation of tyrosine hydroxylase by peroxynitrite. *Journal of Biological Chemistry* **276** 46017–46023. (<https://doi.org/10.1074/jbc.M105564200>)
- Blessing WW & Reis DJ 1982 Inhibitory cardiovascular function of neurons in the caudal ventrolateral medulla of the rabbit: relationship to the area containing A1 noradrenergic cells. *Brain Research* **253** 161–171. ([https://doi.org/10.1016/0006-8993\(82\)90683-7](https://doi.org/10.1016/0006-8993(82)90683-7))
- Blessing WW, Sved AF & Reis DJ 1982 Hypertension with elevated plasma vasopressin after lesions of noradrenergic neurons in the rabbit medulla oblongata. *Transactions of the Association of American Physicians* **95** 79–85.
- Block ML & Hong JS 2007 Chronic microglial activation and progressive dopaminergic neurotoxicity. *Biochemical Society Transactions* **35** 1127–1132. (<https://doi.org/10.1042/BST0351127>)
- Booth JN 3rd, Li J, Zhang L, Chen L, Muntner P & Egan B 2017 Trends in prehypertension and hypertension risk factors in US adults: 1999–2012. *Hypertension* **70** 275–284. (<https://doi.org/10.1161/HYPERTENSIONAHA.116.09004>)
- Brosnihan KB, Li P, Ganten D & Ferrario CM 1997 Estrogen protects transgenic hypertensive rats by shifting the vasoconstrictor-vasodilator balance of RAS. *American Journal of Physiology* **273** R1908–R1915. (<https://doi.org/10.1152/ajpcell.1997.273.6.C1908>)
- Brown GC & Vilalta A 2015 How microglia kill neurons. *Brain Research* **1628** 288–297. (<https://doi.org/10.1016/j.brainres.2015.08.031>)
- Bruno RM, Sudano I, Ghiadoni L, Masi L & Taddei S 2011 Interactions between sympathetic nervous system and endogenous endothelin in patients with essential hypertension. *Hypertension* **57** 79–84. (<https://doi.org/10.1161/HYPERTENSIONAHA.110.163584>)
- Byrne KB, Geraghty DP, Stewart BJ & Burcher E 1994 Effect of contraceptive steroid and enalapril treatment of systolic blood pressure and plasma renin-angiotensin in the rat. *Clinical and Experimental Hypertension* **16** 627–657. (<https://doi.org/10.3109/10641969409067966>)
- Cabassi A, Dumont EC, Girouard H, Bouchard JF, Le Jossec M, Lamontagne D, Besner JG & de Champlain J 2001 Effects of chronic N-acetylcysteine treatment on the actions of peroxynitrite on aortic vascular reactivity in hypertensive rats. *Journal of Hypertension* **19** 1233–1244. (<https://doi.org/10.1097/00004872-200107000-00008>)
- Calippe B, Douin-Echinard V, Laffargue M, Laurell H, Rana-Poussine V, Pipy B, Guery JC, Bayard F, Arnal JF & Gourdy P 2008 Chronic estradiol administration in vivo promotes the proinflammatory response of macrophages to TLR4 activation: involvement of the phosphatidylinositol 3-kinase pathway. *Journal of Immunology* **180** 7980–7988. (<https://doi.org/10.4049/jimmunol.180.12.7980>)
- Cardona-Gomez GP, DonCarlos L & Garcia-Segura LM 2000 Insulin-like growth factor I receptors and estrogen receptors colocalize in female rat brain. *Neuroscience* **99** 751–760. ([https://doi.org/10.1016/S0306-4522\(00\)00228-1](https://doi.org/10.1016/S0306-4522(00)00228-1))
- Chan SH, Hsu KS, Huang CC, Wang LL, Ou CC & Chan JY 2005 NADPH oxidase-derived superoxide anion mediates angiotensin II-induced pressor effect via activation of p38 mitogen-activated protein kinase in the rostral ventrolateral medulla. *Circulation Research* **97** 772–780. (<https://doi.org/10.1161/01.RES.0000185804.79157.C0>)
- Chasan-Taber L, Willett WC, Manson JE, Spiegelman D, Hunter DJ, Curhan G, Colditz GA & Stampfer MJ 1996 Prospective study of oral contraceptives and hypertension among women in the United States. *Circulation* **94** 483–489. (<https://doi.org/10.1161/01.CIR.94.3.483>)
- Cholanian M, Krajewski-Hall SJ, McMullen NT & Rance NE 2015 Chronic oestradiol reduces the dendritic spine density of KNDy (kisspeptin/neurokinin B/dynorphin) neurones in the arcuate nucleus of ovariectomised Tac2-enhanced green fluorescent protein transgenic mice. *Journal of Neuroendocrinology* **27** 253–263. (<https://doi.org/10.1111/jne.12263>)
- Cooper C, Stakkestad JA, Radowski S, Hardy P, Pilate C, Dain MP & Delmas PD 1999 Matrix delivery transdermal 17beta-estradiol for the prevention of bone loss in postmenopausal women. The International Study Group. *Osteoporosis International* **9** 358–366. (<https://doi.org/10.1007/s001980050159>)
- Csakvari E, Kurunczi A, Hoyk Z, Gyenes A, Naftolin F & Parducz A 2008 Estradiol-induced synaptic remodeling of tyrosine hydroxylase immunopositive neurons in the rat arcuate nucleus. *Endocrinology* **149** 4137–4141. (<https://doi.org/10.1210/en.2007-1559>)
- Dammanahalli KJ & Sun Z 2008 Endothelins and NADPH oxidases in the cardiovascular system. *Clinical and Experimental Pharmacology and Physiology* **35** 2–6. (<https://doi.org/10.1111/j.1440-1681.2007.04830.x>)
- Davey C, Servy C, Dendane M, Marin P & Ducrocq C 1997 Oxidation and nitration of catecholamines by nitrogen oxides derived from nitric oxide. *Nitric Oxide* **1** 234–243. (<https://doi.org/10.1006/niox.1997.0123>)
- Demarest KT, Riegler GD & Moore KE 1984 Long-term treatment with estradiol induces reversible alterations in tuberoinfundibular dopaminergic neurons: a decreased responsiveness to prolactin. *Neuroendocrinology* **39** 193–200. (<https://doi.org/10.1159/000123979>)
- Demling J, Fuchs E, Baumert M & Wuttke W 1985 Preoptic catecholamine, GABA, and glutamate release in ovariectomized and ovariectomized estrogen-primed rats utilizing a push-pull cannula technique. *Neuroendocrinology* **41** 212–218. (<https://doi.org/10.1159/000124180>)
- Donahue JE, Stopa EG, Chorsky RL, King JC, Schipper HM, Tobet SA, Blaustein JD & Reichlin S 2000 Cells containing immunoreactive

