Physiology & Behavior 194 (2018) 410–419

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Altered salt taste response and increased tongue epithelium *Scnna1* expression in adult *Engrailed-2* null mice

Ankita Gupta[a,1], Xinyi Li[b], Emanuel DiCicco-Bloom[c], Nicholas T. Bello[a,b,*]

[a] Department of Animal Sciences, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA
[b] Nutritional Sciences Graduate Program, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA
[c] Department of Neuroscience and Cell Biology/Pediatrics, Rutgers Robert Wood Johnson Medical School, Rutgers, The State University of New Jersey, Piscataway, NJ, USA

ARTICLE INFO

Keywords:
Bitter
Sour
Saccharin
Epithelial sodium channel
Davis rig
Sweet

ABSTRACT

Sensory impairments are critical for diagnosing and characterizing neurodevelopmental disorders. Taste is a sensory modality often not well characterized. *Engrailed-2* (*En2*) is a transcription factor critical for neural development, and mice lacking *En2* ($En2^{-/-}$) display signs of impaired social interaction, cognitive processes (e.g., learning and memory, conditioned fear), and neurodevelopmental alterations. As such, $En2^{-/-}$ mice display the behavioral deficits and neural impairments characteristic of the core symptoms associated with autism spectrum disorder (ASD). The objective of this study was to characterize the taste function in $En2^{-/-}$ compared with $En2^{+/+}$ in adult male mice. Measuring taste responsiveness by an automated gustometer, *En2* null mice had decreased lick responses for 1.6 M fructose, whereas they demonstrated an increased taste responsivity (i.e., relative to water) at 0.3 M sodium chloride and 1 M monosodium glutamate. In a separate cohort of mice, $En2^{-/-}$ mice had an increased preference for sodium chloride over a range of concentrations (0.032–0.3 M) compared with $En2^{+/+}$ mice. Regional gene expression of the tongue epithelium demonstrated an increase in *Scnn1a*, *T2R140*, *T1R3*, and *Trpm5* and a decrease in *Pkd1l3* in *En2* null mice. Taken together, such data indicate that deficits in *En2* can produce sensory impairments that can have a measurable impact on taste, particularly salt taste.

1. Introduction

Taste is a sensory modality essential for the ingestion of nutrients and hedonic evaluation of food. Developmental events during childhood and adolescence can alter diet selection and affect preference for certain foods [1]. Picky eating or self-restrictive eating patterns are common in developmental disorders, such as autism spectrum disorder (ASD) [2–4]. Indeed, some level of feeding difficulties occurs in as much as 90% of children diagnosed with ASD and other pervasive developmental disorders [3–5]. Although ASD is a neurodevelopmental disorder that is characterized by deficits in socialization and communication, this disorder can encompass a variety of behavioral and emotional responses [2]. Classification of ASD based on the type of sensory impairment could be beneficial for developing phenotype-based treatments [6]. Compared with other sensory modalities, there are few investigations into the basis for the taste impairments associated with ASD [7]. Previous studies analyzing taste identification accuracy have shown that adolescents with ASD have more difficulty identifying aversive taste stimuli [8]. Another study also found that adults with ASD have difficulty identifying sweet and aversive taste stimuli and often mislabel taste stimuli as salty [9]. Notwithstanding, those few tastes studies that have been performed in ASD subjects have not examined the possible role of heritable factors on their findings [8–10].

Although there are several genetic variations and possible candidate genes implicated in ASD, single nucleotide polymorphisms, *rs1861972* (A/G) and *rs181973*(C/G), in the intronic region of the *Engrailed 2(EN2)* homeobox gene have been associated with ASD in human populations [11–14]. *Engrailed 2* is a transcription factor that is critical for neural development and affects norepinephrine levels in the forebrain and hindbrain [15]. *En2* knockout ($En2^{-/-}$; KO) mice display signs of poor social interaction and cognitive behaviors that are characteristic of ASD [16]. Further, this mouse model exhibits deficits in learning and memory, deformities in cerebellar development, and a decrease in the number of cerebellar Purkinje cells [12, 17–19]. Our laboratory recently reported that a juvenile exposed (postnatal day 21 to 60) to a

