ELSEVIER

Neurotoxicology and Teratology 24 (2002) 5–16

NEUROTOXICOLOGY AND TERATOLOGY

Neurobehavioral effects of dietary soy phytoestrogens

Edwin D. Lephart[a,*], Timothy W. West[a], K. Scott Weber[a], Reuben W. Rhees[a], Kenneth D.R. Setchell[b], Herman Adlercreutz[c], Trent D. Lund[a]

[a]*Neuroscience Center, 633 WIDB, Brigham Young University, Provo, UT 86402, USA*
[b]*Clinical Mass Spectrometry, Children's Hospital Medical Center, Cincinnati, OH 45229, USA*
[c]*Institute for Preventive Medicine, Nutrition, and Cancer, Folkhälsan Research, Center, and Division of Clinical Chemistry, University of Helsinki, Helsinki, Finland*

Received 27 June 2001; received in revised form 14 September 2001; accepted 26 October 2001

Abstract

Phytoestrogens, plant-derived nonsteroidal estrogens found in high abundance in most soy food products, have been studied for their potential beneficial effects against hormone-dependent cancers and age-related diseases. However, little is known about the influence of phytoestrogens on the brain or behavior. This brief review describes mainly our own studies in rodents that have examined the influence of dietary soy isoflavones on certain aspects of brain structure, learning, memory and anxiety along with the brain androgen-metabolizing enzyme, aromatase. These studies used a commercially available diet rich in phytoestrogens (Phyto-rich) vs. a custom diet relatively free of phytoestrogens (Phyto-free). The phytoestrogen content of each diet was determined by high-performance liquid chromatography analysis, circulating plasma phytoestrogen levels were quantified by gas chromatography mass spectroscopy and concentrations of phytoestrogens in specific brain regions were measured by time-resolved fluoroimmunoassay (TR-FIA). Our studies showed that brain aromatase levels were not significantly altered by phytoestrogen diet treatments in perinatal, maternal or adult rats. However, volumes of the sexually dimorphic nucleus of the preoptic area (SDN-POA) were significantly affected by the Phyto-free diet treatment in male rats during adulthood, where SDN-POA volumes were smaller compared to Phyto-rich male values. Additionally, the Phyto-rich diet fed to adult male and female rats produced anxiolytic effects as assessed in the elevated plus maze vs. Phyto-free fed animals. Finally, when learning and memory parameters were examined in a radial arm maze testing visual–spatial memory (VSM), the diet treatments significantly changed the typical sexually dimorphic pattern of VSM. Specifically, adult Phyto-rich fed females outperformed Phyto-free fed females, while in males on the same diets, the opposite pattern of maze performance was observed. When female vs. male performance was compared, Phyto-rich females executed the VSM task in a manner similar to that of Phyto-free fed males, while Phyto-free fed female's VSM was comparable to Phyto-rich males. These results indicate that consumption of dietary phytoestrogens resulting in very high plasma isoflavone levels (in many cases over a relatively short interval of consumption in adulthood) can significantly alter sexually dimorphic brain regions, anxiety, learning and memory. The findings of these studies identify the biological actions of phytoestrogens, specifically isoflavones and their metabolites, found in animal soy-containing diets on brain and behavior and implicate the importance of phytoestrogens given the recognized significance of estrogens in brain and neural disorders, such as Alzheimer's disease, especially in women.

Keywords: Phytoestrogens; Radial arm maze; Elevated plus maze; Brain aromatase; Rat

1. Introduction

Phytoestrogens are represented by hundreds of different types of molecules that are broadly classified as nonsteroidal in configuration with a customary diphenolic structure. They are abundant in fruits, vegetables, legumes, whole grains and especially flaxseed, clover and soy products, and they have many of the physicochemical and physiological properties of estrogens [1,2,4,10,17,18,42,56,61,62,83,86,94,101,102,104,110,117]. The three main classes of phytoestrogens of clinical interest are: (1) isoflavones (derived principally from soybeans and clover), (2) lignans (found in flaxseed in large quantities) and (3) coumestans (derived from sprouting plants like alfalfa) [1,2,4,10,17,42,56,61,62,83,86,94,101,102,104,117]. Of the isoflavones, genistein and daidzein are thought to exert the most potent estrogenic

* Corresponding author. Tel.: +1-801-378-1218.
E-mail address: edwin_lephart@byu.edu (E.D. Lephart).

0892-0362/02/$ – see front matter
PII: S0892-0362(01)00197-0

hormone activity and thus most of the research attention has been directed toward these molecules [2,4,17,42,56–59,83,94,101,102,104]. Apparently, the phenolic B-ring of isoflavones confers binding to estrogen receptors (ER $\beta > \alpha$, although at a lower affinity relative to estradiol) and acts like natural selective estrogen receptor modulators (SERMs) [19,45,47,57–59,108]. In some cases, there is evidence that phytoestrogens act as estrogen agonists at tissue-specific targets, whereas in others, they display antagonistic characteristics comparable to that of tamoxifen, or especially raloxifene, where SERM activity may be sex hormone- and gender-dependent [45,47,86].

Phytoestrogens have been shown in animal models and in limited clinical investigations to be protective in the prevention of: (1) hormone-dependent cancers (e.g., breast and prostate), (2) cardiovascular disease, (3) osteoporosis and (4) to alleviate the symptoms of menopause (such as hot flashes) [1,2,4,5,11,42,56,60,61,83,86,94,101,102,104,110,117,126]. The anticancer effects of phytoestrogens appear to be associated with several possible mechanisms including their ability to inhibit tyrosine kinase(s), growth factors, DNA topoisomerase, steroidogenic enzymes and to act as antioxidant and antiangiogenic agents [1,2,4,5,11,34,56,60,61,83,86,101,102,104].

In reference to the health benefits of soy and cardiovascular protection, in October 1999, the US Food and Drug Administration approved a health "claim" for soy protein (25 g/day) in reducing the risk of coronary heart disease by its hypocholesterolemic effect — specifically food products that contain 6.25 g per serving of soy protein qualified for the health "claim." At the time, there was no recognition of a protective effect from isoflavones due to a paucity of data, but subsequent studies have shown that the cholesterol-lowering effect is influenced in a dose-dependent manner by the presence of isoflavones in the protein matrix [25,117]. Furthermore, data collected thus far in animals and from limited clinical trials show that isoflavones have protective or no effects on bone [8,29,62,93,100]. The synthetic phytoestrogen, ipriflavone, has been reported to increase the rate of bone formation (via a different mechanism than that of estradiol) where it may act analogous to SERMs having antiestrogenic effects on breast tissue, no apparent effect on uterotrophic activity and a proestrogenic effect on bone [8]. However, a recent large study reported that ipriflavone is not effective for osteoporosis [6]. Finally, the focus of soy consumption in addressing the symptoms of menopause is covered below.

Epidemiological data have shown clear relationships between the incidence rates of prostate cancer (PCa), breast cancer (BCa) and soy consumption. In the examination of the use of soy, for example, consumption of tofu in Asian countries can be documented for thousands of years [11], whereas the introduction and growing of soybeans in the USA have taken place only within the last 100 years [11]. Moreover, the awareness of the potential health benefits and consumption of soy products [103] in the USA has only increased within recent years [1,2,4,5,11,42,101,102,104,117]. The relatively high consumption of soy in Japan corresponds to the relatively low incidence of PCa and BCa compared to that in the USA. These correlations, along with the estrogenic nature of the biologically active molecules, have led to the proposal that phytoestrogen-rich diets may explain these observations [1,2,4,5,11,37,44,56,60–62,83,86,101–104]. In the examination of PCa, support for the connection between phytoestrogens and decreased PCa comes from rodent studies where soy diets have been found to be protective against PCa cell growth [5,11,20,22,26,34,44,60,63,82,86,101,104,106,110,120,129]. Additionally, several human studies suggest that phytoestrogens inhibit benign prostatic hyperplasia (BPH) and PCa growth in vitro and in vivo [22,44,110]. In the USA (and in other developed countries), PCa is the second most common cause of cancer death and BPH is potentially a premalignant condition representing a major health concern in men.

Conversely, the benefits of phytoestrogens compared to hormone replacement therapy (HRT) in women have received increased media attention directed mainly at the prevention and treatment of perimenopausal and menopausal symptoms [42,117]. While there is evidence to suggest that phytoestrogens may alleviate many of the symptoms of menopause [42,117], there are abundant data demonstrating the proven health benefits of traditional HRT compared to the relatively small number of clinical studies validating the effectiveness of phytoestrogens in treating menopausal symptoms. Effects on hot flushes are modest in comparison to that observed for HRT, but nevertheless, there is an indication from the relatively low rates of hot flushes in Japan that the lifelong exposure to soy isoflavones may be a more important determinant of the effectiveness in reducing symptoms of the menopause. For example, in several studies, no effects of soy or isoflavone-containing supplements were observed. Ongoing and future clinical trials will provide additional evidence as to whether the use of phytoestrogens will complement the traditional utilization of HRT due to their efficacy, ease of administration and potentially less toxic effects, even though these molecules have been described as endocrine disruptors [42,117,124]. For example, it is not known whether phytoestrogens act as agonists or antagonists with SERMs (such as tamoxifen or raloxifene) that may protect against or promote neoplastic growth [19,42,45,62,117]. Finally, if a dietary approach of treating or preventing these conditions proves successful, this will have global implications for human health benefits associated with dietary soy consumption.