- estrogen receptor-alpha in the human basal forebrain. *Brain Research* **856** 142–151. ([https://doi.org/10.1016/S0006-8993\(99\)02413-0](https://doi.org/10.1016/S0006-8993(99)02413-0))
- Erickson KI, Voss MW, Prakash RS, Chaddock L & Kramer AF 2010 A cross-sectional study of hormone treatment and hippocampal volume in postmenopausal women: evidence for a limited window of opportunity. *Neuropsychology* **24** 68–76. (<https://doi.org/10.1037/a0017292>)
- Espeland MA, Rapp SR, Shumaker SA, Brunner R, Manson JE, Sherwin BB, Hsia J, Margolis KL, Hogan PE, Wallace R *et al.* 2004 Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. *JAMA* **291** 2959–2968. (<https://doi.org/10.1001/jama.291.24.2959>)
- Espeland MA, Shumaker SA, Leng I, Manson JE, Brown CM, LeBlanc ES, Vaughan L, Robinson J, Rapp SR, Goveas JS *et al.* 2013 Long-term effects on cognitive function of postmenopausal hormone therapy prescribed to women aged 50 to 55 years. *JAMA Internal Medicine* **173** 1429–1436. (<https://doi.org/10.1001/jamainternmed.2013.7727>)
- Espeland MA, Rapp SR, Manson JE, Goveas JS, Shumaker SA, Hayden KM, Weitlauf JC, Gaussoin SA, Baker LD, Padula CB *et al.* 2017 Long-term effects on cognitive trajectories of postmenopausal hormone therapy in two age groups. *Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* **72** 838–845. (<https://doi.org/10.1093/geron/algw156>)
- Freudenberger T, Oppermann M, Heim HK, Mayer P, Kojda G, Schrör K & Fischer JW 2010 Proatherogenic effects of estradiol in a model of accelerated atherosclerosis in ovariectomized ApoE-deficient mice. *Basic Research in Cardiology* **105** 479–486. (<https://doi.org/10.1007/s00395-010-0091-6>)
- Fuxe K 1964 Cellular localization of monoamines in the median eminence and the infundibular stem of some mammals. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **61** 710–724. (<https://doi.org/10.1007/BF00342620>)
- Galea LA, Lee TT, Kostaras X, Sidhu JA & Barr AM 2002 High levels of estradiol impair spatial performance in the Morris water maze and increase 'depressive-like' behaviors in the female meadow vole. *Physiology and Behavior* **77** 217–225. ([https://doi.org/10.1016/S0031-9384\(02\)00849-1](https://doi.org/10.1016/S0031-9384(02)00849-1))
- Garcia-Ovejero D, Veiga S, Garcia-Segura LM & DonCarlos LL 2002 Glial expression of estrogen and androgen receptors after rat brain injury. *Journal of Comparative Neurology* **450** 256–271. (<https://doi.org/10.1002/cne.10325>)
- Geraghty DP, Byrne KB, McPherson GA & Burcher E 1990. Renal and myocardial adrenoceptors in steroid contraceptive-induced hypertension in rats. *Clinical and Experimental Pharmacology and Physiology* **17** 567–578.
- Gilbreath ET, MohanKumar SM, Balasubramanian P, Agnew DW & MohanKumar PS 2014 Chronic exposures to low levels of estradiol and their effects on the ovaries and reproductive hormones: comparison with aging. *Endocrine Disruptors* **2** e967127. (<https://doi.org/10.4161/23273739.2014.967127>)
- Gimenez J, Garcia MP, Serna M, Bonacasa B, Carbonell LF, Quesada T & Hernandez I 2006 17Beta-oestradiol enhances the acute hypotensive effect of captopril in female ovariectomized spontaneously hypertensive rats. *Experimental Physiology* **91** 715–722. (<https://doi.org/10.1113/expphysiol.2006.033449>)
- Gleason CE, Dowling NM, Wharton W, Manson JE, Miller VM, Atwood CS, Brinton EA, Cedars MI, Lobo RA, Merriam GR *et al.* 2015 Effects of hormone therapy on cognition and mood in recently postmenopausal women: findings from the randomized, controlled KEEPS-cognitive and affective study. *PLoS Medicine* **12** e1001833; discussion e1001833. (<https://doi.org/10.1371/journal.pmed.1001833>)
- Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J & Zoeller RT 2015 EDC-2: The Endocrine Society's Second Scientific Statement on endocrine-disrupting chemicals. *Endocrine Reviews* **36** E1–E150. (<https://doi.org/10.1210/er.2015-1010>)
- Goya RG, Castro MG & Meites J 1991 Differential effect of aging on serum levels of prolactin and alpha-melanotropin in rats. *Proceedings of the Society for Experimental Biology and Medicine* **196** 218–221. (<https://doi.org/10.3181/00379727-196-43183>)
- Grady D, Yaffe K, Kristof M, Lin F, Richards C & Barrett-Connor E 2002 Effect of postmenopausal hormone therapy on cognitive function: the Heart and Estrogen/progestin Replacement Study. *American Journal of Medicine* **113** 543–548. ([https://doi.org/10.1016/S0002-9343\(02\)01270-6](https://doi.org/10.1016/S0002-9343(02)01270-6))
- Gundlach C, Kohama SG, Mirkes SJ, Garyfallou VT, Urbanski HF & Bethea CL 2000 Distribution of estrogen receptor beta (ERbeta) mRNA in hypothalamus, midbrain and temporal lobe of spayed macaque: continued expression with hormone replacement. *Brain Research: Molecular Brain Research* **76** 191–204. ([https://doi.org/10.1016/S0006-8993\(99\)02475-0](https://doi.org/10.1016/S0006-8993(99)02475-0))
- Guyenet PG 2006 The sympathetic control of blood pressure. *Nature Reviews Neuroscience* **7** 335–346. (<https://doi.org/10.1038/nrn1902>)
- Hayward CS & Kelly RP 1997 Gender-related differences in the central arterial pressure waveform. *Journal of the American College of Cardiology* **30** 1863–1871. ([https://doi.org/10.1016/S0735-1097\(97\)00378-1](https://doi.org/10.1016/S0735-1097(97)00378-1))
- Heiman ML & Ben-Jonathan N 1982a Dopaminergic receptors in the rat anterior pituitary change during the estrous cycle. *Endocrinology* **111** 37–41. (<https://doi.org/10.1210/endo-111-1-37>)
- Heiman ML & Ben-Jonathan N 1982b Rat anterior pituitary dopaminergic receptors are regulated by estradiol and during lactation. *Endocrinology* **111** 1057–1060. (<https://doi.org/10.1210/endo-111-4-1057>)
