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Adult emotionality and neural plasticity as a function of adolescent nutrient supplementation in male rats

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ABSTRACT

The present study explored the effects of supplementing male rats with either choline, omega-3 fatty acids, or phytoestrogens, from weaning into early adulthood, on emotionality and hippocampal plasticity. Because of the neuroprotective properties of these nutrients, we hypothesized that they would positively affect both behavior and hippocampal function when compared to non-supplemented control rats. To test this hypothesis, male Sprague Dawley rats were assigned to one of four nutrient conditions after weaning: 1) control (normal rat chow); 2) choline (supplemented in drinking water); 3) omega 3 fatty acids (daily oral supplements); or 4) phytoestrogens (supplemented in chow). After 4 weeks on their respective diets, a subset of rats began 3 weeks of behavioral testing, while the remaining behaviorally naïve rats were sacrificed after 6 weeks on the diets to assess numbers of adult-born hippocampal neurons using the immature neuron marker, doublecortin. The results revealed that choline supplementation affected emotional functioning; compared to rats in other diet conditions, rats in this group were less anxious in an open field and after exposure to predator odor and showed less behavioral despair after forced swimming. Similar behavioral findings were evident following supplementation with omega-3 fatty acids and phytoestrogen supplementation, though not on all tests and not to the same magnitude. Histological findings followed a pattern consistent with the behavioral findings: choline supplementation, followed by omega-3 fatty acid supplementation, but not phytoestrogen supplementation, significantly increased the numbers of new-born hippocampal neurons. Choline and omega-3 fatty acids have similar biological functions—affecting cell membranes, growth factor levels, and epigenetically altering gene transcription. Thus, the present findings suggest that targeting nutrients with these effects may be a viable strategy to combat adult psychopathologies.

1. Introduction

The developing mammalian brain exhibits significant plasticity and many internal and environmental variables can influence its organization during this time (Caspi et al., 2007; Conner et al., 2009; Meck and Williams, 2003a; Teicher et al., 2012; Zeisel, 2012a). For example, optimal levels of certain nutrients during the prenatal and postnatal periods are critical for normal brain development (Morgane et al., 1993) and contribute to variety in behavioral phenotypes (Long and Benton, 2013; Seshadri et al., 2002), like shaping adult emotional and affective behavior (Glenn et al., 2012). During adolescence, disturbances in emotion, including symptoms of anxiety and depression, become more pronounced in at-risk youth (Merikangas et al., 2010; Zisook et al., 2007) and, in the general population, there are correlations between nutrient intake and cognitive and emotional functioning (Dauncy, 2012; Morris et al., 2003). In the present study, emotional behaviors were examined in young adult male rats that were supplemented with one of three specific nutrients from weaning: choline, omega-3 fatty acids, and phytoestrogens. Previous research suggests that these nutrients are neuroprotective (Morris et al., 2003), particularly in the hippocampus (Denis et al., 2013; Wong-Goodrich et al., 2008a), a brain region that contributes to normal cognitive and emotional functions (McKenzie et al., 2013; Gray and McNaughton, 2003). The hippocampus is markedly sensitive to stress and is adversely affected by it: chronic stress atrophies hippocampal neurons (McEwen, 2000) and decreases adult hippocampal neurogenesis [(Gould and Tanapat, 1999), but see Schoenfeld and Gould, 2012]. These kinds of morphological changes are associated with numerous psychopathologies of mood, including anxiety-related disorders, depression, and schizophrenia (Eisch et al., 2008; McEwen, 2004). Thus, we sought evidence that, in addition to affecting emotional behaviors on tests that index anxiety, fear, and despair, the nutrient supplements may also increase neural plasticity, indexed by numbers of newborn neurons in the dentate gyrus of the hippocampus.