While some studies have examined the effects of phytoestrogens on brain [35,36,45,51,55,69,73,88–90], the influence of these estrogen mimics on brain and behavior is still largely unknown. A recent epidemiological study that created some concern was the observation of deteriorating brain function in Japanese–American men living in Oahu and consuming more than two to three servings of tofu per week. While no direct evidence for an

effect of phytoestrogens was provided by this study, it was indirectly implied by the authors that phytoestrogens were involved. The role of isoflavones on human brain function is clearly an area that is important to study. Given the practical difficulties of examining at the cellular, molecular and physiological level the effects of isoflavones on human brain function, we have turned our focus on the use of animal models to study their effects, even though there may be some limitations to this approach. This report will focus mainly on our studies in rats that have investigated the influence of dietary soy-derived phytoestrogens on certain aspects of brain structure/chemistry, learning, memory, anxiety and the brain androgen-metabolizing enzyme, aromatase.

2. Phytoestrogen diets

Soymeal is a protein source in most commonly used and commercially available rodent diets (ranging from approximately 200 to over 800 μg/g) [18,114]. Therefore, animals ingesting these diets are continually exposed to these hormonally active compounds [18,114]. The estrogenic potency of naturally occurring soy phytoestrogens is significant, and if concentrations are high, these compounds have the potential to trigger many of the biological responses evoked by endogenous steroidal estrogens [18,101,104]. Notably, rather than use a diet that contains only one or a combination of a few phytoestrogens (such as genistein and/or daidzein), throughout our studies, we utilized a commercially available diet (Harlan Teklad 8604, Madison, WI, USA) which was found by high-performance liquid chromatography to contain [24,105] approximately 600 μg/g total phytoestrogens (in the glycoside form), being equivalent to approximately 420 μg/g diet of the aglycones (or total isoflavones). This diet is referred to as the Phyto-rich diet. In this diet, soymeal is the first ingredient listed in the formulation (and the diet does not contain alfalfa). The total isoflavone concentration of the diet we used is similar to that reported in a separate study (approximately 350 μg/g diet of total isoflavones) that was found to have estrogenic activity in rodents [16]. We have compared the influence of this commercial diet to that of a custom diet with very low concentrations of phytoestrogens (Ziegler Brothers, Gardner, PA, USA) [24,105]. This custom diet is referred to, hereafter, as the Phyto-free diet. The exact composition of the two diets: percentage protein, fat, carbohydrate levels, amino acid, vitamin and mineral levels have been published elsewhere [78,122]. For comparison, other commercially available diets, like the Purina no. 5001 diet, contain 810 μg/g, while the NIH-07 diet has 394 μg/g total phytoestrogens [18]. Finally, reference to phytoestrogens in the current presentation will refer to isoflavones derived from soy and does not include the coumestans such as coumestrol that are sometimes present in rodent diets that are formulated with alfalfa.

2.1. Phytoestrogen plasma levels

Using these diets, more than five studies have been conducted by our laboratories examining two different strains of rats (i.e., Sprague–Dawley and Long–Evans animals) [69,78,113,120–122]. In studies with Sprague–Dawley rats fed the Phyto rich diet, the plasma isoflavone concentrations were expectedly 35- to 76-fold higher than values found in animals fed the Phyto-free diet [69,121,122]. With Long–Evans rats, plasma isoflavone concentrations were also higher but the magnitude of the difference between the diet treatments was less [78], suggesting a possible strain difference in the absorption, metabolism or bioavailability. In general, females tended to have slightly higher total plasma isoflavone concentrations than males, although the difference was not statistically significant. However, since male and female plasma isoflavone concentrations were similar with the two diets, the plasma phytoestrogen data for male and female animals were combined (see Fig. 1). Overall, a 20-fold higher circulating isoflavone concentration was observed between animals fed the phyto-rich and phyto-free diets. The major plasma phytoestrogen in the Phyto-rich diet fed animals was equol (a specific metabolite of the soy isoflavone aglycones, daidzein, and presumably genistein) [69,78,121,122]. The plasma phytoestrogen concentrations are similar to that of adults living in Asia (1–2 μM) where the average intake of soy protein is 4–8 g/day or 20–50 g of soy food products [1,2,4,5]. The low levels of plasma phytoestrogens from the Phyto-free diet fed animals can be

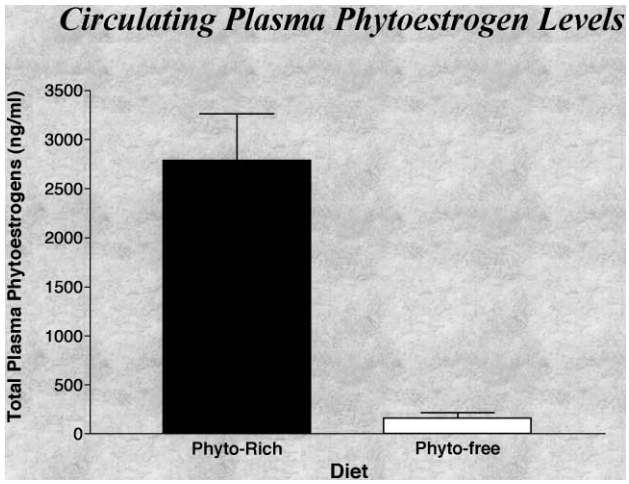

Fig. 1. Circulating plasma phytoestrogen levels in Sprague–Dawley and Long–Evans rats combined from five different studies. The animals were fed (lifelong) either the Phyto-rich diet or the Phyto-free diet from conception to adulthood. Phytoestrogen analysis was performed using gas chromatography mass spectrometry. The phytoestrogen levels (combined across sex vs. diet) are expressed in nanograms per milliliters (ng/ml). Total phytoestrogens = the sum of the three main phytoestrogens (equol, daidzein and genistein) and are expressed as the mean ± S.E.M. Animals fed the Phyto-rich diet displayed significantly higher total phytoestrogen serum levels than those fed the Phyto-free diet.

considered to be reflective of the typical intakes of people in Western populations where soy foods are rarely consumed [1,2,4,5,69,78]. Therefore, we have designed our animal model to mimic the typical exposure levels of phytoestrogens to these two contrasting populations.

2.2. Phytoestrogen brain levels

Evidence showing that phytoestrogens can reach the brain was obtained after intraperitoneal injection of genistein and daidzein to adult Sprague–Dawley rats (Ref. [40]; reviewed by Setchell in Ref. [101]). In that study, rapid appearance of both isoflavones was noted from the greatly increased isoflavone levels in brain tissue. Phytoestrogens, like endogenous estrogens, can clearly enter the lipophilic environment of the brain, and do so rather quickly. In order to determine the influence of dietary soy phytoestrogens on neurobehavioral parameters, we first confirmed that these molecules entered the brain and then we examined the relative phytoestrogen content of specific brain structures. This was accomplished by time-resolved fluoroimmunoassay (TR-FIA) on lyophilized brain tissue samples [119]. In one of our studies, daidzein and genistein levels in the medial basal hypothalamus (MBH) were approximately eightfold higher in Phyto-rich fed animals vs. Phyto-free fed rats [69]. In a more recent study where improved methodologies enabled the detection of the aglycones (daidzein and genistein) plus the metabolite, equol, our MBH results were confirmed along with the finding that the phytoestrogen content of the cerebellum of Phyto-rich fed animals was approximately ninefold higher than in animals fed the Phyto-free diet [71,80]. This is an important finding because the cerebellum has an abundance of estrogen receptor beta located in Purkinje cells and isoflavones have a relatively high affinity for binding to the ERβ receptor subtype [12,95]. Finally, in other as yet unpublished data, it was observed that in the frontal cortical region of the brain, where again estrogen receptor beta is abundant, Phyto- rich fed animals had tissue levels that were approximately 50-fold higher than animals fed the Phyto-free diets [80]. These studies establish conclusively that phytoestrogens are concentrated in specific regions of the brain that are associated with an abundance of ER and this may have relevance to their potential impact on learning and memory.

2.3. Brain aromatase and dietary phytoestrogens

A major androgen-metabolizing enzyme, aromatase cytochrome *P*450, plays a critical role in influencing the genesis of sexually dimorphic brain structures during perinatal development by converting testicular-derived testosterone to estrogen in situ within certain brain structures [9,65,67,70]. The local formation of estrogens (from androgen precursors) via brain aromatase also modulates neuroendocrine functions and regulates sexual behavior in hypothalamic regions of the brain [65,67]. These biological parameters represent important elements in the structure/function relationship of the genesis of the rat CNS perinatally and the morphometric and functional characteristics of hypothalamic brain areas postnatally [28,65,67,70]. It is known that phytoestrogens inhibit aromatase enzyme activity in peripheral endocrine tissue sites during postnatal development (in vitro) [2,3,49,54,56,86,118]. However, the effect of phytoestrogens on brain aromatase has not been previously examined until now. In adult Sprague–Dawley male, perinatal male and female, or maternal rats, brain aromatase enzyme activity was not significantly influenced by dietary phytoestrogens [71,72,120,121]. This suggests that the effects of dietary phytoestrogens in the brain are not at the site of the aromatase enzyme, and hence, alterations in the production of endogenous estrogens in situ in the brain are probably not the location for the primary influences of dietary phytoestrogens in neural tissues. A more reasonable explanation is that phytoestrogens act on estrogen receptors to exert their agonist or antagonist effect(s) that in turn may influence brain structure and function (see Section 3 below).