- Henderson VW, St John JA, Hodis HN, McCleary CA, Stanczyk FZ, Karim R, Shoupe D, Kono N, Dustin L, Allayee H *et al.* 2013 Cognition, mood, and physiological concentrations of sex hormones in the early and late postmenopause. *PNAS* **110** 20290–20295. (<https://doi.org/10.1073/pnas.1312353110>)
- Herbison AE 1997 Noradrenergic regulation of cyclic GnRH secretion. *Reviews of Reproduction* **2** 1–6. (<https://doi.org/10.1530/ror.0.0020001>)
- Herbison AE 2008 Estrogen positive feedback to gonadotropin-releasing hormone (GnRH) neurons in the rodent: the case for the rostral periventricular area of the third ventricle (RP3V). *Brain Research Reviews* **57** 277–287. (<https://doi.org/10.1016/j.brainresrev.2007.05.006>)
- Herbison AE & Pape JR 2001 New evidence for estrogen receptors in gonadotropin-releasing hormone neurons. *Frontiers in Neuroendocrinology* **22** 292–308. (<https://doi.org/10.1006/frne.2001.0219>)
- Hilzendeger AM, Morgan DA, Brooks L, Dellsperger D, Liu X, Grobe JL, Rahmouni K, Sigmund CD & Mark AL 2012 A brain leptin-renin angiotensin system interaction in the regulation of sympathetic nerve activity. *American Journal of Physiology: Heart and Circulatory Physiology* **303** H197–H206. (<https://doi.org/10.1152/ajpheart.00974.2011>)
- Hirooka Y 2008 Role of reactive oxygen species in brainstem in neural mechanisms of hypertension. *Autonomic Neuroscience* **142** 20–24. (<https://doi.org/10.1016/j.autneu.2008.06.001>)
- Hirooka Y 2011 Oxidative stress in the cardiovascular center has a pivotal role in the sympathetic activation in hypertension. *Hypertension Research* **34** 407–412. (<https://doi.org/10.1038/hr.2011.14>)
- Hofer M, Pagliusi SR, Hohn A, Leibrock J & Barde YA 1990 Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *EMBO Journal* **9** 2459–2464. (<https://doi.org/10.1002/j.1460-2075.1990.tb07423.x>)
- Hosli E, Ruhl W & Hosli L 2000 Histochemical and electrophysiological evidence for estrogen receptors on cultured astrocytes: colocalization with cholinergic receptors. *International Journal of Developmental Neuroscience* **18** 101–111. ([https://doi.org/10.1016/S0736-5748\(99\)00074-X](https://doi.org/10.1016/S0736-5748(99)00074-X))
- Huie RE & Padmaja S 1993 The reaction of NO with superoxide. *Free Radical Research Communications* **18** 195–199. (<https://doi.org/10.3109/10715769309145868>)
- Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B & Vittinghoff E 1998 Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* **280** 605–613. (<https://doi.org/10.1001/jama.280.7.605>)
- Hulley S, Furberg C, Barrett-Connor E, Cauley J, Grady D, Haskell W, Knopp R, Lowery M, Satterfield S, Schrott H *et al.* 2002 Noncardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). *JAMA* **288** 58–66. (<https://doi.org/10.1001/jama.288.1.58>)
- Hwang CK, Kim DK & Chun HS 2013 Cholecystokinin-8 induces brain-derived neurotrophic factor expression in noradrenergic neuronal cells. *Neuropeptides* **47** 245–250. (<https://doi.org/10.1016/j.npep.2013.04.002>)
- Imam SZ, el-Yazal J, Newport GD, Itzhak Y, Cadet JL, Slikker W Jr & Ali SF 2001 Methamphetamine-induced dopaminergic neurotoxicity: role of peroxynitrite and neuroprotective role of antioxidants and peroxynitrite

- decomposition catalysts. *Annals of the New York Academy of Sciences* **939** 366–380. (<https://doi.org/10.1111/j.1749-6632.2001.tb03646.x>)
- Ischiropoulos H, Duran D & Horwitz J 1995 Peroxynitrite-mediated inhibition of DOPA synthesis in PC12 cells. *Journal of Neurochemistry* **65** 2366–2372. (<https://doi.org/10.1046/j.1471-4159.1995.65052366.x>)
- Juric DM, Loncar D & Carman-Krzan M 2008 Noradrenergic stimulation of BDNF synthesis in astrocytes: mediation via $\alpha 1$ - and $\beta 2$ -adrenergic receptors. *Neurochemistry International* **52** 297–306. (<https://doi.org/10.1016/j.neuint.2007.06.035>)
- Kastenberger I, Lutsch C & Schwarzer C 2012 Activation of the G-protein-coupled receptor GPR30 induces angiogenic effects in mice, similar to oestradiol. *Psychopharmacology* **221** 527–535. (<https://doi.org/10.1007/s00213-011-2599-3>)
- Kasturi BS, MohanKumar SM, Sirivelu MP & MohanKumar PS 2009 Chronic exposure to low levels of oestradiol-17 β affects oestrous cyclicity, hypothalamic norepinephrine and serum luteinising hormone in young intact rats. *Journal of Neuroendocrinology* **21** 568–577. (<https://doi.org/10.1111/j.1365-2826.2009.01873.x>)
- Kasturi BS, MohanKumar SM, Sirivelu MP, Shin AC & MohanKumar PS 2013 Chronic estradiol-17 β exposure suppresses hypothalamic norepinephrine release and the steroid-induced luteinizing hormone surge: role of nitration of tyrosine hydroxylase. *Brain Research* **1493** 90–98. (<https://doi.org/10.1016/j.brainres.2012.11.031>)
- Keefer DA, Stumpf WE & Petrusz P 1976 Quantitative autoradiographic assessment of 3H-estradiol uptake in immunocytochemically characterized pituitary cells. *Cell and Tissue Research* **166** 25–35. (<https://doi.org/10.1007/BF00215122>)
- Kenny PJ, File SE & Rattray M 2000 Acute nicotine decreases, and chronic nicotine increases the expression of brain-derived neurotrophic factor mRNA in rat hippocampus. *Brain Research: Molecular Brain Research* **85** 234–238. ([https://doi.org/10.1016/S0169-328X\(00\)00246-1](https://doi.org/10.1016/S0169-328X(00)00246-1))
- Kienast T, Hariri AR, Schlagenhauf F, Wrase J, Sterzer P, Buchholz HG, Smolka MN, Gruber G, Cumming P, Kumakura Y *et al.* 2008 Dopamine in amygdala gates limbic processing of aversive stimuli in humans. *Nature Neuroscience* **11** 1381–1382. (<https://doi.org/10.1038/nn.2222>)
- Kishi T & Hirooka Y 2012 Oxidative stress in the brain causes hypertension via sympathoexcitation. *Frontiers in Physiology* **3** 335. (<https://doi.org/10.3389/fphys.2012.00335>)