* Corresponding author at: Department of Animal Sciences, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA.
E-mail address: ntbello@rutgers.edu (N.T. Bello).
[1] North Carolina State College of Veterinary Medicine,1060 William Moore Drive, Raleigh, NC, USA.

https://doi.org/10.1016/j.physbeh.2018.06.030
Received 27 December 2017; Received in revised form 19 June 2018; Accepted 20 June 2018
Available online 25 June 2018
0031-9384/

ketogenic diet improved the autistic-like social impairments and exploratory behaviors associated with adult En2 KO mice [20]. Taken together, this suggests the *En2* KO mouse has a behavioral phenotype that can be a useful model for uncovering the sensory deficits associated with ASD.

The following study was designed to understand the taste sensory impairments in the $En2^{-/-}$ mouse. Specifically, these experiments examined the taste acceptability and preference for established tastes (i.e., salt, sweet, bitter, sour, and umami). In addition, we examined taste-receptor gene expression of the tongue epithelium, which included *Scnn1a* (salty), *Tas1r1* (umami), *Tas1r3* (sweet and umami), *Tas2r116* (bitter), and *Tas2r140* (bitter), as well as taste cell type and taste bud related genes (*Trpm5, Plc-β2*, *Pkd1l3*, and *Kcnq1*) [21–23]. This is the first study to characterize the taste dysfunction associated with *Engrailed 2* gene status. Thus, understanding the impaired sensory processing in $En2^{-/-}$ compared with $En2^{+/+}$ (WT; Wildtype) will help to further characterize the sensory impairments associated with autism-related behaviors and associated genes.

2. Materials and methods

2.1. Mice

$En2^{tm1Alj/tm1Alj}$ mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). These mice were generated on a 129S2/SvPas background as previously described [16]. *En2* heterozygous ($En2^{+/-}$) breeding pairs were placed on a 12:12 h light:dark cycle with lights off at 1800 h. To prevent genetic drift, every ten generations, $En2^{+/-}$ mice were crossed to B6129SF2/J mice for creation of new $En2^{+/-}$ breeding pairs. Pups were kept with the dam until weaning at postnatal day (PND) 21. After weaning, male juveniles were housed 2–4 mice per cage. All experiments were conducted using adult (≥ PND 56) *En2* WT and KO male littermates. Mice were fed standard chow (Purina Mouse Diet 5015, 25.34% fat, 19.81% protein, 54.86% CHO, 3.7 Kcal/g) and water was available at all times, unless otherwise noted. Genomic DNA from ear clippings was used to genotype animals, following previously published protocol [20]. All animal work was conducted according to relevant national and international guidelines. Animal care protocol was approved by the Institutional Care and Use Committee at Rutgers University (OLAW #A3262-01, protocol#13-001).

2.2. Taste responsivity assessment

A brief access taste assessment was performed as previously described, but with slight modifications [24, 25]. An automated gustometer (Davis Rig; Dilog Instruments, Tallahassee, Florida) was used to measure the number of licks for the different concentrations of taste stimuli for each animal [24] [26] [27]. During the light cycle (~ 0900 h) the mouse was placed in the plexiglass testing chamber (6″W × 11″H × 10″D) with access to a single spout of water. The floor was composed of a stainless-steel grid, and both the chamber and floor could be sanitized. The chamber was connected to a motor-driven bottle rack that could hold up to 16 bottles at a time. Throughout the study a maximum of 7 bottles were used. Each mouse was given a 5 min acclimatization to the gustometer chamber before initiating a taste session. A session was initiated with the opening of the shutter and presentation of a tube spout with a tube containing a single concentration of a taste stimulus. The concentration was presented for 5 s. The intertrial interval (ITI) was 8 s. There were 15 presentations of each tube with a maximum of 105 trials occurring over a period of 25 min.