3. Phytoestrogens alter brain morphology

Phytoestrogens have been shown to alter brain structure. The sexually dimorphic nucleus in the preoptic area of the hypothalamus (SDN-POA), which is normally two to five times larger in male than female rats, is significantly affected by the perinatal administration of genistein [9,35,36,73,127]. In two different studies, the neonatal exposure of genistein to gonadectomized females resulted in significantly increased SDN-POA volumes [35,36]. However, intact females injected with genistein showed a non-significant decrease in SDN-POA volumes [35], suggesting that phytoestrogens have differential effects on SDN-POA volumes that are dependent on the hormonal status of the model tested (i.e., intact vs. ovariectomized).

In our study, we tested the ability of phytoestrogens to influence SDN-POA volumes in mature rats using a change in diet during adulthood. Adult Long–Evans rats were time-mated within their respective diet treatments so that the offspring of these pairings would be exposed solely to the Phyto-rich diet. At 75 days of age, approximately one-half the total number of male or female (random cycling) rats was kept on the original Phyto-rich diet (long term) and the other half was assigned to the Phyto-free diet (short term). The animals remained on the diet treatments until 120 days of age when brain tissue was collected and SDN-POA volumes were determined (see Fig. 2). Since almost all commercially available rodent diets contain substantial levels of phytoestrogens [18,114], and because phytoestrogen-free diets are rarely used to rear animals, no animals were exposed to the Phyto-free diet during development in this experimental design. The SDN-POA volumes of male and female rats, as influenced by the diet treatments, are

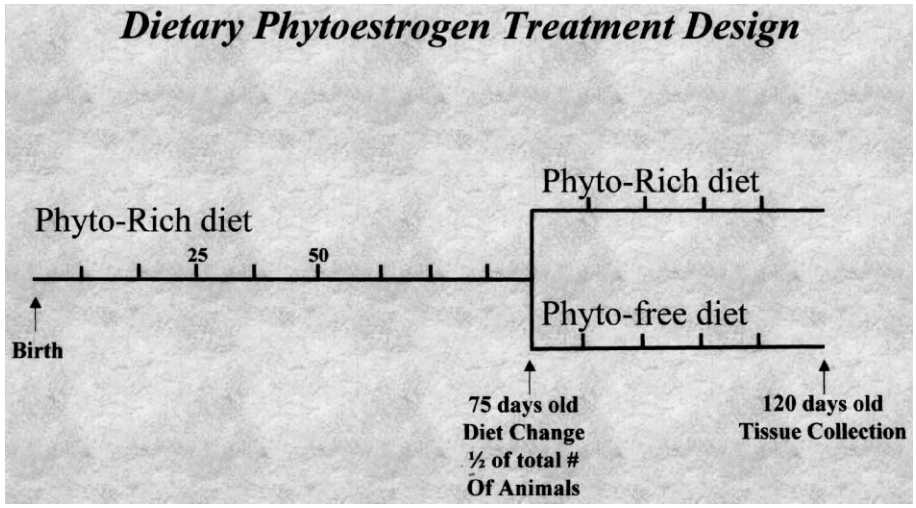

Fig. 2. Dietary soy phytoestrogen treatment design. Animals were obtained at 50 days of age and placed on the Phyto-rich diet (containing 600 μg/g diet) [71,78,122]. At 80–85 days of age, female rats were mated with breeder males on the Phyto-rich diet so that the offspring of these pairings would be exposed solely to this diet. At 75 days of age, approximately one-half the total number of male or female (random cycling) rats was kept on the original Phyto-rich diet (long-term) and the other half was assigned to the Phyto-free diet (short term). The animals remained on the diet treatments until 120 days of age.

displayed in Fig. 3. The methods for the quantification of SDN-POA volumes are outlined, as follows. At 120 days of age, all female and male animals were weighed, anesthesized with ketamine and sacrificed. All animals were then perfused transcardially with isotonic saline, then with 10% formaldehyde. Brains were removed and stored in formaldehyde for 10 days to allow the tissue to become fixed. Brains were sectioned at 50 μm using a series 1000 cryostat (Zeiss, Thornwood, NY, USA), following which the sections were stained with Thionin. With the aid of a microprojector (McBain Instruments, Los Angeles, CA USA) at $\times 10$ magnification, the SDN-POA sections were traced by two investigators without knowledge of the diet treatment or sex of the brain samples. The areas of each tracing were then quantified with a BioQuant scanner (R&M Biometrics, Nashville, TN, USA) in conjunction with an Dell PC optiplex GX1 and the SDN-POA volumes were averaged and calculated using established methods [79].

In agreement with all previous findings, the SDN-POA volumes of males were significantly greater (approximately twofold) than females when both genders consumed a phytoestrogen-rich diet. However, unexpectedly, Phyto-rich male SDN-POA volumes were found to be significantly greater than Phyto-free fed males. The inverse of this relationship was observed in female animals, with the Phyto-rich fed females having smaller, although not statistically significant ($P < .07$), SDN-POA volumes compared to Phyto-free fed females. With a lack of estrogenic stimulus in the diet, there were no significant differences in SDN-POA volumes of Phyto-free fed males compared to Phyto-free fed female SDN-POA values. Furthermore, no significant gender differences were identified for total brain weight between the diets [78], suggesting that the neural structural changes are specific only to hormone-sensitive brain areas.

Consistent with the findings for SDN-POA volumes, similar differences were observed in another sexually dimorphic nucleus, the anteroventral periventricular nucleus (AVPV) [68,72]. Due to the nature of AVPV volume

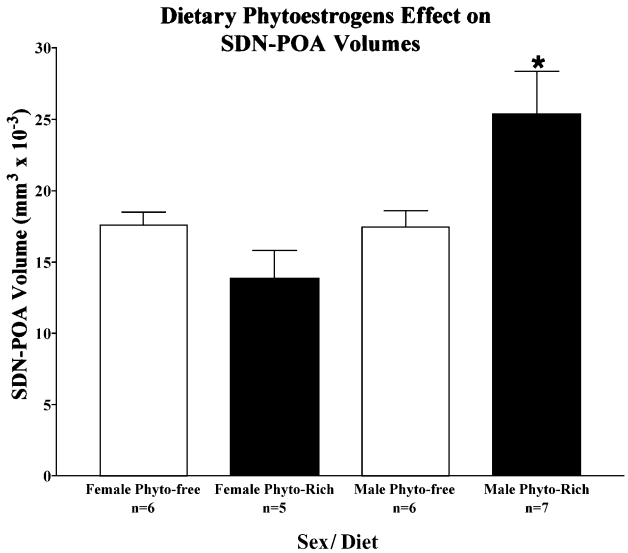

Fig. 3. Volumes of the SDN-POA (±S.E.M.) in male and female Long–Evans rats. The Phyto-rich refers to animals exposed to the Phyto-rich diet from conception until time of sacrifice at 120 days of age. Phyto-free refers to animals exposed to the Phyto-rich diet from conception until 75 days of age and then placed on the Phyto-free diet from 75 days of age until sacrifice at 120 days of age. * The SDN-POA volumes of males fed the Phyto-rich diet (Male Phyto-Rich) were significantly larger than females fed the Phyto-rich diet (Female Phyto-Rich) or males switched to the Phyto-free diet (Male Phyto-Free). The larger SDN-POA volumes of females switched to the Phyto-free (Female Phyto-Free) diet approached significance ($P < .07$) when compared to the volumes of females fed the Phyto-rich diet (Female Phyto-Rich).

characteristics, where females display approximately two-fold larger AVPV volumes compared to males, the phytoestrogen diet treatments resulted in opposite, but significant, effects in both males and females. In brief, Phyto-free females AVPV volumes were smaller and male Phyto-free values were significantly larger compared to Phyto-rich female and male values, respectively [72].