The dietary nutrients under investigation in the present study have related functions, but distinct mechanisms of action. Choline is an

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essential nutrient with various biological roles critical to healthy brain function: it is the precursor to the neurotransmitter, acetylcholine (Zeisel, 2006; Blusztajn and Wurtman, 1983), promotes cell membrane integrity through its conversion to phosphatidylcholine (Zeisel, 2006; Blusztajn and Mellot, 2012), participates in signaling pathways at the cell membrane (Sanders and Zeisel, 2007), and is an important source of methyl groups, which are critical mediators of epigenetic modifications to gene expression (Zeisel, 2006; Niculescu and Zeisel, 2002). Prenatal choline availability is integral in normal brain development, both during neural tube closure in early gestation, and later during the development of the basal forebrain-hippocampal cholinergic system (Sanders and Zeisel, 2007; Monk et al., 2012). Choline supplementation during late pregnancy increases the expression of neurotrophic factors (Glenn et al., 2007; Glenn et al., 2008), alters the expression of genes through methylation reactions (Zeisel, 2006; Mehedint and Zeisel, 2013; Niculescu et al., 2006), and increases hippocampal neurogenesis prenatally (Craciunescu et al., 2003) and in adulthood (Glenn et al., 2007). Our recent work also suggests that choline may modulate emotional behaviors; female rats supplemented with choline prenatally (gestation day 10 to birth) or postnatally (during the period after weaning and extending past adolescence: postnatal days 25–50) had less anxiety- and depressive-like behaviors (Glenn et al., 2012). These behaviors are often accompanied by alterations in hippocampal neurogenesis (Eisch and Petrik, 2012), suggesting it may be a biological basis of these alterations. Thus, there is compelling evidence that increased choline availability offers the brain protection from environmental insults, including stress (Corriveau and Glenn, 2012).

Like choline, omega-3 fatty acids also contribute to the integrity of neuronal plasma membranes, increase the expression of neurotrophic factors (Gomez-Pinilla, 2008), and alter DNA methylation patterns integral for gene expression (Dhobale and Joshi, 2012). The omega-3 fatty acid, docasahexaenoic acid (DHA), helps maintain ionic permeability of the membrane and thus synaptic function (Gomez-Pinilla, 2008), and, consistent with the neuroprotective properties of omega-3 fatty acids, normalizes dysregulation of neurotrophic factors by traumatic brain injury (Wu et al., 2011). Omega-3 fatty acids also regulate cognitive and emotional functioning in humans (Gomez-Pinilla, 2008; Freeman et al., 2006; Hibbeln, 1998; Moriguchi et al., 2000) and in animal models (Vines et al., 2012), and, like choline, may exert these effects through actions on hippocampal plasticity. The biological functions of phytoestrogens differ from those of choline and omega-3 fatty acids; their primary mechanism of action is via estrogen receptor binding. In this way, they affect not only the reproductive system, but also the hypothalamic–pituitary–adrenal (HPA) axis, and the many brain regions rich in estrogen receptors, including the hippocampus (Walf and Frye, 2008). Much nutritional phytoestrogen research employs soy, as it contains isoflavones, a sub-class of phytoestrogens (Hartley et al., 2003). As a common source of dietary protein, isoflavones are frequently found in commercial rodent chows and in many processed human foods, making them an especially physiologically relevant phytoestrogen (Hartley et al., 2003).