- Kishi T, Hirooka Y, Kimura Y, Ito K, Shimokawa H & Takeshita A 2004 Increased reactive oxygen species in rostral ventrolateral medulla contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats. *Circulation* **109** 2357–2362. (<https://doi.org/10.1161/01.CIR.0000128695.49900.12>)
- Kiss A, Delattre AM, Pereira SI, Carolino RG, Szawka RE, Anselmo-Franci JA, Zanata S M & Ferraz A C 2012. 17 β -estradiol replacement in young, adult and middle-aged female ovariectomized rats promotes improvement of spatial reference memory and an antidepressant effect and alters monoamines and BDNF levels in memory- and depression-related brain areas. *Behavioural Brain Research* **227** 100–108. (<https://doi.org/10.1016/j.bbr.2011.10.047>)
- Kohama SG, Brown SA, Finch CE & McNeill TH 1992 Chronic estradiol administration did not cause loss of hypothalamic LHRH or TIDA neurons in young or middle-aged C57BL/6J mice. *Brain Research* **574** 341–344. ([https://doi.org/10.1016/0006-8993\(92\)90838-Z](https://doi.org/10.1016/0006-8993(92)90838-Z))
- Kohama SG, Goss JR, McNeill TH & Finch CE 1995 Glial fibrillary acidic protein mRNA increases at proestrus in the arcuate nucleus of mice. *Neuroscience Letters* **183** 164–166. ([https://doi.org/10.1016/0304-3940\(94\)11141-5](https://doi.org/10.1016/0304-3940(94)11141-5))
- Konno S, Hirooka Y, Kishi T & Sunagawa K 2012 Sympathoinhibitory effects of telmisartan through the reduction of oxidative stress in the rostral ventrolateral medulla of obesity-induced hypertensive rats. *Journal of Hypertension* **30** 1992–1999. (<https://doi.org/10.1097/HJH.0b013e328357fa98>)
- Langub MC Jr & Watson RE Jr 1992 Estrogen receptor-immunoreactive glia, endothelia, and ependyma in guinea pig preoptic area and median eminence: electron microscopy. *Endocrinology* **130** 364–372. (<https://doi.org/10.1210/endo.130.1.1727710>)
- Lawson DM, Haisenleder DJ & Marshall JC 1993 A comparison of the temporal effects of estradiol and diethylstilbestrol on pituitary content of DNA, prolactin mRNA and prolactin and on serum prolactin levels in ovariectomized Holtzman rats. *Life Sciences* **53** 1267–1272. ([https://doi.org/10.1016/0024-3205\(93\)90571-J](https://doi.org/10.1016/0024-3205(93)90571-J))
- Legan SJ & Callahan WH 1999 Suppression of tonic luteinizing hormone secretion and norepinephrine release near the GnRH neurons by estradiol in ovariectomized rats. *Neuroendocrinology* **70** 237–245. (<https://doi.org/10.1159/000054482>)
- Legan SJ & Tsai HW 2003 Oestrogen receptor- α and - β immunoreactivity in gonadotropin-releasing hormone neurones after ovariectomy and chronic exposure to oestradiol. *Journal of Neuroendocrinology* **15** 1164–1170. (<https://doi.org/10.1111/j.1365-2826.2003.01115.x>)
- Li LH, Wang ZC, Yu J & Zhang YQ 2014. Ovariectomy results in variable changes in nociception, mood and depression in adult female rats. *PLoS One* **9** e94312. (<https://doi.org/10.1371/journal.pone.0094312>)
- Lim KG, Isles CG, Hodsmen GP, Lever AF & Robertson JW 1987 Malignant hypertension in women of childbearing age and its relation to the contraceptive pill. *BMJ* **294** 1057–1059. (<https://doi.org/10.1136/bmj.294.6579.1057>)
- Lima R, Wofford M & Reckelhoff JF 2012 Hypertension in postmenopausal women. *Current Hypertension Reports* **14** 254–260. (<https://doi.org/10.1007/s11906-012-0260-0>)
- Linzmayr L, Semlitsch HV, Saletu B, Bock G, Saletu-Zyhlarz G, Zoghalmi A, Gruber D, Metka M, Huber J, Oettel M *et al.* 2001 Double-blind, placebo-controlled psychometric studies on the effects of a combined estrogen-progestin regimen versus estrogen alone on performance, mood and personality of menopausal syndrome patients. *Arzneimittelforschung* **51** 238–245. (<https://doi.org/10.1055/s-0031-1300030>)
- Liu F, Benashski SE, Xu Y, Siegel M & McCullough LD 2012. Effects of chronic and acute oestrogen replacement therapy in aged animals after experimental stroke. *Journal of Neuroendocrinology* **24** 319–330. (<https://doi.org/10.1111/j.1365-2826.2011.02248.x>)
- Long JA & Evans HM 1922 The oestrous cycle in the rat and its associated phenomena. In *Memoirs of the University of California*, University of California Press, Berkeley, CA, USA pp 5–137.
- Loomis ED, Sullivan JC, Osmond DA, Pollock DM & Pollock JS 2005 Endothelin mediates superoxide production and vasoconstriction through activation of NADPH oxidase and uncoupled nitric oxide synthase in the rat aorta. *Journal of Pharmacology and Experimental Therapeutics* **315** 1058–1064. (<https://doi.org/10.1124/jpet.105.091728>)
- Lu KH, Hopper BR, Vargo TM & Yen SS 1979 Chronological changes in sex steroid, gonadotropin and prolactin secretions in aging female rats displaying different reproductive states. *Biology of Reproduction* **21** 193–203. (<https://doi.org/10.1095/biolreprod21.1.193>)
- Lu JK, Gilman DP, Meldrum DR, Judd HL & Sawyer CH 1981 Relationship between circulating estrogens and the central mechanisms by which ovarian steroids stimulate luteinizing hormone secretion in aged and young female rats. *Endocrinology* **108** 836–841. (<https://doi.org/10.1210/endo-108-3-836>)
- Luine VN, Richards ST, Wu VY & Beck KD 1998 Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. *Hormones and Behavior* **34** 149–162. (<https://doi.org/10.1006/hbeh.1998.1473>)
- Lund TD, Rovis T, Chung WC & Handa RJ 2005. Novel actions of estrogen receptor- β on anxiety-related behaviors. *Endocrinology* **146** 797–807. (<https://doi.org/10.1210/en.2004-1158>)
- Lynch AM, Murphy KJ, Deighan BF, O'Reilly JA, Gun'ko YK, Cowley TR, Gonzalez-Reyes RE & Lynch MA 2010 The impact of glial activation in the aging brain. *Aging and Disease* **1** 262–278.