It is intriguing to consider how phytoestrogens may exert their effects especially since the measured parameters are hormonally sensitive. (I) Phytoestrogens may disrupt the in situ conversion of testosterone to estradiol via aromatase in brain. This possibility is unlikely since no significant differences were observed in brain aromatase enzymatic activity of either Sprague–Dawley or Long–Evans males or Sprague–Dawley females fed a Phyto-rich diet during perinatal development or in adults [71,72,120,121] (see Section 2.3). (II) Phytoestrogens may alter aromatase activity in the periphery (gonads) and thus alter circulating steroid hormone levels. In males, this possibility has been addressed but is not supported from our studies since circulating serum testosterone or estradiol levels were not altered by the diet treatments in Long–Evans rats [78]. (III) Phytoestrogens may decrease vs. enhance prolonged cell death mechanisms, via its estrogenic action, by blocking or promoting Bcl-2 and Bax apoptotic pathways [32,41]. Additionally, the hormonal action of estrogens may be mediated by the differential expression of estrogen receptors β vs. α in brain structures that activate or inhibit cell death mechanisms [32,41]. In support of this, we have shown that phytoestrogens significantly decrease brain calcium-binding protein in the hypothalamic area in perinatal or adult Sprague–Dawely rats [71,113], suggesting that alterations may exist in neuroprotective mechanisms with dietary phytoestrogen consumption [48,71]. (IV) The action of phytoestrogens may be tissue site-specific. Phytoestrogens may act both as agonists and/or antagonists in a site-specific manner, similar to the hormonal action of SERMs [19,45,47]—a hypothesis that is not incompatible with the previous possibilities (I–III). For example, women with BCa taking tamoxifen appear to develop depression more often compared to women with BCa not taking tamoxifen [21] and women who responded well to antidepressants before tamoxifen treatment became nonresponders or needed higher doses of antidepressants during tamoxifen treatment [45]. This suggests an antiestrogen effect or antagonistic effect on estrogen hormone action on the brain. Conversely, raloxifene in the brain appears to act as an agonist where it stimulates neurite growth in NFG-treated PC 12 cells; however, no cognitive alterations have been reported with this drug [45,87,128]. Our results support the hypothesis that phytoestrogens act as agonists in intact male animals and, in general, act as antagonists in intact females for several reproductive endocrine parameters. We postulate that phytoestrogens probably are agonists in ovariectomized rats. For example, phytoestrogens mimic estrogen hormone action by decreasing prostate and body weight in males and females while delaying the onset of puberty presumably by blocking estrogen hormone action without impacting cyclicity [72,78]. Our findings are similar to that of a recent study (using a similar concentration of total isoflavones in the diet) that demonstrated high estrogenic activity from dietary phytoestrogens that interfered with a normal uterine response to exogenously administered estradiol [16].

Phytoestrogens have similar effects to estrogens in increasing the volume of the SDN-POA of males while having the opposite effect in decreasing SDN-POA volumes of intact females. The opposite effect was observed in the AVPV brain region [71,77,79]. Coverage of this mechanism has been recently reviewed by Woodson and Gorski [127] where the estrogenic hormone actions have opposite effects in the SDN-POA and AVPV brain regions by sex. These results indicate that phytoestrogens have considerable effects on hormonally sensitive somatic, reproductive organ and neuroendocrine parameters. More importantly, the dimorphic expression of the sexually dimorphic nuclear structures (such as the SDN-POA and AVPV) in rats published thus far may be due, in part, to the presence of dietary soy-derived phytoestrogens in commercially available animal diets.

4. Phytoestrogens and anxiety

Experimental evidence has shown that the ovarian hormones, estradiol and progesterone, alter behavioral indices of anxiety in male and female rats [31,38,50,53,75,130]. High levels of these hormones, present during proestrus or following exogenous hormone injections in ovariectomized females, exert an antianxiety effect in behavioral paradigms such as the elevated plus maze [31,50,91,130].

In addition to endogenous hormones, a number of plant extracts have been shown to have anxiolytic properties [7,52,84,92,115]. Several reports suggest that natural compounds extracted from plants such as *Cassia siamea* [115], the *Eurycoma longifolia* Jack root [7], the *Azadirachta indica* leaf [52] and the *Ziziphus jujuba* seed [92] have anxiolytic effects.

Since estrogens are associated with anxiety and phytoestrogens act as estrogen mimics, we designed a study to determine the effects of dietary soy-derived phytoestrogens (i.e., lifelong exposure to either the Phyto-600 or the Phyto-free diet) on anxiety in adult male and random cycling female Long–Evans rats in a well- validated behavioral test utilizing the elevated plus maze that measures anxiety-related behavior [91]. This behavioral test relies on the inherent conflict between exploration of a novel environment and avoidance of its aversive features [50,53,91]. Normally, animals spend little time and make few entries into the open arms of the maze compared to the closed arms of the maze [50,53,84,92]. However, when animals are treated with clinically effective anxiolytics, the amount of time spent in the open arms and the number of entries into

open arms increase [50,53,84,92]. In addition to synthetic anxiolytic drugs, anxiety, as it is expressed in the plus maze, has also been shown to be affected by steroid hormones [23,31,50,53,75,115] and plant derivatives [7,52,75,114].

Fig. 4 represents dietary soy phytoestrogen's influence on anxiety behavior, as expressed in the elevated plus maze. Typically, rats avoid spending time on or entering into open arms. However, males and females fed the Phyto-rich diet spent significantly more time on and made significantly more entries into the open arms than did males or females fed the Phyto-free diet. The behavioral effects of phytoestrogens seen in this experiment may be mediated by changes in $GABA_A$ receptors and/or the effects of these chemical messengers on influencing GABA action [13,14,39,64,69,75,130]. Known anxiolytics, such as the benzodiazepines, bind the $GABA_A$ receptor and produce antianxiety behavioral effects as seen in plus- maze behavior [39,64,130]. Estrogens, progesterone and androgens (especially progesterone and androgen metabolites) have also been shown to interact with $GABA_A$ by increasing benzodiazepine binding sites on the GABA receptor, increasing the number of $GABA_A$ receptors and enhancing GABA-activated chloride ion flux [39,64,130]. Perhaps phytoestrogens act directly or indirectly through this same system, or phytoestrogens may alter the metabolism or production of anxiolytic androgens or progestins. Notably, the activation of $GABA_A$ receptors or the enhanced action of GABA via sex steroids is usually associated with decreased anxiety, sedation and a decline in locomotor behavior [64,68]. However, the actions of sex steroid hormones and especially estrogen-like molecules such as phytoestrogens represent complex effects on non-reproductive behaviors such as anxiety and locomotion [31,50,53,84]. Thus, phytoestrogens may produce anxiolytic effects that decrease anxiety without causing sedative effects that in turn increase locomotor and exploratory behavior that traditionally are associated with the hormonal action of sex steroids.

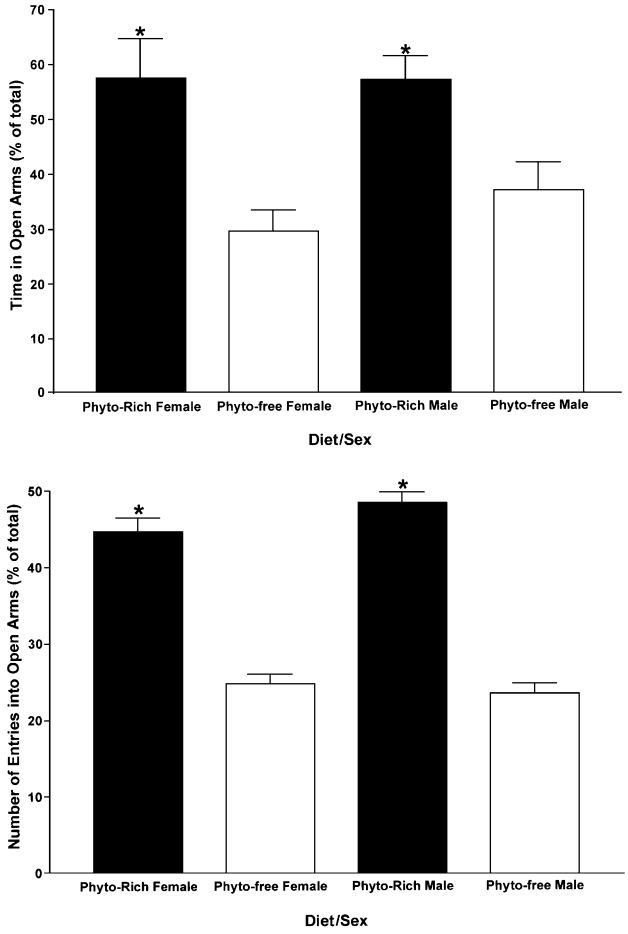

Fig. 4. Dietary soy phytoestrogens (lifelong) influence on the percentage of time spent in the open arms (left panel) (total time in maze = 5 min) and the percentage of entries into the open arms (right panel) of an elevated plus maze. Male and female Long–Evans rats were exposed lifelong to either a diet rich in phytoestrogens (Phyto-rich) or a diet low in phytoestrogens (Phyto-free) (Phyto-Rich Female, $n = 12$; Phyto-Free Female, $n = 14$; Phyto-Rich Male, $n = 12$; Phyto-Free Male, $n = 10$; data represent mean ± S.E.M.). * Phyto-rich fed males and females spent significantly more time on and made significantly more entries into open arms than did Phyto-free fed males or Phyto-free females.

