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Acute dietary tryptophan manipulation differentially alters social behavior, brain serotonin and plasma corticosterone in three inbred mouse strains

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ABSTRACT

Clinical evidence indicates brain serotonin (5-HT) stores and neurotransmission may be inadequate in subpopulations of individuals with autism, and this may contribute to characteristically impaired social behaviors. Findings that depletion of the 5-HT precursor tryptophan (TRP) worsens autism symptoms support this hypothesis. Yet dietetic studies show and parents report that many children with autism consume less TRP than peers. To measure the impact of dietary TRP content on social behavior, we administered either diets devoid of TRP, with standard TRP (0.2 g%), or with 1% added TRP (1.2 g%) overnight to three mouse strains. Of these, BTBRT^{+}*Itpr3*^{tf}/J and 129S1/SvImJ consistently exhibit low preference for social interaction relative to C57BL/6. We found that TRP depletion reduced C57BL/6 and 129S social interaction preference, while TRP enhancement improved BTBR sociability ($p < 0.05$; $N = 8$–10). Subsequent marble burying did not differ among diets or strains. After behavior tests, brain TRP levels and plasma corticosterone were higher in TRP enhanced C57BL/6 and BTBR, while 5-HT levels were reduced in all strains by TRP depletion ($p < 0.05$; $N = 4$–10). Relative hyperactivity of BTBR and hypoactivity of 129S, evident in self-grooming and chamber entries during sociability tests, were uninfluenced by dietary TRP. Our findings demonstrate mouse sociability and brain 5-HT turnover are reduced by acute TRP depletion, and can be enhanced by TRP supplementation. This outcome warrants further basic and clinical studies employing biomarker combinations such as TRP metabolism and 5-HT regulated hormones to characterize conditions wherein TRP supplementation may best ameliorate sociability deficits.

1. Introduction

Sociability deficits, specifically interpersonal interaction impairments such as social anxiety, withdrawal, inattentiveness, or lack of social motivation are characteristic of autism spectrum disorders. Serotonin (5-HT) system dysfunctions are implicated in some forms of autism, and may contribute to characteristic social interaction impairments (Lam et al., 2006; Rubin et al., 2013; Yang et al., 2014). During fetal and juvenile brain development, 5-HT plays many critical roles (Daws and Gould, 2011). Clinical and basic research findings indicate that 5-HT-regulated brain developmental trajectories are disrupted in autism, either via deficient or excessive central 5-HT availability (Chandana et al., 2005; Azmitia et al., 2011; Madden and Zup, 2014; Yang et al., 2014).

Among individuals with autism, brain 5-HT availability and neurotransmission are variable, since a diverse range of genetic and environmental risk factors can manifest in common core behavioral deficits (Unwin et al., 2013; Whitehouse and Stanley, 2013). Yet subpopulations with distinct autism phenotypes can be identified, including a group with physiological markers and behavioral symptoms consistent with central hyposerotonemia (Brune et al., 2006; McNamara et al., 2008; Veenstra-VanderWeele et al., 2012). Such markers comprise reduced 5-HT transporter binding in frontal cortex (Makkonen et al., 2008; Nakamura et al., 2010), low oxytocin and low melatonin levels (Alabdali et al., 2014; Ruggeri

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et al., 2014). Selective 5-HT reuptake inhibitors (SSRIs) improve autism symptoms in some patients (West et al., 2009; Kumar et al., 2012; Hollander et al., 2012; Politte et al., 2014),[1] suggesting 5-HT neurotransmission may be reduced and/or brain 5-HT depleted.

Tryptophan (TRP) is the essential amino acid 5-HT precursor. Acute TRP depletion can be used to assess the prognosis of patients with depression to benefit from 5-HT-based treatments (Delgado, 2006; Toker et al., 2010). With TRP depletion, depression symptoms worsen and cognitive functions decline in patients that responded favorably to SSRI treatments, or in individuals with high 5-HT turnover rates (Bell et al., 2001; Delgado, 2006; Feder et al., 2011). TRP depletion in individuals with autism likewise worsens core behavior symptoms, indicating heightened sensitivity to fluctuations in TRP and 5-HT availability (McDougle et al., 1993, 1996). On the other hand, increasing dietary TRP intake ameliorated autism symptoms in a case study (Beretich, 2009). This presents a paradox, in light of reports that many individuals with autism prefer foods with relatively low TRP content or have aversions to high dietary protein content (Kidd, 2002; Arnold et al., 2003; Herndon et al., 2009; Hyman et al., 2012; Johnson et al., 2014).

Given this, we tested the hypothesis that acute dietary TRP depletion should impair social behavior, while TRP enhancement might improve it. Inbred mice expressing the high-functioning TRP hydroxylase 2 (Tph2) enzyme isoform to convert TRP to 5-HT, with well-characterized sociability phenotypes (Carneiro et al., 2009; Moy et al., 2007) were used. These included socially deficient BTBR T+ Itpr3tf/J (BTBR) and 129S1/SvImJ (129S), and relatively gregarious C57BL/6J (C57) mice. Preferences for social interaction and novelty, chamber entries, self-grooming during sociability tests and subsequent marble burying were compared among strains and overnight TRP diet treatments. After behavior tests, brain TRP, 5-HT turnover and plasma corticosterone (CORT) – since it can be suppressed by central 5-HT transmission (Gould et al., 2014) – were measured in tissues collected from all strain × diet treatment groups to assess their central 5-HT status.