- MacLennan AH, Henderson VW, Paine BJ, Mathias J, Ramsay EN, Ryan P, Stocks NP & Taylor AW 2006 Hormone therapy, timing of initiation, and cognition in women aged older than 60 years: the REMEMBER pilot study. *Menopause* **13** 28–36. (<https://doi.org/10.1097/01.gme.0000191204.38664.61>)
- Madden CJ & Sved AF 2003 Rostral ventrolateral medulla C1 neurons and cardiovascular regulation. *Cellular and Molecular Neurobiology* **23** 739–749. (<https://doi.org/10.1023/A:1025000919468>)
- Maeda T, Ikegami H, Sakata M, Yamaguchi M, Wada K, Koike K, Adachi K, Kurachi H, Hirota K & Miyake A 1996 Intraventricular administration of estradiol modulates rat prolactin secretion and synthesis. *Journal of Endocrinological Investigation* **19** 586–592. (<https://doi.org/10.1007/BF03349022>)
- Maki PM, Gast MJ, Vieweg AJ, Burriss SW & Yaffe K 2007 Hormone therapy in menopausal women with cognitive complaints: a randomized,

- double-blind trial. *Neurology* **69** 1322–1330. (<https://doi.org/10.1212/01.wnl.0000277275.42504.93>)
- Maki PM, Dennerstein L, Clark M, Guthrie J, LaMontagne P, Fornelli D, Little D, Henderson VW & Resnick SM 2011 Perimenopausal use of hormone therapy is associated with enhanced memory and hippocampal function later in life. *Brain Research* **1379** 232–243. (<https://doi.org/10.1016/j.brainres.2010.11.030>)
- Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, Trevisan M, Black HR, Heckbert SR, Detrano R *et al.* 2003 Estrogen plus progestin and the risk of coronary heart disease. *New England Journal of Medicine* **349** 523–534. (<https://doi.org/10.1056/NEJMoa030808>)
- Marder K & Sano M 2000 Estrogen to treat Alzheimer's disease: too little, too late? So what's a woman to do? *Neurology* **54** 2035–2037. (<https://doi.org/10.1212/WNL.54.11.2035>)
- Masana Y, Wanaka A, Kato H, Asai T & Tohyama M 1993 Localization of trkB mRNA in postnatal brain development. *Journal of Neuroscience Research* **35** 468–479. (<https://doi.org/10.1002/jnr.490350503>)
- McAsey ME, Cady C, Jackson LM, Li M, Randall S, Nathan BP & Struble RG 2006. Time course of response to estradiol replacement in ovariectomized mice: brain apolipoprotein E and synaptophysin transiently increase and glial fibrillary acidic protein is suppressed. *Experimental Neurology* **197** 197–205. (<https://doi.org/10.1016/j.expneurol.2005.09.008>)
- McEwen BS, Akama KT, Spencer-Segal JL, Milner TA & Waters EM 2012 Estrogen effects on the brain: actions beyond the hypothalamus via novel mechanisms. *Behavioral Neuroscience* **126** 4–16. (<https://doi.org/10.1037/a0026708>)
- Menani JV, Vieira AA, Colombari DSA, De Paula PM, Colombari E & De Luca LA Jr 2014 Preoptic-periventricular integrative mechanisms involved in behavior, fluid-electrolyte balance, and pressor responses. In *Neurobiology of Body Fluid Homeostasis: Transduction and Integration*, Eds L A De Luca jr, J V Menani and A K Johnson. Boca Raton (FL): CRC Press/Taylor & Francis; 2014. Chapter 4.
- Merlio JP, Ernfors P, Jaber M & Persson H 1992 Molecular cloning of rat trkC and distribution of cells expressing messenger RNAs for members of the trk family in the rat central nervous system. *Neuroscience* **51** 513–532. ([https://doi.org/10.1016/0306-4522\(92\)90292-A](https://doi.org/10.1016/0306-4522(92)90292-A))
- Miranda RC, Sohrabji F & Toran-Allerand CD 1993 Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. *PNAS* **90** 6439–6443. (<https://doi.org/10.1073/pnas.90.14.6439>)
- Mittelman-Smith MA, Williams H, Krajewski-Hall SJ, Lai J, Ciofi P, McMullen NT & Rance NE 2012 Arcuate kisspeptin/neurokinin B/dynorphin (KNDy) neurons mediate the estrogen suppression of gonadotropin secretion and body weight. *Endocrinology* **153** 2800–2812. (<https://doi.org/10.1210/en.2012-1045>)
- MohanKumar SM & MohanKumar PS 2004 Aging alters norepinephrine release in the medial preoptic area in response to steroid priming in ovariectomized rats. *Brain Research* **1023** 24–30. (<https://doi.org/10.1016/j.brainres.2004.06.040>)
- Mohankumar PS, Thyagarajan S & Quadri SK 1994 Correlations of catecholamine release in the medial preoptic area with proestrous surges of luteinizing hormone and prolactin: effects of aging. *Endocrinology* **135** 119–126. (<https://doi.org/10.1210/endo.135.1.8013343>)
- Mohankumar SM, Kasturi BS, Shin AC, Balasubramanian P, Gilbreath ET, Subramanian M & Mohankumar PS 2011 Chronic estradiol exposure induces oxidative stress in the hypothalamus to decrease hypothalamic dopamine and cause hyperprolactinemia. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **300** R693–R699. (<https://doi.org/10.1152/ajpregu.00481.2010>)
- Mora S, Dussaubat N & Diaz-Veliz G 1996. Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. *Psychoneuroendocrinology* **21** 609–620.
- Morel GR, Caron RW, Console GM, Soaje M, Sosa YE, Rodriguez SS, Jahn GA & Goya RG 2009 Estrogen inhibits tuberoinfundibular dopaminergic neurons but does not cause irreversible damage. *Brain Research Bulletin* **80** 347–352. (<https://doi.org/10.1016/j.brainresbull.2009.08.026>)
- Morgan MA & Pfaff DW 2001. Effects of estrogen on activity and fear-related behaviors in mice. *Hormones and Behavior* **40** 472–482. (<https://doi.org/10.1006/hbeh.2001.1716>)
- Mueller EE & Nistico G 1989 *Brain Messengers and the Pituitary*. San Diego: Academic Press.
