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Sodium butyrate attenuates social behavior deficits and modifies the transcription of inhibitory/excitatory genes in the frontal cortex of an autism model

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ABSTRACT

The core behavioral symptoms of Autism Spectrum Disorders (ASD) include dysregulation of social communication and the presence of repetitive behaviors. However, there is no pharmacological agent that is currently used to target these core symptoms. Epigenetic dysregulation has been implicated in the etiology of ASD, and may present a pharmacological target. The effect of sodium butyrate, a histone deacetylase inhibitor, on social behavior and repetitive behavior, and the frontal cortex transcriptome, was examined in the BTBR autism mouse model. A 100 mg/kg dose, but not a 1200 mg/kg dose, of sodium butyrate attenuated social deficits in the BTBR mouse model. In addition, both doses decreased marble burying, an indication of repetitive behavior, but had no significant effect on self-grooming. Using RNA-seq, we determined that the 100 mg/kg dose of sodium butyrate induced changes in many behavior-related genes in the prefrontal cortex, and particularly affected genes involved in neuronal excitation or inhibition. The decrease in several excitatory neurotransmitter and neuronal activation marker genes, including cFos Grin2b, and Adra1, together with the increase in inhibitory neurotransmitter genes Drd2 and Gabrg1, suggests that sodium butyrate promotes the transcription of inhibitory pathway transcripts. Finally, DMCM, a GABA reverse agonist, decreased social behaviors in sodium-butyrate treated BTBR mice, suggesting that sodium butyrate increases social behaviors through modulation of the excitatory/inhibitory balance. Therefore, transcriptional modulation by sodium butyrate may have beneficial effects on autism related behaviors.

1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by social interaction and communication deficits, and repetitive or stereotyped behaviors. ASD affects approximately 1 in 68 children with a ratio of 4:1 affected male to female (Liu and Takumi, 2014). However, the determination of specific molecular mechanisms in the brain that are involved in the development of autistic behavior has remained elusive. Dynamic changes in histone modification are considered to play a primary role in the development of the nervous system and the development of mammalian behaviors (Rangasamy et al., 2013). Histone modifications and DNA methylation are the main epigenetic mechanisms that modify gene transcription. Different subtypes of histone modifications, including acetylation, methylation, and ubiquitination, define a specific epigenetic state that balances between gene silencing and gene activation (Sailaja et al., 2012). Histone acetylation in promoter regions is usually associated with activation of gene transcription, while the effect of histone methylation is highly dependent on the histone region which is modified. Histone acetylation is performed by Histone acetylases, while Histone Deacetylases (HDACs) removes acetyl groups from the histones.

Recently, several lines of evidence have led to the conclusion that both DNA methylation and histone modifications are involved in the etiology of ASD. Neuronal H3K4 methylation was found to be dysregulated in the brain of individuals with autism (Shulha et al., 2012). HDAC4 gene expression levels were also found to be upregulated in the prefrontal cortex of individuals with autism (Nardone et al., 2014). DNA methylation levels were also found to be altered in the brain of individuals with autism (Ladd-Acosta

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et al., 2014; Nardone et al., 2014).

Several studies suggest that HDAC inhibitors (HDACis) have potential as a therapeutic agent against cognitive impairments (Gräff and Tsai, 2013a, 2013b; Takuma et al., 2014). HDACi administration has beneficial effects in models of neurodegenerative diseases, such as Alzheimer's Disease (Guan et al., 2009) and Huntington's Disease (Gundersen and Blendy, 2009), and HDAC inhibitors increased social behaviors in prairie voles (Wang et al., 2013). Sodium butyrate (SB) is a widely known HDAC inhibitor which is often used in psychobiology studies, due to its ability to cross the blood brain barrier. SB attenuates memory deficits and improves cognitive function in several animal models (Fischer et al., 2007; Takuma et al., 2014). In addition, multiple studies have determined a memory-enhancing effect of SB in Alzheimer's Disease mice models (Govindarajan et al., 2011; Kilgore et al., 2010), and a separate study determined a similar effect in a rat model aging-related memory decline (Reolon et al., 2011). In addition, SB has antidepressant effects (Steckert et al., 2013), and may attenuate anxiety like behavior (Gundersen and Blendy, 2009). SB has an established role in the inhibition of most class I and II (Wei et al., 2014) HDACs.

The frontal cortex has primary roles in high-order cognitive and emotional behaviors, including social behavior, and recent studies have highlighted morphological, transcriptional, and epigenetic dysregulation in the frontal cortex of individuals diagnosed with autism. Separate studies reported an enlarged frontal cortex (Carper and Courchesne, 2005) and an increase in neuronal number in the frontal cortex (Courchesne et al., 2011). Converging evidence also suggests over connectivity within the frontal cortex, with parallel decreases in connectivity in other brain regions (Courchesne and Pierce, 2005). In a whole throughput gene transcription study, Voineagu et al. documented an increase in immune response gene expression, and a decrease in synaptic gene expression, in the frontal cortex of individuals with autism (Voineagu et al., 2011). In epigenetic studies, dysregulation in both H3K4 methylation patterns and DNA methylation patterns have been identified in the frontal cortex (Nardone et al., 2014; Shulha et al., 2012).

