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Interaction of bisphenol A (BPA) and soy phytoestrogens on sexually dimorphic sociosexual behaviors in male and female rats

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ABSTRACT

Concerns have been raised regarding the potential for endocrine disrupting compounds (EDCs) to alter brain development and behavior. Developmental exposure to bisphenol A (BPA), a ubiquitous EDC, has been linked to altered sociosexual and mood-related behaviors in various animal models and children but effects are inconsistent across laboratories and animal models creating confusion about potential risk in humans. Exposure to endocrine active diets, such as soy, which is rich in phytoestrogens, may contribute to this variability. Here, we tested the individual and combined effects of low dose oral BPA and soy diet or the individual isoflavone genistein (GEN; administered as the aglycone genistin (GIN)) on rat sociosexual behaviors with the hypothesis that soy would obfuscate any BPA-related effects. Social and activity levels were unchanged by developmental exposure to BPA but soy diet had sex specific effects including suppressed novelty preference, and open field exploration in females. The data presented here reinforce that environmental factors, including anthropogenic chemical exposure and hormone active diets, can shape complex behaviors and even reverse expected sex differences.

Introduction

Bisphenol A (BPA), a monomer used in epoxy sealants and plastics, has become ubiquitous in our environment and bodies, prompting concerns about its potential health impacts especially on neural development and behavior (Beronius et al., 2010; Chapin et al., 2008; FAO/WHO, 2011; FDA, 2012). Understanding how environmental factors, including endocrine disrupting chemicals (EDCs) such as BPA, contribute to impairments in affective and reciprocal social behaviors, are of considerable interest because they are features of psychosocial disorders for which incidence is rapidly rising, including autism spectrum disorders (ASDs) (Aguiar et al., 2010; Gore et al., 2014). Elevated gestational urinary BPA concentrations have been associated with adverse behavioral outcomes in children, including social deficits, hyperactivity, anxiety, and executive function deficits (Mustieles et al., 2015) suggesting the possibility that early life BPA exposure could impact behaviors related to emotionality. Numerous animal-based experiments have generated corroborating evidence for disruptions of sexual and mood-related behavior, most notably heightened anxiety, but supporting experimental evidence for impacts on social investigation and affiliation is extremely limited and highly discordant across animal models (Cox et al., 2010; Jasarevic et al., 2011; Sullivan et al., 2014; Wolstenholme et al., 2013). Diet is one factor thought to contribute to this variability, particularly hormonally active diets such as soy (Thigpen et al., 2013). Here we examined the impact of developmental, oral BPA exposure (at a dose considered human-relevant) alone and in combination with a soy rich diet or the individual soy phytoestrogen, genistein (GEN) on social investigation and exploratory behavior. A final group was exposed to BPA in peripubertal development and then placed on a soy diet at weaning to establish the impact of sequential exposure.

Soy contains numerous phytoestrogens, many of which are endocrine active but GEN is arguably the most well studied (Patisaul and Jefferson, 2010). That soy-based diets can confound EDC studies has been known for decades, but remains generally unrecognized by most researchers (Thigpen et al., 1999; Thigpen et al., 2013). The need to more comprehensively establish how hormonally active diets influence neural development and behavior (independently and in the presence of BPA and other ubiquitous anthropogenic EDCs) is pressing because of rapidly increasing soy consumption rates (Patisaul and Jefferson, 2010), particularly of soy-based infant formula.

The studies herein were undertaken to test the hypothesis that developmental, low dose BPA exposure has sex specific effects on social and exploratory behaviors, and that a soy rich diet or the isoflavone,

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GEN may modify these effects depending on timing of consumption. While the impacts of developmental BPA exposure on anxiety-related behaviors have been explored in some depth by us and others in a variety of species, impacts on social interactions have only been assessed in a handful of studies (with mixed results) and potential interactions with diet are virtually unknown. Thus the present studies fill a critical data gap and, importantly, incorporated research design recommendations for EDC studies on behavior and the sexual differentiation of the nervous system (Beronius et al., 2013; Li et al., 2008) including robust sample sizes (Chadman et al., 2009), controlling for possible litter effects, and minimizing exogenous EDC exposures which could confound study outcomes.

Materials and methods

Subjects

Animal care and use

Complete study design details are described in a prior study in which we used the same animals (Cao et al., 2015). Briefly, 54 pairs of Wistar rats were obtained and their offspring tested for these experiments. Rats were housed at the Biological Resource Facility of North Carolina State University (NCSU; 23 °C, 50% average relative humidity and 12:12 h reverse light: dark cycle; lights off at 10:00), according to the applicable portions of the Animal Welfare Act and the U.S. Department of Health and Human Services Guide for the Care and use of Laboratory Animals. All aspects of this study were approved by the Institutional Animal Care and Use Committee of NCSU. As in our prior studies (Cao et al., 2012; Patisaul et al., 2009), and in accordance with recommended practices for EDC research (Beronius et al., 2013; Li et al., 2008), rats were housed in conditions specifically designed to minimize unintended EDC exposure including thoroughly washed polysulfone (BPA-free) caging with glass water bottles and wood chip (not corn cob) bedding.