- Nakashima A, Hayashi N, Kaneko YS, Mori K, Sabban EL, Nagatsu T & Ota A 2009 Role of N-terminus of tyrosine hydroxylase in the biosynthesis of catecholamines. *Journal of Neural Transmission* **116** 1355–1362. (<https://doi.org/10.1007/s00702-009-0227-8>)
- Nelson JF, Felicio LS, Osterburg HH & Finch CE 1981 Altered profiles of estradiol and progesterone associated with prolonged estrous cycles and persistent vaginal cornification in aging C57BL/6J mice. *Biology of Reproduction* **24** 784–794. (<https://doi.org/10.1095/biolreprod24.4.784>)
- Okada M, Hayashi N, Kometani M, Nakao K & Inukai T 1997 Influences of ovariectomy and continuous replacement of 17beta-estradiol on the tail skin temperature and behavior in the forced swimming test in rats. *Japanese Journal of Pharmacology* **73** 93–96. (<https://doi.org/10.1254/jjp.73.93>)
- Olatunji LA & Soladoye AO 2006 The effect of nifedipine on oral contraceptive-induced hypertension in rats. *Nigerian Postgraduate Medical Journal* **13** 277–281.
- Olatunji LA & Soladoye AO 2008 Oral contraceptive-induced high blood pressure is prevented by renin-angiotensin suppression in female rats but not by sympathetic nervous system blockade. *Indian Journal of Experimental Biology* **46** 749–754.
- Olatunji LA, Seok YM, Igundu A, Kang SH & Kim IK 2016. Combined oral contraceptive-induced hypertension is accompanied by endothelial dysfunction and upregulated intrarenal angiotensin II type 1 receptor gene expression. *Naunyn Schmiedeberg's Archives of Pharmacology* **389** 1147–1157. (<https://doi.org/10.1007/s00210-016-1272-0>)
- Oliveira-Sales EB, Nishi EE, Carillo BA, Boim MA, Dolnikoff MS, Bergamaschi CT & Campos RR 2009 Oxidative stress in the sympathetic premotor neurons contributes to sympathetic activation in renovascular hypertension. *American Journal of Hypertension* **22** 484–492. (<https://doi.org/10.1038/ajh.2009.17>)
- Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ & Scanlan TS 1997 Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. *Science* **277** 1508–1510. (<https://doi.org/10.1126/science.277.5331.1508>)
- Patriarca MT, Barbosa de Moraes AR, Nader HB, Petri V, Martins JR, Gomes RC & Soares JM Jr 2013 Hyaluronic acid concentration in postmenopausal facial skin after topical estradiol and genistein treatment: a double-blind, randomized clinical trial of efficacy. *Menopause* **20** 336–341. (<https://doi.org/10.1097/GME.0b013e318269898c>)
- Pereira RI, Casey BA, Swibas TA, Erickson CB, Wolfe P & Van Pelt RE 2015 Timing of estradiol treatment after menopause may determine benefit or harm to insulin action. *Journal of Clinical Endocrinology and Metabolism* **100** 4456–4462. (<https://doi.org/10.1210/jc.2015-3084>)
- Perez de la Mora M, Gallegos-Cari A, Crespo-Ramirez M, Marcellino D, Hansson AC & Fuxe K 2012 Distribution of dopamine D(2)-like receptors in the rat amygdala and their role in the modulation of unconditioned fear and anxiety. *Neuroscience* **201** 252–266. (<https://doi.org/10.1016/j.neuroscience.2011.10.045>)
- Radi R 2013 Protein tyrosine nitration: biochemical mechanisms and structural basis of functional effects. *Accounts of Chemical Research* **46** 550–559. (<https://doi.org/10.1021/ar300234c>)
- Rance NE 2009 Menopause and the human hypothalamus: evidence for the role of kisspeptin/neurokinin B neurons in the regulation of estrogen negative feedback. *Peptides* **30** 111–122. (<https://doi.org/10.1016/j.peptides.2008.05.016>)
- Rance N, Wise PM, Selmanoff MK & Barraclough CA 1981 Catecholamine turnover rates in discrete hypothalamic areas and associated changes in median eminence luteinizing hormone-releasing hormone and serum gonadotropins on proestrus and diestrus day 1. *Endocrinology* **108** 1795–1802. (<https://doi.org/10.1210/endo-108-5-1795>)
- Raudaskoski T, Tomas C & Laatikainen T 1999 Insulin sensitivity during postmenopausal hormone replacement with transdermal estradiol and intrauterine levonorgestrel. *Acta Obstetrica et Gynecologica Scandinavica* **78** 540–545. (<https://doi.org/10.1080/j.1600-0412.1999.780612.x>)
- Raymond V, Beaulieu M, Labrie F & Boissier J 1978 Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. *Science* **200** 1173–1175. (<https://doi.org/10.1126/science.418505>)
- Resnick SM & Henderson VW 2002 Hormone therapy and risk of Alzheimer disease: a critical time. *JAMA* **288** 2170–2172. (<https://doi.org/10.1001/jama.288.17.2170>)

- Sakata K & Duke SM 2014 Lack of BDNF expression through promoter IV disturbs expression of monoamine genes in the frontal cortex and hippocampus. *Neuroscience* **260** 265–275. (<https://doi.org/10.1016/j.neuroscience.2013.12.013>)
- Santin AP & Furlanetto TW 2011 Role of estrogen in thyroid function and growth regulation. *Journal of Thyroid Research* **2011** 7.
- Sarkar DK, Gottschall PE & Meites J 1984 Decline of tuberoinfundibular dopaminergic function resulting from chronic hyperprolactinemia in rats. *Endocrinology* **115** 1269–1274. (<https://doi.org/10.1210/endo-115-4-1269>)
- Schally AV, Arimura A, Kastin AJ, Matsuo H, Baba Y, Redding TW, Nair RM, Debeljuk L & White WF 1971 Gonadotropin-releasing hormone: one polypeptide regulates secretion of luteinizing and follicle-stimulating hormones. *Science* **173** 1036–1038. (<https://doi.org/10.1126/science.173.4001.1036>)
- Shelton KA, Cline JM & Cann JA 2013. 17 β -Estradiol reduces atherosclerosis without exacerbating lupus in ovariectomized systemic lupus erythematosus-susceptible LDLr(-/-) mice. *Atherosclerosis* **227** 228–235. (<https://doi.org/10.1016/j.atherosclerosis.2012.12.031>)
- Shen M, Kumar SP & Shi H 2014 Estradiol regulates insulin signaling and inflammation in adipose tissue. *Hormone Molecular Biology and Clinical Investigation* **17** 99–107. (<https://doi.org/10.1515/hmbci-2014-0007>)
- Shenoy V, Grobe JL, Qi Y, Ferreira AJ, Fraga-Silva RA, Collamat G, Bruce E & Katovich M J 2009. 17 β -Estradiol modulates local cardiac renin-angiotensin system to prevent cardiac remodeling in the DOCA-salt model of hypertension in rats. *Peptides* **30** 2309–2315. (<https://doi.org/10.1016/j.peptides.2009.09.005>)
- Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Lane DS, Fillit H, Stefanick ML, Hendrix SL, Lewis CE *et al.* 2004 Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. *JAMA* **291** 2947–2958. (<https://doi.org/10.1001/jama.291.24.2947>)
- Sierra A, Gottfried-Blackmore A, Milner TA, McEwen BS & Bulloch K 2008 Steroid hormone receptor expression and function in microglia. *Glia* **56** 659–674. (<https://doi.org/10.1002/glia.20644>)
- Simpkins JW 1983 Hormone and neurotransmitter changes. In *Neuroendocrinology of Aging*, Ed J Meites Plenum Press: NY, USA pp 41–59.