Animal models of autism include BTBR T+tf/J (BTBR), which exhibits several autism-like behavioral phenotypes, including social interaction deficits, impaired communication and repetitive behavior (Langley et al., 2015; Martin et al., 2014; McFarlane et al., 2008). While no animal model can fully recapitulate all symptoms of a human neurodevelopmental disorder, BTBR mice strain displays phenotypic traits of all diagnostic symptoms of autism. BTBR is an inbred mouse strain, and unlike genetic models, it is not clear what is the exact genetic aberration in this strain that is responsible for the autism-like phenotype. BTBR mice have a mutation in the autism-related gene Disc1, and a nonsynonymous SNP in the geneKmo (Meyza et al., 2013). However, it is not clear if these genetic changes have any relation to their autism-like behavior. Due to the consistent replication of the autism-like phenotype in several laboratories, BTBR mice are often used in pharmacological studies related to autism. In early work on BTBR mouse, researchers also found an abnormality of the corpus callosum, which is an anatomical anomaly also present in some autistic individuals (McFarlane et al., 2008).

In this study we test the hypothesis that chronic treatment with the HDAC inhibitor sodium butyrate will improve behavior deficits in the BTBR mouse model for ASD. Here we show that chronic treatment with SB during adulthood can attenuate social deficits in the three chamber test and social odor test, while having no side-effects on locomotor or anxiety-like behaviors. Whole genome gene expression has been employed to reveal molecular mechanisms in the frontal cortex which are likely to be responsible for the effects of sodium butyrate on behavior.

2. Materials and methods

2.1. Mice handling

Mice were housed according to the FELSA guidelines. All mice were bred and maintained in a vivarium at 22 °C and 50% humidity in a 12 h light/dark cycle, with food and water available *ad libitum*. BTBR T+tf/J strain was donated by Dr. Tali Kimchi (Weizmann Institute of Science). All experimentation performed in this study was approved by the Bar Ilan University Institutional Animal Care and Use Committee (IACUC) in protocol number 27-7-2013.

2.2. Drug administration

Sodium butyrate (Sigma and Cayman chemical company) was suspended in PBS and administrated in two concentrations: 100 mg/kg and 1200 mg/kg. PBS was administered as control. Drugs were administered by an intraperitoneal injection once a day for 10 days. Last injection was given 60 min before the beginning of the experiment.

Separate groups of mice were used for each behavioral test and for each molecular test (RNA-seq, cFos, etc.). Separate groups were necessary to ensure that each test was carried out at the exact same time point, 60 min following the tenth administration of sodium butyrate.

DMCM (methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate, Santa Cruz) was dissolved in 100% DMSO (Dimethyl Sulfoxide, Sigma) and diluted further with PBS. All experimental mice received 9 days injection of SB 100 mg/kg and on the day of the experiment the mice were divided in to two groups. The first group injection containing both SB and DMCM. The final injection was administrated 30 min before the beginning of the experiment.

2.2.1. Social interaction

The three chamber paradigm was performed as previously described (McFarlane et al., 2008). Apparatus is a Non-Glare Perspex box (60 × 40 cm) with two partitions that divide the apparatus to three chambers, Left, Center and Right (20 × 40 cm). The mouse is placed in the middle chamber for habituation (5 min) when the entry for both side chambers is barred. Test mouse was then allowed to explore the whole arena for ten minutes, where they freely choose between interacting with a novel mouse in one chamber, or stay in an empty chamber (social test). Immediately following this test, a second stranger mouse is introduced to the empty chamber, and the test mouse is allowed ten minutes to freely choose between interacting with the novel or familiar mouse. Time spent in each chamber is measured by Ethovision. The experiments were recorded with the Panasonic WV-CL930 camera and with the Ganz IR 50/50 Infrared panel. The recorded movement of the mice was analyzed by the Ethovision XT 10/11 (Noldus) software.

2.2.2. Social odor test

The test was performed in the same apparatus as the social interaction test. The mouse is placed in the middle chamber for habituation (5 min) when the entry for both side chambers is barred. Test mouse was then allowed to explore the whole arena for ten minutes, where they freely choose between the odor of unfamiliar bedding in one chamber, or stay in an empty chamber with clean bedding (social odor test). Immediately following this test, novel bedding was introduced to the empty chamber, and the test mouse is allowed ten minutes to freely choose between the novel or familiar odor. Time spent in each chamber is measured by Ethovision.