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Research report

Low dietary soy isoflavonoids increase hippocampal spine synapse density in ovariectomized rats

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ABSTRACT

High dietary intake of plant estrogens (phytoestrogens) can affect brain structure and function. The effects of phytoestrogen intake within the range of normal animal and human dietary consumption, however, remain uncertain. The aim of the present study was to determine the effects of the isoflavonoids present in a standard low phytoestrogen laboratory rat chow on spine synapse density in the stratum radiatum of area CA1 of the hippocampus.

Weanling rats (22 days old) were fed either standard chow (Teklad 2018), a nutritionally comparable diet without soy (Teklad 2016) or a custom diet containing Teklad 2016 supplemented with the principal soy isoflavonoids, daidzein and genistein, for 40 days. Rats were ovariectomized at 54 days of age. Eight days later, spine synapse density on the apical dendrites of hippocampal pyramidal neurons in the stratum radiatum of area CA1 was measured by electron microscopic stereological analysis. Animals maintained on Teklad 2016 exhibited an approximately 60% lower CA1 spine synapse density than animals consuming Teklad 2018. Replacing genistein and daidzein in Teklad 2016 returned synapse density to levels indistinguishable from those in animals on Teklad 2018.

These results indicate that the isoflavonoids in a standard laboratory rat diet exert significant effects on spine synapse density in the CA1 region of the hippocampus. Since changes in spine synapse density in this region of the hippocampus have been linked to cognitive performance and mood state, these data suggest that even relatively low daily consumption of soy phytoestrogens may be sufficient to influence hippocampal function.

1. Introduction

A number of studies have suggested that women may benefit from eating foods rich in phytoestrogens, or taking soy-based supplements (which contain the isoflavonoids genistein and daidzein) to minimize the symptoms associated with the loss of ovarian estrogens at menopause, including depression and cognitive dysfunction (Franco et al., 2016; Pitkin, 2012; Soni et al., 2014). Work in laboratory animals supports this hypothesis, demonstrating estrogen-like effects of isoflavonoids in rats (Lephart et al., 2002; Luine et al., 2006; Lund et al., 2001; Pisani et al., 2012). However, not all the reported effects on cognitive function have been positive, in either animals (Neese et al., 2012) or humans (Henderson et al., 2012; Soni et al., 2014; St John et al., 2014; Zhao and Brinton, 2007). This may in part be because isoflavonoids do not have the same receptor specificity as the principal mammalian estrogen, estradiol. While estradiol acts through at least three different receptor systems [estrogen receptor (ER)α, ERβ and the G-protein coupled transmembrane estrogen receptor GPR30 (Prossnitz and Barton, 2011)], genistein and daidzein are partially selective for ERβ and GPR30 (Kajta et al., 2013; Thomas and Dong, 2006) and have lower affinity for ERα (Casanova et al., 1999).

In addition to their potential use as a dietary supplement, phytoestrogens are also important in laboratory animal studies when phytoestrogens and other xenobiotic estrogens (Brown and Setchell, 2001) may be present in the food supply. Many commercial animal foods contain soy meal, contributing significant quantities of isoflavonoids, including genistein and daidzein. Studies comparing the effect of high and low phytoestrogen diets suggest that these compounds exert significant estrogenic effects (Lee et al., 2009; Lund et al., 2001; Patisaul and Jefferson, 2010; Whitten et al., 2002), raising the potential for confounding effects in studies of endocrine function and behavior (Jensen and

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Ritskes-Hoitinga, 2007; Thigpen et al., 2013, 2004). However, since some of these studies used diets with a relatively high isoflavonoid content (>300 mg/kg; Bu et al., 2005; Lee et al., 2009; Lund et al., 2001) it remains uncertain whether similar effects may also occur in animals fed using standard diets, with isoflavonoid contents in the 100–300 mg/kg range (Jensen and Ritskes-Hoitinga, 2007). Interpretation of the data is also complicated by the fact that substituting other foodstuffs for phytoestrogen-rich components in the diet may potentially have effects related to their nutritional value, rather than their activity as estrogens.

The present study was performed to determine whether the phytoestrogens present in a standard rat chow influence the density of spine synapses in area CA1 of the hippocampus. Spine and spine synapse formation in this region of the brain are highly sensitive to estrogen and have been linked to estradiol-induced changes in both cognitive performance (Frankfurt and Luine, 2015; Phan et al., 2011) and mood state (Hajszan and MacLusky, 2006; Hajszan et al., 2005, 2010). We compared spine synapse density in ovariectomized rats fed either Teklad 2018 (a widely used rodent diet with reduced alfalfa and soy content); a nutritionally comparable soy-free diet (Teklad 2016); or Teklad 2016 supplemented with genistein and daidzein to levels similar to those present in Teklad 2018. The results suggest that even relatively low levels of dietary isoflavonoids may have positive effects on hippocampal neuroplasticity.