- Singh M, Meyer EM & Simpkins JW 1995 The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. *Endocrinology* **136** 2320–2324. (<https://doi.org/10.1210/endo.136.5.7720680>)
- Singh M, Setalo G Jr, Guan X, Warren M & Toran-Allerand CD 1999 Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. *Journal of Neuroscience* **19** 1179–1188. (<https://doi.org/10.1523/JNEUROSCI.19-04-01179.1999>)
- Sirivelu MP, Burnett R, Shin AC, Kim C, MohanKumar PS & MohanKumar SM 2009a Interaction between GABA and norepinephrine in interleukin-1 β -induced suppression of the luteinizing hormone surge. *Brain Research* **1248** 107–114. (<https://doi.org/10.1016/j.brainres.2008.10.057>)
- Sirivelu MP, Shin AC, Perez GI, MohanKumar PS & MohanKumar SM 2009b Effect of L-dopa on interleukin-1 β -induced suppression of luteinizing hormone secretion in intact female rats. *Human Reproduction* **24** 718–725. (<https://doi.org/10.1093/humrep/den434>)
- Sirivelu MP, MohanKumar PS & MohanKumar SM 2012 Differential effects of systemic interleukin-1 β on gene expression in brainstem noradrenergic nuclei. *Life Sciences* **90** 77–81. (<https://doi.org/10.1016/j.lfs.2011.10.006>)
- Skovlund CW, Morch LS, Kessing LV & Lidegaard O 2016 Association of hormonal contraception with depression. *JAMA Psychiatry* **73** 1154–1162. (<https://doi.org/10.1001/jamapsychiatry.2016.2387>)
- Soderstrom I, Strand M, Ingridsson AC, Nasic S & Olsson T 2009 17 β -Estradiol and enriched environment accelerate cognitive recovery after focal brain ischemia. *European Journal of Neuroscience* **29** 1215–1224. (<https://doi.org/10.1111/j.1460-9568.2009.06662.x>)
- Sohrabji F, Miranda RC & Toran-Allerand CD 1995 Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. *PNAS* **92** 11110–11114. (<https://doi.org/10.1073/pnas.92.24.11110>)
- Sophonsritsuk A, Appt SE, Clarkson TB, Shively CA, Espeland MA & Register TC 2013. Differential effects of estradiol on carotid artery inflammation when administered early versus late after surgical menopause. *Menopause* **20** 540–547. (<https://doi.org/10.1097/GME.0b013e31827461e0>)
- Steger RW, Huang HH, Chamberlain DS & Meites J 1980 Changes in control of gonadotropin secretion in the transition period between regular cycles and constant estrus in aging female rats. *Biology of Reproduction* **22** 595–603. (<https://doi.org/10.1093/biolreprod/22.3.595>)
- Stocker SD, Meador R & Adams JM 2007 Neurons of the rostral ventrolateral medulla contribute to obesity-induced hypertension in rats. *Hypertension* **49** 640–646. (<https://doi.org/10.1161/01.HYP.0000254828.71253.dc>)
- Stone DJ, Song Y, Anderson CP, Krohn KK, Finch CE & Rozovsky I 1998 Bidirectional transcription regulation of glial fibrillary acidic protein by estradiol in vivo and in vitro. *Endocrinology* **139** 3202–3209. (<https://doi.org/10.1210/endo.139.7.6084>)
- Su JD, Qiu J, Zhong YP & Chen YZ 2001 Expression of estrogen receptor-alpha and -beta immunoreactivity in the cultured neonatal suprachiasmatic nucleus: with special attention to GABAergic neurons. *Neuroreport* **12** 1955–1959. (<https://doi.org/10.1097/00001756-200107030-00036>)
- Subramanian M, Balasubramanian P, Garver H, Northcott C, Zhao H, Haywood JR, Fink GD, MohanKumar SM & MohanKumar PS 2011 Chronic estradiol-17 β exposure increases superoxide production in the rostral ventrolateral medulla and causes hypertension: reversal by resveratrol. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **300** R1560–R1568. (<https://doi.org/10.1152/ajpregu.00020.2011>)
- Subramanian M, Hahn-Townsend C, Clark KA, MohanKumar SM & MohanKumar PS 2015 Chronic estrogen exposure affects gene expression in the rostral ventrolateral medulla of young and aging rats: Possible role in hypertension. *Brain Research* **1627** 134–142. (<https://doi.org/10.1016/j.brainres.2015.09.007>)
- Subramanian M, MohanKumar SM, Balasubramanian P, Northcott CA, Garver H, Fink GD & MohanKumar PS 2017 Chronic exposure to low doses of estradiol-17 β increases blood pressure in young female rats: a possible role for central Endothelin-1. *Scientific Reports* **7** 139. (<https://doi.org/10.1038/s41598-017-00213-9>)
- Surace MJ & Block ML 2012 Targeting microglia-mediated neurotoxicity: the potential of NOX2 inhibitors. *Cellular and Molecular Life Sciences* **69** 2409–2427. (<https://doi.org/10.1007/s00018-012-1015-4>)
- Tsai HW & Legan SJ 2001 Chronic elevation of estradiol in young ovariectomized rats causes aging-like loss of steroid-induced luteinizing hormone surges. *Biology of Reproduction* **64** 684–688. (<https://doi.org/10.1095/biolreprod64.2.684>)
- Tsai HW & Legan SJ 2002 Loss of luteinizing hormone surges induced by chronic estradiol is associated with decreased activation of gonadotropin-releasing hormone neurons. *Biology of Reproduction* **66** 1104–1110. (<https://doi.org/10.1095/biolreprod66.4.1104>)
- Turko IV & Murad F 2002 Protein nitration in cardiovascular diseases. *Pharmacological Reviews* **54** 619–634. (<https://doi.org/10.1124/pr.54.4.619>)
- Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV *et al.* 2012 Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine Reviews* **33** 378–455. (<https://doi.org/10.1210/er.2011-1050>)
- Viant MR, Millam JR, Delany ME & Fry DM 2000 Regulation of brain-derived neurotrophic factor messenger RNA levels in avian hypothalamic slice cultures. *Neuroscience* **99** 373–380. ([https://doi.org/10.1016/S0306-4522\(00\)00167-6](https://doi.org/10.1016/S0306-4522(00)00167-6))
- Walf AA & Frye CA 2009. Estradiol enhances sociosexual behavior and can have proliferative effects in ovariectomized rats. *Age* **31** 221–229. (<https://doi.org/10.1007/s11357-008-9079-7>)
- Walf AA & Frye CA 2010a. Estradiol reduces anxiety- and depression-like behavior of aged female mice. *Physiology & Behavior* **99** 169–174. (<https://doi.org/10.1016/j.physbeh.2009.09.017>)
- Walf AA & Frye CA 2010b. Raloxifene and/or estradiol decrease anxiety-like and depressive-like behavior, whereas only estradiol increases carcinogen-induced tumorigenesis and uterine proliferation among ovariectomized rats. *Behavioural Pharmacology* **21** 231–240.