Unlike research on choline and omega-3 fatty acids, work examining the behavioral and neural impacts of phytoestrogens has produced contradictory findings. For example, one study found that male rats fed a soy-rich diet, compared with rats fed a soy-free diet, were more anxious in a social interaction test and on an elevated plus maze, and had increased levels of corticosterone and vasopressin (Hartley et al., 2003); another study found that male rats fed a soy-rich diet were less anxious than controls in the elevated plus maze (Lund and Lephart, 2001). In contrast to these conflicting findings of phytoestrogen supplementation in males, phytoestrogen supplementation appears to have a reliable anxiolytic effect on females, both ovariectomized and intact (Lund and Lephart, 2001; Lephart et al., 2002; Rodríguex-Landa et al., 2009). This sexually dimorphic response to phytoestrogens also occurs on tests of learning and memory: phytoestrogen supplementation improved female rats' performance on the radial arm maze for visual–spatial memory, but disrupted male performance in the same task (Lephart et al., 2002). Research at the molecular level has also yielded mixed results. Phytoestrogens may activate estrogen receptors α and β (ERα and ERβ), but the β receptor is strongly activated by isoflavone phytoestrogens found in soy (Lephart et al., 2002). Administration of the ERβ agonist diarylpropionitrile (DPN), subcutaneously or orally, had no effect on measures of anxiety in one study (Patisaul et al., 2009), while a separate study found that injecting ERβ agonists, including DPN, directly into the hippocampus decreased anxiety-related behaviors in rats (Walf and Frye, 2007). Therefore, it is evident that the effects of phytoestrogens on emotional behavior and hippocampal function are uncertain and the lack of congruent data in the literature warrants further research to better characterize the role of phytoestrogens in the regulation of emotional behaviors and hippocampal plasticity.

In order to better understand the impact of essential dietary nutrients on specific emotional behaviors, the present study compared the effects of nutrient supplementation of either choline, omega-3 fatty acids, or phytoestrogens with regular rat chow. The diets began right after weaning and continued into young adulthood. A subset of adult rats from each diet condition underwent behavioral tests and the remaining rats served as cage controls for assays of hippocampal plasticity. The emotional behaviors under investigation were 1) anxiety, assessed in the open field and on a test of predator odor; 2) fear, assessed in the presence of predator odor; and 3) despair, assessed in the forced swim test. Hippocampal plasticity was assessed with unbiased stereological procedures to estimate numbers of new immature neurons, marked by immunostaining for the microtubule-associated protein doublecortin (DCX). Overall, our findings support choline's role in buffering against anxiogenic experiences and provide compelling evidence that phytoestrogens and omega-3 fatty acids may also possess similar anxiolytic properties. It is expected that this work will open a line of research surrounding the potential for nutritional therapies to be used as putative interventions and preventions for emotional disorders.

2. Material and methods

2.1. Animals and diets

The subjects were 60 male Sprague Dawley rats (CD strain; Charles River Breeders, Raleigh, NC) that arrived in the colony post-weaning on postnatal day (PD) 23. Rats were housed in individually ventilated clear polycarbonate cages (30.8 × 30.8 × 18.7 cm; Thoren Caging Systems, Hazleton, PA) in a colony with a 12:12 h light–dark cycle with lights on at 08:00 h; the colony temperature was 21 ± 2 °C with 40–60% humidity. After acclimating to colony conditions for 2 days, rats were placed on one of 4 dietary nutrient protocols that continued for the duration of the study. The protocols were: 1) CONTROL (n = 14)—commercially available rat chow (2016 Teklad Global 16% Protein Rodent Diet; Harlan Laboratories) without alfalfa or soybean meal, and thus little to no phytoestrogen content and non-detectable to 20 mg/kg isoflavone concentrations, was available to rats ad libitum along with free access to drinking water flavored with 50 mM saccharin; 2) CHOLINE (n = 15)—rat chow, as described for the CONTROL protocol, was available ad libitum along with choline-supplemented drinking water containing 25 mM choline chloride and 50 mM saccharin; 3) OMEGA (n = 15)—rat chow and saccharin-sweetened drinking water was available ad libitum, as described for the CONTROL rats, and was combined with daily oral supplements of fish oil (3 g/kg; Sigma Aldrich Chemical Co., St. Louis, MI) containing 20–31% omega-3 fatty acids as triglycerides; and 4) PHYTO (n = 16)—commercially available rat chow (8640 Teklad 22/5 Rodent Diet; Harlan Laboratories), containing 350–650 mg/kg isoflavone concentration and thus rich in phytogestrogens, was available ad libitum along with free access to saccharin-sweetened drinking water.