2. Methods

2.1. Animals

Female Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were kept in individual cages on a 12-h light, 12-h dark cycle and provided with unlimited access to water and food. Animal protocols used in this study were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory animals and approved by the Institutional Animal Care and Use Committee of Yale University. The complete experimental protocol is summarized in Table 1. Rats arrived at the age of 22 days and were housed until the age of 54 days, when they were sexually mature (first estrus occurs in this strain of rats at 33–34 days of age; (Brown et al., 1994). The animals were maintained during the 40 day period of study with minimal experimental manipulation, to minimize potential interference from stress which is known to interfere with both pubertal development (Holder and Blaustein, 2014) and the effects of estrogen on hippocampal neuroplasticity (Frick et al., 2004; Scharfman et al., 2007).

Table 1
Summary of the experimental protocol.

Weaning	OVX	CA1 spine density assessment	# of animals
Diets introduced			
22 days	54 days	62 days	
Diet compositions:			
Group 1 Teklad 2018 total isoflavonoids 232 mg/kg			4
Group 2 Teklad 2016 (isoflavonoids undetectable)			4
Group 3 Teklad 2016 + 112 mg/kg genistein & 108 mg/kg daidzein			4

Animals were received from the supplier and immediately switched onto one of the three test diets. After ovariectomy (OVX) at 54 days, the animals were maintained on their regular schedule for a further 8 days, and then perfused for CA1 spine synapse density assessment.

The experiment was performed on three groups of rats, each consisting of 4 animals. Rats in the control group were fed a normal soy meal-containing diet (Teklad 2018; Envigo Research Indianapolis, Indiana, USA). In this diet, isoflavonoid contents (daidzein + genistein aglycone equivalents) range from 150 to 250 mg/kg, which is in the mid-range of isoflavonoid contents of commercial soy-containing laboratory rodent diets (Jensen and Ritskes-Hoitinga, 2007). The second group of rats (phytoestrogen reduced) was fed a nutritionally comparable diet (Teklad 2016) containing no soybean meal, in which isoflavonoid levels typically are below 20 mg/kg. Rats in the third experimental group were fed a special diet based on Teklad 2016, but with 220 mg/kg of pure isoflavonoids (112 mg/kg genistein and 108 mg/kg daidzein) mixed into the food at the time of milling. The content of isoflavones in the diet was provided by the manufacturer, based on data generated by Dr. Patricia Murphy at Iowa State University using previously described chromatographic procedures (Wang and Murphy, 1994). The basic nutrient contents of the non-supplemented Teklad 2016 and 2018 diets are summarized in Table 2.

The animals were fed the test diets, with food and water ad libitum, from the age of 22 days until the end of the experiment, 40 days later. At 54 days of age, they were anesthetized with 4 ml/kg b.wt. of a mixture of ketamine (25 mg/ml), xylazine (1.3 mg/ml), and acepromazine (0.25 mg/ml), and then ovariectomized (OVX). Following ovariectomy, the animals were allowed to recover for a further 8 days. At 62 days of age, under deep ether anaesthesia, the vascular system was perfused via the left ventricle with 50–80 ml of heparinized saline followed by 200 ml of fixative containing 4% paraformaldehyde, 0.1% glutaraldehyde in 0.1 M, pH 7.4 phosphate buffer (PB). Following perfusion, selected of the brain areas were dissected out and immersed in the same fixative for an additional 24 h. One hundred micron thick sections were prepared on a vibratome perpendicular to the longitudinal axis of the hippocampal formation, dehydrated in graded series of ethanol (70% contained 1% uranyl acetate) and flat embedded in Durcupan.

2.2. Determination of spine synapse density

Spine synapses were counted in all animal groups according to our standard protocol, using unbiased stereological methods (Leranth et al., 2004). Briefly, to assess possible changes in tissue volume, a correction factor was calculated assuming that the hormonal treatments did not alter the total number of pyramidal neurons (Rusakov et al., 1997). In all hippocampi, ten disector pairs (pairs of adjacent 2 μm toluidine blue-stained semithin sections mounted on slides) were sampled and analyzed using the technique of Braendgaard and Gundersen (1986). The pyramidal cell density value (D) was calculated using a formula: D = N/sT, where

Table 2
Macronutrient contents of the two diets used in this study.

Macronutrient Content	Units	Teklad 2018	Teklad 2016
Crude Protein	%	18.6	16.4
Fat	%	6.2	4
Crude Fiber	%	3.5	3.3
Ash	%	5.3	4.9
Insoluble Fiber	%	14.7	15.2
Carbohydrate (available)	%	44.2	48.5
Energy Density	kcal/g (kJ/g)	3.1/(13.0)	3.0/(12.6)
Calories from Protein	%	24	22
Calories from Fat	%	18	12
Calories from Carbohydrate	%	58	66

Details of the other components of these two diets are provided on the manufacturer's Web sites: (http://www.envigo.com/resources/data-sheets/2018-datasheet-0915.pdf; http://www.envigo.com/resources/data-sheets/2016-datasheet-0915.pdf).