- Wang Q & Zheng JQ 1998 cAMP-mediated regulation of neurotrophin-induced collapse of nerve growth cones. *Journal of Neuroscience* **18** 4973–4984. (<https://doi.org/10.1523/JNEUROSCI.18-13-04973.1998>)
- West AE, Chen WG, Dalva MB, Dolmetsch RE, Kornhauser JM, Shaywitz AJ, Takasu MA, Tao X & Greenberg ME 2001 Calcium regulation of neuronal gene expression. *PNAS* **98** 11024–11031. (<https://doi.org/10.1073/pnas.191352298>)
- Westerhaus MJ & Loewy AD 1999 Sympathetic-related neurons in the preoptic region of the rat identified by viral transneuronal labeling. *Journal of Comparative Neurology* **414** 361–378. ([https://doi.org/10.1002/\(SICI\)1096-9861\(19991122\)414:3<361::AID-CNE6>3.0.CO;2-X](https://doi.org/10.1002/(SICI)1096-9861(19991122)414:3<361::AID-CNE6>3.0.CO;2-X))
- Wetmore C, Ernfors P, Persson H & Olson L 1990 Localization of brain-derived neurotrophic factor mRNA to neurons in the brain by in situ hybridization. *Experimental Neurology* **109** 141–152. ([https://doi.org/10.1016/0014-4886\(90\)90068-4](https://doi.org/10.1016/0014-4886(90)90068-4))
- Wharton W, Baker LD, Gleason CE, Dowling M, Barnet JH, Johnson S, Carlsson C, Craft S & Asthana S 2011 Short-term hormone therapy with transdermal estradiol improves cognition for postmenopausal women with Alzheimer's disease: results of a randomized controlled trial. *Journal of Alzheimer's Disease* **26** 495–505. (<https://doi.org/10.3233/JAD-2011-110341>)
- Whitmer RA, Quesenberry CP, Zhou J & Yaffe K 2011 Timing of hormone therapy and dementia: the critical window theory revisited. *Annals of Neurology* **69** 163–169. (<https://doi.org/10.1002/ana.22239>)
- Wing LY, Chen YC, Shih YY, Cheng JC, Lin YJ & Jiang MJ 2009. Effects of oral estrogen on aortic ROS-generating and -scavenging enzymes and atherosclerosis in apoE-deficient mice. *Experimental Biology and Medicine* **234** 1037–1046. (<https://doi.org/10.3181/0811-RM-332>)
- Winklewski PJ, Radkowski M, Wszedybyl-Winklewska M & Demkow U 2015 Brain inflammation and hypertension: the chicken or the egg? *Journal of Neuroinflammation* **12** 85. (<https://doi.org/10.1186/s12974-015-0306-8>)
- Wise PM 1984 Estradiol-induced daily luteinizing hormone and prolactin surges in young and middle-aged rats: correlations with age-related changes in pituitary responsiveness and catecholamine turnover rates in microdissected brain areas. *Endocrinology* **115** 801–809. (<https://doi.org/10.1210/endo-115-2-801>)
- Wise PM, Rance N & Barraclough CA 1981 Effects of estradiol and progesterone on catecholamine turnover rates in discrete hypothalamic regions in ovariectomized rats. *Endocrinology* **108** 2186–2193. (<https://doi.org/10.1210/endo-108-6-2186>)
- Woods JW 1988 Oral contraceptives and hypertension. *Hypertension* **11** II11–II15.
- Wu H, Friedman WJ & Dreyfus CF 2004 Differential regulation of neurotrophin expression in basal forebrain astrocytes by neuronal signals. *Journal of Neuroscience Research* **76** 76–85. (<https://doi.org/10.1002/jnr.20060>)
- Xu Y, Sheng H, Bao Q, Wang Y, Lu J & Ni X 2016. NLRP3 inflammasome activation mediates estrogen deficiency-induced depression- and anxiety-like behavior and hippocampal inflammation in mice. *Brain, Behavior, and Immunity* **56** 175–186. (<https://doi.org/10.1016/j.bbi.2016.02.022>)
- Yan QS, Zheng SZ & Yan SE 2004 Prenatal cocaine exposure decreases brain-derived neurotrophic factor proteins in the rat brain. *Brain Research* **1009** 228–233. (<https://doi.org/10.1016/j.brainres.2004.02.052>)
- Young CN & Davisson RL 2015 Angiotensin-II, the brain, and hypertension: an update. *Hypertension* **66** 920–926. (<https://doi.org/10.1161/HYPERTENSIONAHA.115.03624>)
- Young NA, Wu LC, Burd CJ, Friedman AK, Kaffenberger BH, Rajaram MV, Schlesinger LS, James H, Shupnik MA & Jarjour WN 2014 Estrogen modulation of endosome-associated toll-like receptor 8: an IFN α -independent mechanism of sex-bias in systemic lupus erythematosus. *Clinical Immunology* **151** 66–77. (<https://doi.org/10.1016/j.clim.2014.01.006>)
- Zandi PP, Carlson MC, Plassman BL, Welsh-Bohmer KA, Mayer LS, Steffens DC, Breitner JC & Cache County Memory Study Investigators 2002 Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. *JAMA* **288** 2123–2129. (<https://doi.org/10.1001/jama.288.17.2123>)
- Zhang Z, Qin P, Deng Y, Ma Z, Guo H, Guo H, Hou Y, Wang S, Zou W, Sun Y *et al.* 2018 The novel estrogenic receptor GPR30 alleviates ischemic injury by inhibiting TLR4-mediated microglial inflammation. *Journal of Neuroinflammation* **15** 206. (<https://doi.org/10.1186/s12974-018-1246-x>)

Received 5 March 2018

First decision 3 April 2018

Revised manuscript received 29 August 2018

Accepted 17 September 2018