Exposure

On gestational day 1 (GD 1), dams were randomly assigned to six exposure groups (n = 9 per group; Table 1): Soy-Free (soy-free diet; Teklad 2020), Soy (soy diet; custom soy diet, Harlan), BPA + Soy-Free (soy-free diet plus water containing BPA), BPA + Soy (soy diet plus water containing BPA), BPA/GIN + Soy-Free (soy-free diet plus water containing BPA and genistin (GIN) daily via food treat) and BPA/Soy-Free/Soy PND 21 (soy-free diet plus water containing BPA then pups switched to soy diet on PND 21). Exposure continued until weaning (postnatal day (PND) 21), with the exception of the group switched from soy-free (Soy-Free) to soy-based diet on PND 21 (BPA/Soy-Free/Soy PND 21). The soy diet was a custom diet (Harlan Laboratories, Madison, WI) with maximum similarity to Teklad 2020 with isoflavone levels ~ 400 mg/kg diet. Effects on growth, body weight, age at puberty and other developmental parameters for these animals are detailed in a prior publication (Cao et al., 2015).

BPA (Sigma-Aldrich, St. Louis, MO) was administered via drinking water, as described previously (Cao et al., 2012), at 2 mg/L of water; to produce serum levels in the human range (<4 ng/ml serum) (Cao et al., 2015). GEN levels (dams and pups) in the soy-fed group were 3–31 ng/ml (Cao et al., 2015). Genistin (GIN; the glycosylated form of GEN found in food (Jefferson et al., 2009)) was administered (40 mg GIN (LC Laboratories, Woburn, MA)) on a peanut butter (Skippy Natural Creamy, Englewood Cliffs, NJ) covered Mini Nilla Wafer (Nabisco, East Hanover, NJ) (1 wafer/day/dam), daily through PND 21 (resulting serum GEN levels in the range of 18–48 ng/ml) (Cao et al., 2015). This range is approximately equivalent to vegetarians but below that of soy formula-fed infants (Patisaul and Jefferson, 2010). The detected range was wide but not atypical for a single measurement taken from animals given free access to the diet. This assessment was intended to be a survey of internal levels and not a formal pharmacokinetic-pharmacodynamic (PPK)-type analysis.

Behavior

Two tests were conducted: social choice (juveniles) and open field (adults). All testing was conducted within the first 4 h of the dark cycle under red-light, video recorded, and scored using TopScan (Clever Sys Inc.) software as we have done previously (Rebuli et al., 2015; Sullivan et al., 2014) using published testing methodologies (Crawley et al., 2007; Insel et al., 1999; Winslow and Insel, 2002). Test order was randomized among subjects and a randomly selected subset of videos was scored by hand by an independent observer to validate the computer scoring (Pearson Correlation r = 0.98). Juveniles (PND 24–28) were tested for 20 min in a three-chambered social choice apparatus (Sullivan et al., 2011). Familiar animals were same-sex sibling cage-mates and novel animals were age and sex-matched conspecifics. No more than two animals per sex per litter were used and sample size was within the range recommended for behavioral studies (Chadman et al., 2009). Final animal numbers were as follows: Females: Soy-Free n = 15, Soy n = 10, BPA + Soy-Free n = 15, BPA + Soy n = 16, BPA/GIN + Soy-Free n = 11, and BPA + Soy-Free/Soy PND 21 n = 12. Males: Soy-Free n = 9, Soy n = 16, BPA + Soy-Free n = 12, BPA + Soy n = 19, BPA/GIN + Soy-Free n = 11, and BPA + Soy-Free/Soy PND 21 n = 13.

Beginning on PND 60, the rats were then subjected to a standard 20 minute open field (OF) test (Sullivan et al., 2014). Adult females were tested in estrus (assessed by vaginal cytology (Becker et al., 2005)) by PND 110. Distance traveled, entries and time in the center, edges and corners were quantified using TopScan and validated by an independent observer (Pearson Correlation r = 0.98). No more than two animals per sex per litter were used. Final animal numbers were as follows: Females: Soy-Free n = 17, Soy n = 16, BPA + Soy-Free n = 17, BPA + Soy n = 15, BPA/GIN + Soy-Free n = 13, and BPA + Soy-Free/Soy PND 21 n = 15. Males: Soy-Free n = 16, Soy n = 19, BPA + Soy-Free n = 15, BPA + Soy n = 15, BPA/GIN + Soy-Free n = 12, and BPA + Soy-Free/Soy PND 21 n = 14.

Statistical analysis

To probe for baseline behavioral sex differences, *t*-tests were used to compare males and females within the Soy-Free and Soy groups (not exposed to BPA). For each task, evidence of expected sex differences (as discerned from prior, published literature) in the soy-free group

Table 1
Experimental design and groups.

Exposure group	Water (through PND 21)		GIN through PND 21	Soy-free diet		Soy diet	
	Vehicle	BPA		Before PND 21	After PND 21	Before PND 21	After PND 21
Soy-Free	+	−	−	+	+	−	−
Soy	+	−	−	−	−	+	+
BPA + Soy-Free	−	+	−	+	+	−	−
BPA + Soy	−	+	−	−	−	+	+
BPA/GIN + Soy-Free	−	+	+	+	+	−	−
BPA/Soy at PND 21	−	+	−	+	−	−	+