5. Phytoestrogens and visual–spatial memory (VSM)

A type of cognition that is dependent on gonadal hormones is VSM, which refers to the ability to represent, transform, generate and recall symbolic, nonlinguistic information. Generally, VSM refers to the ability to imagine what irregular figures would look like if they were rotated in space, the ability to discern the relationship between shapes and objects or the ability to recall the location of objects or places [74]. In learning and memory tasks that require the use of visual spatial cues, males typically acquire and exhibit performance superior to females [28,30,76,98,99]. This sex difference in rats has been attributed to the hormonal influence of estrogens. For example, exogenous estradiol administered to intact male rats inhibited memory, whereas estrogen replacement to ovariectomized rats enhanced memory [27,28,30,76,125]. Since estrogen hormone action appears to influence VSM, we studied the influence of phytoestrogens on VSM utilizing radial arm maze methods to examine varying aspects of memory. In one preliminary study, where animals were fed the Phyto-rich or Phyto-free diets lifelong (from conception to time of testing), female rats fed the Phyto-rich diet acquired the maze significantly faster than females fed the Phyto-free diet. However, in males, the opposite effect occurred with Phyto-free fed males outperforming Phyto-rich fed males [80]. There were no significant diet effects observed in working memory.

In another preliminary study, male and female rats were fed the Phyto-rich diet lifelong and tested for acquisition and working memory, then at 75 days of age, one- half of the total number of rats was either kept on their original diet

Table 1
VSM (reference) performance after the diet change in Long–Evans male and female rats

Diet	Trials 13–15, mean (±S.E.M.)
Females	
Lifelong Phyto-Rich ($n=5$)	2.40 (0.30)*
Phyto-Rich to Phyto-Free ($n=6$)	1.88 (0.16)
Males	
Lifelong Phyto-Rich ($n=7$)	1.66 (0.14)
Phyto-Rich to Phyto-Free ($n=6$)	2.56 (0.11)**

The diet treatment design is the same as that employed earlier as seen in Fig. 3 (i.e., the Phyto-Rich refers to animals exposed to the Phyto-rich diet from conception until time of sacrifice at 120 days of age; Phyto-Free refers to animals exposed to the Phyto-rich diet from conception until 75 days of age and then placed on the Phyto-free diet from 75 days of age until sacrifice at 120 days of age). The number of correct choices in the four-arm task (averaging the last three trials) is expressed as the mean ± S.E.M.

* Lifelong Phyto-Rich fed females made significantly more correct choices than Phyto-rich fed females changed to the Phyto-free diet or males fed the Lifelong Phyto-Rich diet ($P<.05$).

** Phyto-Rich males changed to the Phyto-free diet made significantly more correct choices than Lifelong Phyto-Rich fed males or females fed the Phyto-rich diet that were changed to the Phyto-free diet ($P<.05$).

(Phyto-rich) or changed to the Phyto-free diet. After the diet switch, the animals were tested on a task testing reference memory (associated with the frontal cortex). Phyto-rich fed males acquired the maze significantly faster and outperformed females (on the working memory task) fed the Phyto-rich diet, before the diet switch. However, after the diet switch, in the reference memory task, Phyto-rich females performed significantly better than females switched to the Phyto-free diet and surprisingly, males switched to the Phyto-free diet significantly outperformed males fed the Phyto-rich diet lifelong [77,80] (see Table 1). These results suggest that dietary phytoestrogens significantly sex-reversed the normal sexually dimorphic expression of VSM. Specifically, in tasks requiring the use of reference, but not working, memory, VSM was enhanced in females fed the Phyto-rich diet, whereas in males, VSM was inhibited by the same diet. Males fed the Phyto-rich diet showed a decline in VSM in our study. While it is important to establish the effects of dietary phytoestrogens on memory, the true significance of these findings needs to be investigated further in order to determine the effects of phytoestrogens and the time of exposure on cognition. However, the present findings correspond with a recent study where tofu consumption, in aged men, resulted in loss of brain weight and increased dementia [123]. Finally, because reference memory is thought to be mediated by the frontal cortex, we have examined in preliminary studies the expression of calbindin (a calcium-binding protein thought to play a neuroprotective role against programmed cell death) [48,66,111] in this brain region along with cyclooxygenase-2 (COX-2; an inflammatory factor prevalent in Alzheimer's disease). In males fed the Phyto-rich diet, frontal cortical levels of calbindin were significantly decreased, whereas COX-2 levels were significantly increased compared to Phyto-free male values. These recent data suggest that dietary soy phytoestrogens may alter VSM in Phyto-rich fed males via increased expression of COX-2 and decreased calbindin levels in the frontal cortex [71].

6. Summary

Phytoestrogens have commanded a great deal of scientific investigative attention in recent years due to their potential for protecting against many hormone-dependent diseases. While some studies have examined the influence of phytoestrogens on brain, the effects of these natural estrogen mimics on brain and behavior remain largely unknown. Most of the research has been performed in rats, where the effects of soy isoflavones on certain aspects of brain structure, learning, memory, anxiety and the brain androgen-metabolizing enzyme, aromatase, have been documented. Whether these observations hold true for the human brain remains to be established and will only become apparent from long-term clinical studies. One point that needs clarification relates to the timing of brain development in the rat, which differs from that in the human.

These findings from these animal studies indicate that dietary soy-derived phytoestrogens can significantly influence brain and behavior parameters (see summary in Table 2). Increased awareness and understanding of brain plasticity (and behavior) is evolving with the advancements in technology and exchange of scientific information [2,15,18,27,33,40,43,45,47,51,62,71,78,81,96,97,104,112,114,116,123,127]. Since all commercially available rodent diets use soy as the main source of protein in their formulations, it is intriguing to consider the possible impact dietary phytoes-

Table 2
Summary of dietary soy phytoestrogen effects on neurobehavioral parameters

	Phyto-rich		Phyto-free	
	Male	Female	Male	Female
Body weight	⇓	⇓	⇑	⇑
Brain aromatase	NSC	NSC	NSC	NSC
SDN-POA	⇑	⇓	⇓	⇑
AVPV	⇓	⇑	⇑	⇓
Anxiety* (lifelong)	⇓	⇓	–	–
VSM				
Acquisition* (lifelong)	⇓	⇑	⇑	⇓
Reference (after diet switch in the four-arm task)	⇓	⇑	⇑	⇓

SDN-POA = sexually dimorphic nucleus of the preoptic area volumes; AVPV = anteroventral periventricular nucleus volumes; NSC = no significant change; (–) = compared to Phyto-rich animals.

* In the experiments testing anxiety and acquisition, the animals were exposed to the diets lifelong while all other experiments utilized the dietary exposure (of diet change) outlined in Fig. 2.

trogens may have on brain development, neural function and the expression of behavior. This is significant in light of the influence estrogens have not only on reproductive function, but cognition and memory. This is especially true in postmenopausal women where a lack of estrogen increases the incidence of Alzheimer's disease [2,42,45,46,56,85,104, 107,109,117].

Acknowledgments

This work was supported, in part, by grants from the National Science Foundation, IBN-9507972 (to E.D.L.), BYU Research Office 19-223566 (to E.D.L.), BYU Research Office (ORCA to K.S.W.), BYU Dean's Neuroscience Graduate Fellowship (to T.D.L. and K.S.W.) and The Commission of the European Communities, contract QLK1-CT-2000-00266 (to H.A.).