Beginning at PND 63, male mice, cohorts of WT (n = 9) and KO (n = 17), were water restricted for 24 h prior to water training for 4 consecutive days. Mice were only allowed access to water during the training sessions. Specifically, on days 1 and 2 of behavioral water training, mice were allowed free access to 2 bottles of water for 15 min each. On days 3 and 4, each animal was given access to 7 bottles of water for a total of 25 min with a presentation time of 5 s for each bottle and ITI of 8 s for a total of 105 trials. After day 4, animals were given water ad libitum in their home cages. The first round of taste stimuli sessions started the following week. For the testing sessions, mice were tested with 7 different taste stimuli at 6 different concentrations under a partial caloric and water restriction (See Supplemental Table 1). Mice were individually housed and were given ~ 1 g standard chow and ~ 2 mL water, 24 h prior to each test day. The taste stimuli included: sucrose (sweet disaccharide: 0, 0.01, 0.03, 0.1, 0.3, 1 and 1.5 M), fructose (sweet monosaccharaide: 0, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 M), sodium chloride (salty: 0, 0.01, 0.03, 0.06, 0.1, 0.3 and 1 M), monosodium glutamate (umami: 0, 0.01, 0.03, 0.06, 0.1, 0.3 and 1 M), alanine (sweet amino acid: 0, 0.01, 0.03, 0.06, 0.1, 0.3 and 1 M), citric acid (sour: 0, 0.001, 0.003, 0.006, 0.01, 0.03, 0.1 M) and quinine-HCL (bitter: 0, 10^{-5}, 3×10^{-5}, 10^{-4}, 3×10^{-4}, 10^{-3}, 3×10^{-3} M). Mice were given an intervening day of chow and water ad libitum between taste stimuli. Concentrations were presented in a standardized design to minimize contrast and order effects (i.e., non-ascending/non-descending fashion). All taste stimuli were prepared on the day of the experiment using filtered tap water (see Supplemental Table 2). This was continued daily over a period of 6 weeks (40 days) so that each mouse was exposed to all the taste stimuli twice in the same sequence (Supplemental Tables 1 & 2). Only data from the second session were used for the analysis. A total of 2 WT and 1 KO mice were excluded from the data set because they failed to lick any of the taste stimuli at any concentration during the sessions. We did include in the data set mice that did not lick at a concentration of a taste stimulus, their recorded lick response was “0”.

2.3. Taste preference assessment

In a separate naïve cohort of WT (n = 10) and KO (n = 16) male mice (PND 56 to 70), a two-bottle choice test was performed as previously described but with slight modifications [28]. Mice were individually housed and tested with 4 different taste stimuli at different concentrations. Testing was performed in ascending order. Taste stimuli tested were sodium saccharin (artificial sweetener: 0, 0.0025 and 0.01 M), sodium chloride (salty: 0, 0.032, 0.075, 0.15, 0.3 and 0.6 M), monosodium glutamate (umami: 0, 0.01, 0.032, 0.1, 0.32 and 0.6 M) and quinine-HCl (bitter: 0, 3.2×10^{-5}, 10^{-4}, 3×10^{-4} M).

Animals were allowed access to the two bottles for 48 h. One bottle was filtered tap water and the other contained one concentration of the taste stimuli. After a period of 24 h, the positions of the bottles were switched in order to avoid place preference. Animals were given an intervening day of ad libitum water after each concentration of taste stimulus. Percent preference was expressed by taking the volume of taste stimuli consumed divided by the total volume of fluid consumed (taste stimulus and water) multiplied by 100. A taste stimulus with a preference score > 50 was considered to be *preferred*, < 50 was *avoided*, and equal to 50 was *indifferent* relative to water (i.e., no more or less palatable).

2.4. Tissue dissections for quantitative real-time PCR (qPCR)

In a separate cohort of WT (n = 12) and KO (n = 8) male mice (PND 60) tongues were removed following decapitation, transferred to Sorenson's phosphate buffer, and were stored in RNAlater (ThermoFisher Scientific, Inc.) overnight at 4 °C. The tongue epithelium was microdissected under a dissection microscope for the circumvallate papillae (CV), folate papillae (FO), and fungiform papillae (FU) regions following regional delineations [29]. To ensure consistency between regions and sample collections, the same lab member performed all the tongue tissue dissections. The individual performing the dissections was blinded to genotype of the mouse throughout the entire experiment. The dissected tissue was stored in −80 °C until RNA extraction. Total RNA was extracted using RNAqueous®-Micro Total RNA Isolation kit