2. Methods

2.1. Mice and acute dietary tryptophan manipulation

All procedures involving live mice were approved by the UTHSCSA Institutional Animal Care and Use Committee, and were in accordance with current NIH guidelines. Mice tested were fifth and sixth generation male offspring bred in the laboratory animal facilities at The University of Texas Health Science Center at San Antonio (UTHSCSA), San Antonio, TX. BTBR, C57 and 129S, founders came from Jackson Laboratory (Bar Harbor, ME, USA).

Mice were maintained at 22–25 °C on 14:10 light dark cycles, with lights on at 0700 h, and *ad libitum* access to Teklad LM-485 mouse/rat irradiated food pellets (#7912, Harlan, Madison, WI) and water in cages lined with wood-chip bedding that were changed bi-weekly. Mice were weaned at postnatal days 20–22 and were housed in same-sex groups of 3–5 per cage. Dietary TRP manipulations and behavior tests were conducted in 3–4 month-old males. For 24–30 h prior to behavior testing (beginning 0900 or 1000 h), mice had *ad libitum* access to purified ingredient or "open source" standard diets with either a) control levels of TRP (2.1 g/kg or 0.2% = green pellets), b) diet devoid of TRP (-TRP, 0% = red), or c) diet with 1% added TRP (+TRP 12.6 g/kg = yellow) from Research Diets Inc. (New Brunswick, NJ). Nutritional information for open-standard purified diets and the Teklad chow the mice were reared and maintained on are provided in Table 1.

2.2. Sociability tests, self grooming during tests and subsequent marble burying

Preference tests for social interaction and social novelty were performed in three chambered testing arenas between 9:00 and 16:00 h CST, as described in prior studies (Gould et al., 2011, 2014; Silverman et al., 2010; Yang et al., 2011). Conditioning and sociability tests were conducted under low red lighting (16 lux). A daily experiment schedule is provided, and sociability-testing arena illustrated in the online supplement (Appx A.1.a and b). Subject mice (4–6 tested in different arenas at the same time, 1 per treatment group) were brought from housing to the testing room 30 min prior to testing 24, 26 or 28 h (typically 0900, 1100 or 1400 h CST) after diets were administered to acclimate. Next, subjects acclimated to and explored the sociability arenas for 20 min. Then, subjects were confined to the center chamber for ≥1 min while pre-conditioned 'strangers' (novel male 129S mice, 8–10 weeks old) and novel objects (empty wire cages) were placed on either end chamber. Stimulus placement for testing was randomized and counter-balanced among groups. Ten min tests were videorecorded for subsequent data collection. Preference for social interaction was tested in the first 10 min with a stranger mouse in a cup cage at one end and an empty cage at the other end chamber. Then subjects were re-confined in the center while new strangers (stranger #2) were placed under empty cages and old strangers (stranger #1) were re-positioned in the arena (Appendix A.1.b). Preference for social novelty was measured in the second 10 min phase. Between subjects strangers were returned to home cages, and arenas cleaned with 70% ethanol and dried with paper towels.

Data collected by treatment-blind observers from 10 to 11 min videorecordings of social interaction and novelty preference tests included time spent in chambers, sniffing and grooming. Chamber dwelling was tracked as subjects entered new chambers by recording the time and each chamber entered and into a spreadsheet, subtracting the exit times to determine each dwelling duration, and adding durations separately for each chamber and each test phase. Sniffing was recorded by timer when a subject directed its nose toward strangers or novel objects (cup cages) from a distance of <1 cm, and ended when they turned their head or stepped away.

Table 1
Comparison of nutrient content of sustaining mouse chow that was given prior to study to the experimental open standard diet that was acutely administered before testing.

Dietary component	Teklad LM-485 (irradiated 7012, Harlan)	Exp. open standard (A11022501, research diets)
Macronutrients (% kcal)		
Protein	25	18
Carbohydrates	58	66
Fat	17	16
Fiber (g%)	14%	10%
Essential[a] L-Amino Acids (g%)		
Arginine	1.2	0.6
Histidine	0.5	0.4
Isoleucine	0.8	0.7
Leucine	1.7	1.5
Lysine	1.0	1.3
Methionine	0.4	0.5
Phenylalanine	0.9	0.8
Threonine	0.8	0.7
Tryptophan	0.3	0.2 (−TRP 0, +TRP 1.2)
Valine	0.9	0.9
Vitamins (IU/g)		
A	30	4000
D	2.4	1000
E	0.2	50
Vitamins (mg/kg)		
Menadione (K3)	80	0.5
B-complex		
Thiamine (B1)	95	6
Riboflavin (B2)	14	6
Niacin (B3)	100	30
Pantothenate (B5)	87	16
Pyroxidine (B6)	17	7
Biotin (B7)	0.8	0.2
Folate (B9)	7	2
Cobaltamin (B12)	0.09	0.01
Minerals (g%)		
Calcium	1.0	0.9
Phosphorus	0.7	0.3
Sodium	0.3	1.7
Potassium	0.8	0.6
Chloride	0.5	1.0
Magnesium	0.2	0.5
Minerals (mg/kg)		
Zinc	63.0	29.0
Manganese	93.0	59.0
Copper	23.0	6.0
Iodine	3.0	0.2
Iron	240.0	37.0

[a] For juvenile mice, per John and Bell, 1976.

[1] However, parallel benefits of SSRI treatment are frequently muted or absent in children with autism (Henry et al., 2009; Williams et al., 2013; Politte et al., 2014).