References

[1] H. Adlercreutz, Evolution, nutrition, intestinal microflora, and prevention of cancer: A hypothesis, Proc. Soc. Exp. Biol. Med. 217 (1997) 241–246.
[2] H. Adlercreutz, Epidemiology of phytoestrogens, Baillieres Clin. Endocrinol. Metab. 12 (1998) 605–623.
[3] H. Adlercreutz, K. Bannwart, T. Wahala, G. Mäkelä, T. Brunow, P.J. Hase, J.R. Arosemena, J.T. Kellis Jr., L.E. Vickery, Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens, J. Steroid Biochem. Mol. Biol. 44 (1994) 147–153.
[4] H. Adlercreutz, W. Mazur, Phyto-oestrogens and Western diseases, Ann. Med. 29 (1997) 95–120.
[5] H. Adlercreutz, W. Mazur, P. Bartels, V. Elomaa, S. Watanabe, K. Wahala, M. Landstrom, E. Lundin, A. Bergh, J.E. Damber, P. Aman, A. Widmark, A. Johansson, J.X. Zhang, G. Hallmans, Phytoestrogens and prostate disease, J. Nutr. 130 (2000) 658S–659S.
[6] P. Alexandersen, A. Toussaint, C. Christiansen, J.P. Devogelaer, C. Roux, J. Fechtenbaum, C. Gennari, J.Y. Reginster, Ipriflavone in the treatment of postmenopausal osteoporosis: A randomized controlled trial, JAMA, J. Am. Med. Assoc. 285 (11) (2001) 1482–1488.
[7] H.H. Ang, H.S. Cheang, Studies on the anxiolytic activity of *Eurycoma longifolia* Jack roots in mice, Jpn. J. Pharmacol. 79 (1999) 497–500.
[8] B.H. Arjmandi, R.S. Birnbum, S. Juma, E. Barengolts, S.C. Kukreja, The synthetic phytoestrogen, Ipriflavone, and estrogen prevent bone loss by different mechanisms, Calcif. Tissue Int. 66 (2000) 61–65.
[9] A.P. Arnold, R.A. Gorski, Gonadal steroid induction of structural sex differences in the central nervous system, Annu. Rev. Neurosci. 7 (1984) 413–422.
[10] J. Ashby, H. Tinwell, J. Odum, I. Kimber, A.N. Brooks, I. Pate, C.C. Boyle, Diet and the aetiology of temporal advances in human and rodent sexual development, J. Appl. Toxicol. 20 (5) (2000) 343–347.
[11] S. Barnes, Evolution of the health benefits of soy isoflavones, Proc. Soc. Exp. Biol. Med. 217 (1998) 386–392.
[12] S.M. Belcher, Regulated expression of estrogen receptor alpha and beta mRNA in granule cell development of the rat cerebellum, Dev. Brain Res. 115 (1999) 57–69.
[13] D. Bitran, R.J. Hilvers, C.A. Frye, M.S. Erskine, Chronic anabolic–androgenic steroid treatment affects brain $GABA_A$ receptor-gated chloride ion transport, Life Sci. 58 (1996) 573–583.
[14] D. Bitran, C.K. Kellogg, R.J. Hilvers, Treatment with an anabolic–androgenic steroid affects anxiety-related behavior and alters the sensitivity of cortical $GABA_A$ receptors in the rat, Horm. Behav. 27 (1993) 568–583.
[15] G.J. Bloch, R.A. Gorski, Estrogen/progesterone treatment in adulthood affects the size of several components of the medial preoptic area in the male rat, J. Comp. Neurol. 275 (1988) 613–622.
[16] H. Boettger-Tong, L. Murthy, C. Chiappetta, J.L. Kirkland, B. Adlercreutz, H. Adlercreutz, G.M. Stancel, S. Mäkelä, A case of a laboratory animal feed with high estrogenic activity and its impact on in vivo responses to exogenously administered estrogens, Environ. Health Perspect. 106 (1998) 369–372.
[17] R.B. Bradbury, D.E. White, Estrogens and related substances in plants, Vitam. Horm. 12 (1954) 207–233.
[18] N.M. Brown, K.D. Setchell, Animal models impacted by phytoestrogens in commercial chow: Implications for pathways influenced by hormones, Lab. Invest. 81 (2001) 735–747.
[19] A. Brzezinski, A. Debi, Phytoestrogens: The "natural" selective estrogen receptor modulators? Eur. J. Obstet. Gynecol. Reprod. Biol. 85 (1999) 47–51.
[20] A. Bylund, J.X. Zhang, A. Bergh, J.E. Damber, A. Widmark, A. Johansson, H. Adlercreutz, P. Aman, M.J. Shepherd, G. Hallmans, Rye bran and soy protein delay growth and increase apoptosis of human LNCap prostate adenocarcinoma in nude mice, Prostate 42 (2000) 304–314.
[21] C.K. Cathcart, S.E.P. Jones, C.S. Pumproy, Clinical recognition and management of depression in node negative breast cancer patients with tamoxifen, Breast Cancer Res. Treat. 27 (1993) 277–281.
[22] Y.H. Choi, W.H. Lee, K.Y. Park, I. Zhang, p53-independent induction of p21 (WAF1/CIPI), reduction of cyclin B1 and G2/M arrest by the isoflavone genistein in human prostate carcinoma cells, Jpn. J. Cancer Res. 91 (2000) 164–173.
[23] P. Chopin, M. Briley, The benzodiazepine antagonist flumazenil blocks the effects of CCK receptor agonist and antagonists in the elevated plus-maze, Psychopharmacology 110 (1993) 409–414.
[24] L. Coward, N.C. Barnes, K.D.R. Setchell, S. Barnes, Genistein, daidzein, and their β-glycoside conjugates: Antitumor isoflavones in soybean foods from American and Asian diets, J. Agric. Food Chem. 41 (1993) 1961–1967.
[25] J.R. Crouse III, T. Morgan, J.G. Terry, J. Ellis, M. Vitolins, G.L. Burke, A randomized trial comparing the effect of casein with that of soy protein content varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins, Arch. Intern. Med. 159 (17) (1999) 2070–2076.
[26] A. Dalu, J.F. Haskel, L. Coward, C.A. Lamartiniere, Genistein, a component of soy, inhibits the expression of the EGF and ErbB2/Neu receptors in the rat dorsolateral prostate, Prostate 37 (1998) 36–43.
[27] J.M. Daniel, A.J. Fader, A.L. Spencer, G.P. Dohanich, Estrogen enhances performance of female rats during acquisition of a radial arm maze, Horm. Behav. 32 (1997) 217–225.
[28] E.C. Davis, J.E. Shryne, R.A. Gorski, A revised critical period for the sexual differentiation of the sexually dimorphic nucleus of the preoptic area in the rat, Neuroendocrinology 62 (1995) 579–585.
[29] S.R. Davis, F.S. Dalais, E.R. Simpson, A.L. Murkies, Phytoestrogens in health and disease, Recent Prog. Horm. Res. 54 (1999) 185–210.
[30] J.L.M. Dawson, Y.M. Cheung, R.T.S. Lau, Developmental effects of neonatal sex hormones on spatial and activity skills in the white rat, Biol. Psychol. 3 (1975) 213–229.
[31] G. Diaz-Veliz, T. Alarcon, C. Espinoza, N. Dussaubat, S. Mora, Ketanserin and anxiety levels: Influence of gender, estrous cycle, ovariectomy and ovarian hormones in female rats, Pharmacol., Biochem. Behav. 58 (1997) 637–642.
[32] D.B. Dubal, P.J. Shughrue, M.E. Wilson, I. Merchenthaler, P.M. Wise, Estradiol modulates bcl-2 in cerebral ischemia: A potential role for estrogen receptors, J. Neurosci. 19 (1999) 6385–6393.
[33] P.S. Eriksson, E. Perfilieva, T. Bjork-Eriksson, A.M. Alborn,

C. Nordborg, D.A. Peterson, F.H. Gage, Neurogenesis in the adult human hippocampus, Nat. Med. 4 (1998) 1313–1317.
[34] B.A. Evans, K. Griffiths, M.S. Morton, Inhibition of 5α-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids, J. Endocrinol. 147 (1995) 295–302.
[35] K.A. Faber, C.L. Hughes Jr., The effect of neonatal exposure to diethylstilbestrol, genistein, and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat, Biol. Reprod. 45 (1991) 649–653.
[36] K.A. Faber, C.L. Hughes Jr., Dose–response characteristics of neonatal exposure to genistein on pituitary responsiveness to gonadotropin releasing hormone and volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in postpubertal castrated female rats, Reprod. Toxicol. 7 (1993) 35–39.
[37] L. Fioravanti, V. Cappelletti, P. Miodini, E. Ronchi, M. Brivio, G. Di Fronzo, Genistein in the control of breast cancer cell growth: Insights into the mechanism of action in vitro, Cancer Lett. 130 (1998) 143–152.
[38] L.J. Forman, V. Tingle, S. Estilow, J. Cater, The response to analgesia testing is affected by gonadal steroids in the rat, Life Sci. 45 (1989) 447–454.
[39] C.A. Frye, S.M. Petralia, M.E. Rhodes, Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3α, 5α-THP, Pharmacol., Biochem. Behav. 67 (2000) 1–10.
[40] P.H. Gamache, T.J. Maher, K.D.R. Setchell, T.-H. Wu, I.N. Acworth, The transfer of non-steroidal dietary estrogens into brain, Soc. Neurosci. Abstr. 22 (1996) 1967.
[41] L.M. Garcia-Segura, P. Cardona-Gomez, F. Naftolin, J.A. Chowen, Estradiol upregulates Bcl-2 expression in adult brain neurons, NeuroReport 9 (1998) 593–597.
[42] M.G. Glazier, M.A. Bowman, A review of the evidence for the use of phytoestrogens as a replacement for traditional estrogen replacement therapy, Arch. Intern. Med. 161 (2001) 1161–1172.
[43] E. Gould, P. Tanapat, T. Rydel, N. Hastings, Regulation of hippocampal neurogenesis in adulthood, Biol. Psychiatry 48 (2000) 715–720.
[44] K. Griffiths, L. Denis, A. Turkes, P.S. Morton, Phytoestrogens and diseases of the prostate gland, Baillieres Clin. Endocrinol. Metab. 12 (1998) 625–647.
[45] U. Halbreich, L.S. Kahn, Selective oestrogen receptor modulators—current and future brain and behaviour applications, Expert Opin. Pharmacother. 1 (2000) 1385–1398.
[46] V.W. Henderson, Estrogen, cognition, and a woman's risk of Alzheimer's disease, Am. J. Med. 103 (1997) 11S–18S.
[47] T. Hol, M.B. Cox, H.U. Bryant, M.W. Draper, Selective estrogen receptor modulators and postmenopausal women's health, J. Women's Health 6 (1997) 523–531.
[48] A.M. Iacopino, E.M. Quintero, E.K. Miller, Calbindin-D_{28K} a potential neuroprotective protein, Neurodegeneration 3 (1994) 1–20.
[49] A.R. Ibrahim, Y.J. Abul-Hajj, Aromatase inhibition by flavonoids, J. Steroid Biochem. Mol. Biol. 37 (1990) 257–260.
[50] J.T. Imhof, Z.M.I. Coelho, M.L. Schmitt, G.S. Morato, A.P. Carobrez, Influence of gender and age on performance of rats in the elevated plus maze apparatus, Behav. Brain Res. 56 (1993) 177–180.
[51] D.A. Jacob, J.L. Temple, H.B. Patisaul, L.J. Young, E.F. Rissman, Coumestrol antagonizes neuroendocrine actions of estrogen via the estrogen receptor alpha, Exp. Biol. Med. 226 (2001) 301–306.
[52] A.K. Jaiswal, S.K. Bhattacharya, S.B. Acharya, Anxiolytic activity of *Azadirachta indica* leaf extract in rats, Indian J. Exp. Biol. 32 (1994) 489–491.
[53] A.L. Johnston, S.E. File, Sex differences in animal tests of anxiety, Physiol. Behav. 49 (1991) 250–254.
[54] J.T. Kellis Jr., L.E. Vickery, Inhibition of human estrogen synthetase (aromatase) by flavones, Science 225 (1984) 1032–1034.
[55] H. Kim, H. Xia, L. Li, J. Gewin, Actions of dietary soy vs. Premarin in mammalian brain, Annu. Meet. Am. Chem. Soc. 221, AGFD-79, 2001, p. 79.
[56] D.C. Knight, J.A. Eden, A review of the clinical effects of phytoestrogens, Obstet. Gynecol. 87 (1996) 897–904.
[57] G.G. Kuiper, B. Carlsson, K. Grandien, E. Enmark, J. Haggblad, S. Nilsson, J.A. Gustafsson, Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta, Endocrinology 138 (1997) 863–870.
[58] G.G. Kuiper, J.G. Lemmen, B. Carlsson, J.C. Corton, S.H. Safe, P.T. van der Saag, B. van der Burg, J.A. Gustafsson, Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta, Endocrinology 139 (1998) 4252–4263.
[59] G.G. Kuiper, P.J. Shughrue, I. Merchenthaler, J.A. Gustafsson, The estrogen receptor beta subtype: A novel mediator of estrogen action in neuroendocrine systems, Front. Neuroendocrinol. 19 (1998) 253–286.
[60] N.B. Kumar, K. Besterman-Dahan, Nutrients in the chemoprevention of prostate cancer: Current and future prospects, Cancer Control 6 (1999) 580–586.
[61] M.S. Kurzer, X. Xu, Dietary phytoestrogens, Annu. Rev. Nutr. 17 (1997) 353–381.
[62] J. Lampe, M. Messina, Are phytoestrogens nature's cure for what ails us? A look at the research, J. Am. Diet. Assoc. 98 (1998) 974–976.
[63] M. Landstrom, J.X. Zhang, G. Hallmans, P. Aman, A. Bergh, J.E. Damber, W. Mazur, K. Wahala, H. Adlercreutz, Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats, Prostate 36 (1998) 151–161.
[64] E.D. Lephart, A review of brain 5α-reductase: Cellular, enzymatic, and molecular perspectives and implications for biological function, Mol. Cell. Neurosci. 4 (1993) 473–484.
[65] E.D. Lephart, A review of brain aromatase cytochrome *P*450, Brain Res. Rev. 22 (1996) 1–26.
[66] E.D. Lephart, Dimorphic expression of calbindin-D28K in the medial basal hypothalamus from perinatal male and female rats, Dev. Brain Res. 96 (1996) 281–284.
[67] E.D. Lephart, Molecular aspects of brain aromatase cytochrome *P*450, J. Steroid Biochem. Mol. Biol. 61 (1997) 375–380.
[68] E.D. Lephart, H. Adlercreutz, T.D. Lund, Dietary soy phytoestrogen effects on brain structure and aromatase in Long–Evans rats, NeuroReport 12 (2001) 3451–3455.
[69] E.D. Lephart, D.R. Ladle, N.A. Jacobson, R.W. Rhees, Inhibition of brain 5α- reductase in pregnant rats. Effects on enzymatic and behavioral activity, Brain Res. 739 (1996) 356–360.
[70] E.D. Lephart, E.R. Simpson, M.J. McPhaul, M.W. Kilgore, J.D. Wilson, S.R. Ojeda, Brain aromatase cytochrome *P*-450 messenger RNA levels and enzyme activity during prenatal and perinatal development in the rat, Mol. Brain Res. 16 (1992) 187–192.
[71] E.D. Lephart, J.M. Thompson, K.D.R. Setchell, H. Adlercreutz, K.S. Weber, Phytoestrogens decrease brain calcium-binding proteins but do not alter hypothalamic androgen metabolizing enzymes in adult male rats, Brain Res. 859 (2000) 123–131.
[72] E.D. Lephart, T.W. West, L.Y. Tian, L.H. Bu, D.L. Simmons, H. Adlercreutz, T.D. Lund, Neurobehavorial effects of dietary soy phytoestrogens I, Soc. Neurosci. Abstr. 27 (2001).
[73] J.R. Levy, K.A. Faber, L. Ayyash, C.L. Hughes, The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats, Proc. Soc. Exp. Biol. Med. 208 (1995) 60–66.
[74] M.C. Linn, A.C. Petersen, Emergence and characterization of sex differences in spatial ability: A meta-analysis, Child Dev. 56 (1985) 1479–1498.
[75] A.B. Lucion, H. Charchat, G.A.M. Pereira, A.A. Rasia-Filho, Influence of early postnatal gonadal hormones on anxiety in adult male rats, Physiol. Behav. 60 (1996) 1419–1423.
[76] V.N. Luine, M. Rodriguez, Effects of estradiol on radial arm maze performance of young and aged rats, Behav. Neural Biol. 62 (1994) 230–236.
[77] T.D. Lund, E.D. Lephart, Dietary soy phytoestrogens produce anxiolytic effects in the elevated plus-maze, Brain Res. 913 (2001) 180–184.

[78] T.D. Lund, R.W. Rhees, K.D.R. Setchell, E.D. Lephart, Altered sexually dimorphic nucleus of the preoptic area (SDN-POA) volume in adult Long–Evans rats by dietary soy phytoestrogens, Brain Res. 914 (2001) 92–99.
[79] T.D. Lund, D.L. Salyer, D.E. Fleming, E.D. Lephart, Pre- or postnatal testosterone and flutamide effects on sexually dimorphic nuclei of the rat hypothalamus, Dev. Brain Res. 120 (2000) 261–266.
[80] T.D. Lund, T.W. West, L.Y. Tian, L.H. Bu, D.L. Simmons, K.D.R. Setchell, H. Adlercreutz, E.D. Lephart, Visual spatial memory is enhanced in females (but not in males) by dietary soy phytoestrogens, BMC Neuroscience 2 (2001) 20.
[81] S.S. Magavi, B.R. Leavitt, J.D. Macklis, Induction of neurogenesis in the neocortex of adult mice, Nature 405 (2000) 951–955.
[82] S.I. Mäkelä, L.H. Pylkkänen, R.S. Santti, H. Adlercreutz, Dietary soybean may be antiestrogenic in male mice, J. Nutr. 125 (1995) 437–445.
[83] W. Mazur, H. Adlercreutz, Overview of naturally occurring endocrine-active substances in the human diet in relation to human health, Nutrition 16 (2000) 654–658.
[84] M.M. McCarthy, S. Schwartz-Giblin, S. Wang, Does estrogen facilitate social behavior by reducing anxiety? Ann. N.Y. Acad. Sci. 807 (1997) 541–542.
[85] B.S. McEwen, Clinical review 108: The molecular and neuroanatomical basis for estrogen effects in the central nervous system, J. Clin. Endocrinol. Metab. 84 (1999) 1790–1797.
[86] A.L. Murkies, G. Wilcox, S.R. Davis, Phytoestrogens — review, J. Clin. Endocrinol. Metab. 83 (1998) 297–303.
[87] T. Nickelsen, E.G. Lufkin, B.L. Riggs, D.A. Cox, T.H. Crook, Raloxifene hydrochloride, a selective estrogen receptor modulator: Safety assessment of effects on cognitive function and mood in postmenopausal women, Psychoneuroendocrinology 24 (1999).
[88] Y. Pan, M. Anthony, T.B. Clarkson, Effect of estradiol and soy phytoestrogens on choline acetyltransferase and nerve growth factor mRNAs in the frontal cortex and hippocampus of female rats, Proc. Soc. Exp. Biol. Med. 221 (1999) 118–125.
[89] Y. Pan, M. Anthony, T.B. Clarkson, Evidence for up-regulation of brain-derived neurotrophic factor mRNA by soy phytoestrogens in the frontal cortex of retired breeder female rats, Neurosci. Lett. 261 (1999) 17–20.
[90] H.B. Patisaul, P.L. Whitten, L.J. Young, Regulation of estrogen receptor beta mRNA in the brain: Opposite effects of 17beta-estradiol and the phytoestrogen, coumestrol, Mol. Brain Res. 67 (1999) 165–171.
[91] S. Pellow, P. Chopin, S.E. File, M. Briley, Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat, J. Neurosci. Methods 14 (1985) 149–167.
[92] W.H. Peng, M.T. Hsieh, Y.S. Lee, Y.C. Lin, J. Liao, Anxiolytic effect of seed of *Ziziphus jujuba* in mouse models of anxiety, J. Ethnopharmacol. 72 (2000) 435–441.
[93] S.M. Potter, J.A. Baum, H. Teng, R.J. Stillman, N.F. Shay, J.W. Erdman Jr., Soy protein and isoflavones: Their effects on blood lipids and bone density in postmenopausal women, Am. J. Clin. Nutr. 68 (1998) 1375S–1379S.
[94] K.R. Price, G.R. Fenwick, Naturally occurring oestrogens in foods — a review, Food Addit. Contam. 2 (1985) 73–106.
[95] R.H. Price Jr., R.J. Handa, Expression of estrogen receptor-beta protein and mRNA in the cerebellum of the rat, Neurosci. Lett. 288 (2000) 115–118.
[96] K.N. Prince, J.S. Prince, E.W. Kinghorn, D.E. Fleming, R.W. Rhees, Effects of sexual behavioral manipulation on brain plasticity in adult rats, Brain Res. Bull. 47 (1998) 349–355.
[97] R.W. Rhees, H.N. Al-Saleh, E.W. Kinghorn, D.E. Fleming, E.D. Lephart, Relationship between sexual behavior and sexually dimorphic structures in the anterior hypothalamus in control and prenatally stressed male rats, Brain Res. Bull. 50 (1999) 193–199.
[98] R.L. Roof, Neonatal exogenous testosterone modifies sex difference in radial arm and Morris water maze performance in prepubescent and adult rats, Behav. Brain Res. 53 (1993) 1–10.
[99] R.L. Roof, M.D. Havens, Testosterone improves maze performance and induces development of male hippocampus in females, Brain Res. 14 (1992) 310.
[100] M.D. Scheiber, J.H. Liu, M.T. Subbiah, R.W. Rebar, K.D. Setchell, NAMS fellowship findings*: Dietary inclusion of whole soy foods results in significant reductions in clinical risk factors for osteoporosis and cardiovascular disease in normal menopausal women, Menopause 8 (2001) 384–392.
[101] K.D.R. Setchell, Phytoestrogens: Biochemistry, physiology and implications for human health of soy isoflavones, Am. J. Clin. Nutr. 129 (1998) 1333S–1346S.
[102] K.D.R. Setchell, H. Adlercreutz, Mammalian lignans and phytoestrogens recent studies on their formation, metabolism and biological role in health and disease, in: I.R. Rowland (Ed.), Role of the Gut Flora in Toxicity and Cancer, Academic Press, New York, 1988, pp. 315–345.
[103] K.D.R. Setchell, S.P. Borriello, P. Hulme, D.N. Kirk, M. Axelson, Nonsteroidal estrogens of dietary origin: Possible roles in hormone-dependent disease, Am. J. Clin. Nutr. 40 (1984) 569–578.
[104] K.D.R. Setchell, A. Cassidy, Dietary isoflavones—biological effects and relevance to human health, J. Nutr. 129 (1999) 758S–767S.
[105] K.D.R. Setchell, M.B. Welsh, C.K. Lim, High-performance liquid chromatographic analysis of phytoestrogens in soy protein preparations with ultraviolet, electrochemical and thermospray mass spectrometric detection, J. Chromatogr. 386 (1987) 315–323.
[106] O.P. Sharma, H. Adlercreutz, J.D. Strandberg, B.R. Zirkin, D.S. Coffey, L.L. Ewing, Soy of dietary source plays a preventing role against the pathogenesis of prostatitis in rats, J. Steroid Biochem. Mol. Biol. 43 (1992) 4557–4564.
[107] B.B. Sherwin, Estrogen and cognitive function in women, Proc. Soc. Exp. Biol. Med. 217 (1998) 17–22.
[108] P.J. Shughrue, M.V. Lane, I. Merchenthaler, Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system, J. Comp. Neurol. 388 (1997) 507–525.
[109] J.W. Simpkins, P.S. Green, K.E. Gridley, M. Singh, N.C. de Fiebre, G. Rajakumar, Role of estrogen replacement therapy in memory enhancement and the prevention of neuronal loss associated with Alzheimer's disease, Am. J. Med. 103 (1997) 19S–25S.
[110] F.O. Stephens, The rising incidence of breast cancer in women and prostate cancer in men. Dietary influences: A possible preventative role for nature's sex hormone modifiers: The phytoestrogens, Oncol. Rep. 6 (1999) 865–870.
[111] E. Stuart, E.D. Lephart, Dimorphic expression of medial basal hypothalamic–preoptic area calbindin-D28K mRNA during perinatal development and adult brain tissue distribution of calbindin-D28K mRNA in Sprague–Dawley rats, Mol. Brain Res. 73 (1999) 60–67.
[112] P. Tanapat, N.B. Hastings, A.J. Reeves, E. Gould, Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat, J. Neurosci. 19 (1999) 5792–5801.
[113] H. Taylor, E.M. Quintero, A.M. Iacopino, E.D. Lephart, Phytoestrogens alter hypothalamic calbindin-D28k levels during prenatal development, Dev. Brain Res. 114 (1999) 277–281.
[114] J.E. Thigpen, K.D.R. Setchell, K.B. Ahlmark, J. Locklear, T. Spahr, G.F. Caviness, M.F. Goelz, J.K. Haseman, R.R. Newbold, D.B. Forsythe, Phytoestrogen content of purified, open- and closed-formula laboratory diets, Lab. Anim. Sci. 49 (1999) 530–536.
[115] W. Thongsaard, C. Deachapunya, S. Pongsakorn, E.A. Boyd, G.W. Bennett, C.A. Marsden, Barakol: A potential anxiolytic extracted from *Cassia siamea*, Pharmacol., Biochem. Behav. 53 (1996) 753–758.
[116] A.D. Tramontin, E.A. Brenowitz, Seasonal plasticity in the adult brain, Trends Neurosci. 23 (2000) 251–258.
[117] A. Vincent, L.A. Fitzpatrick, Soy Isoflavones: Are they useful in menopause? Mayo Clin. Proc. 75 (2000) 1174–1184.
[118] C. Wang, T. Mäkelä, T. Hase, H. Adlercreutz, M.S. Kurzer, Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes, J. Steroid Biochem. Mol. Biol. 50 (1994) 205–212.

[119] G.J. Wang, L. Oldrich, R. Hampl, U. Mariko, N. Al-Maharik, K. Stumpf, M. Heikki, K. Wahala, H. Adlercreutz, Time-resolved fluoroimmunoassay of plasma daidzein and genistein, Steroids 65 (2000) 339–348.
[120] K.S. Weber, N.A. Jacobson, K.D.R. Setchell, E.D. Lephart, Brain aromatase and 5α-reductase, regulatory behaviors and testosterone levels in adult rats on phytoestrogen diets, Proc. Soc. Exp. Biol. Med. 22 (1999) 131–135.
[121] K.S. Weber, K.D.R. Setchell, E.D. Lephart, Maternal and perinatal brain aromatase: Effects of dietary soy phytoestrogens, Dev. Brain Res. 126 (2001) 217–221.
[122] K.S. Weber, K.D.R. Setchell, D.M. Stocco, E.D. Lephart, Dietary soy-phytoestrogens decrease testosterone levels and prostate weight, without altering LH, prostate 5α-reductase or testicular StAR levels in adult male Sprague–Dawley rats, J. Endocrinol. 170 (2001) 591–599.
[123] L.R. White, H. Petrovitch, G.W. Ross, K. Masaki, J. Hardman, J. Nelson, D. Davis, W. Markesbery, Brain aging and midlife tofu consumption, J. Am. Coll. Nutr. (2000) 242–245.
[124] P.L. Whitten, F. Naftolin, Reproductive actions of phytoestrogens, Baillieres Clin. Endocrinol. Metab. 12 (1998) 667–690.
[125] C.L. Williams, A.M. Barnett, W.H. Meck, Organizational effects of early gonadal secretions on sexual differentiation in spatial memory, Behav. Neurosci. 104 (1990) 84–97.
[126] H. Wiseman, J. O'Reilly, Influence of the dietary flavonoid quercetin on the cardioprotective antioxidant action of oestrogen and phytoestrogens, Biochem. Soc. Trans. 25 (1997) 108S.
[127] J.C. Woodson, R.A. Gorski, Structural sex differences in the mammalian brain: Reconsidering the male/female dichotomy, in: A. Matsumoto (Ed.), Sexual Differentiation of the Brain, CRC Press, Boca Raton, FL, 2000, pp. 230–247.
[128] K. Yaffe, K. Krueger, S. Sarkar, D. Grady, E. Barrett-Connor, D.A. Cox, T. Nickelsen, Cognitive function in postmenopausal women treated with raloxifene, N. Engl. J. Med. 344 (2001) 1207–1213.
[129] J.X. Zhang, G. Hallmans, M. Landström, A. Bergh, J.E. Damber, P. Aman, H. Adlercreutz, Soy and rye diets inhibit the development of Dunning R3327 prostatic adenocarcinoma in rats, Cancer Lett. 114 (1997) 313–314.
[130] B. Zimmerberg, M.J. Farley, Sex differences in anxiety behavior in rats: Role of gonadal hormones, Physiol. Behav. 54 (1993) 1119–1124.