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Short communication

Ketogenic diet restores aberrant cortical motor maps and excitation-to-inhibition imbalance in the BTBR mouse model of autism spectrum disorder

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HIGHLIGHTS

- The BTBR mouse has lower movement thresholds and larger motor maps relative to control mice.
- The high-fat low-carbohydrate ketogenic diet raised movement thresholds and reduced motor map size in BTBR mice.
- The ketogenic diet normalizes movement thresholds and motor map size to control levels.

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ABSTRACT

Autism spectrum disorder (ASD) is an increasingly prevalent neurodevelopmental disorder characterized by deficits in sociability and communication, and restricted and/or repetitive motor behaviors. Amongst the diverse hypotheses regarding the pathophysiology of ASD, one possibility is that there is increased neuronal excitation, leading to alterations in sensory processing, functional integration and behavior. Meanwhile, the high-fat, low-carbohydrate ketogenic diet (KD), traditionally used in the treatment of medically intractable epilepsy, has already been shown to reduce autistic behaviors in both humans and in rodent models of ASD. While the mechanisms underlying these effects remain unclear, we hypothesized that this dietary approach might shift the balance of excitation and inhibition towards more normal levels of inhibition. Using high-resolution intracortical microstimulation, we investigated basal sensorimotor excitation/inhibition in the BTBR T+Itpr^{tf}/J (BTBR) mouse model of ASD and tested whether the KD restores the balance of excitation/inhibition. We found that BTBR mice had lower movement thresholds and larger motor maps indicative of higher excitation/inhibition compared to C57BL/6J (B6) controls, and that the KD reversed both these abnormalities. Collectively, our results afford a greater understanding of cortical excitation/inhibition balance in ASD and may help expedite the development of therapeutic approaches aimed at improving functional outcomes in this disorder.

Autism spectrum disorder (ASD) is a behaviorally-defined neurodevelopmental disorder characterized by decreased social interaction, abnormal communication and repetitive behaviors [1]. There has been a six-fold increase in the incidence of ASD over the past few decades, and this dramatic expansion is thought to arise from growing clinical awareness and clearer diagnostic criteria, as well as genetic and environmental influences [2]. Autism spectrum disorder is a life-long condition with high treatment costs for individual families and with greater social ramifications. Thus, accelerated research into the etiology and treatment of ASD is necessary to stem the tide of the current ASD epidemic.

Although not a hallmark diagnostic criterion, motor deficits are prevalent in ASD. These manifest as abnormalities in gait, coordination, skilled movements, motor learning and production of gestures [3,4]. Motor difficulties also shed light on broader neurodevelopmental disorders with implications for social, communicative and learning abilities. In support of this, individuals with ASD display

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increased white matter volume in the primary motor cortex and mini-column abnormalities [3] as well as decreased segregation between upper and lower limb control [4]. Finally, in the valproic acid (VPA) rat model of ASD, significant structural abnormalities in layer II pyramidal neurons from motor cortex have been shown [5]. Taken together, there is increasing evidence for a neuroanatomical basis for abnormal motor function in ASD.

In the present study, we investigated basal network excitability in the BTBR T + Itpr^{tf}/J (BTBR) mouse model of ASD [6] using standard intracortical microstimulation (ICMS) techniques which have been used to establish cortical motor maps that can be modulated by alterations in γ-aminobutyric acid (GABA)-mediated and/or glutamatergic neurotransmission [7,8]. We hypothesized that BTBR mice might display larger basal motor maps expression and decreased movement thresholds compared to control C57BL/6J (B6) animals, indicating an increase in excitation/inhibition in this cortical network. Further, given prior evidence that the high-fat, low-carbohydrate ketogenic diet (KD) – traditionally used in the treatment of medically refractory epilepsy – has been shown to reduce cortical network excitability in human neocortex [9], we examined whether KD treatment in BTBR mice would alter their baseline motor map size and restore normal cortical excitation-to-inhibition balance.

A breeding colony of C57BL/6J (B6) and BTBR T + Itpr3^{tf}/J (BTBR) mice (obtained from Jackson Labs) was established at the University of Calgary. Pups were weaned on postnatal day 21 (P21) and then housed by sex and strain up to five per cage, with a 12:12 h light-to-dark cycle. Food and water were available *ad libitum* except during periods of fasting. Only male mice were used in this study. All animals were handled according to the Canadian Council for Animal Care (CCAC) guidelines, and all procedures were approved by the Health Sciences Animal Care Committee at the University of Calgary.

After weaning, mice were placed on standard rodent diet (SD) for two days until P23. Thereafter, half of the B6 and BTBR animals continued with SD. The other half were fasted overnight for 12 h but with access to water; these mice were then started on an experimental KD (BioServ F3666, Flemington, New Jersey, USA) consisting of a 6.3:1 ratio of fats to carbohydrate plus protein. The KD was made available *ad libitum* from a glass container. Both B6 and BTBR animals were fed the KD until 5–7 weeks of age, at which time ICMS experiments commenced. Four experimental groups were used: (1) B6 mice on SD (n = 8); (2) B6 mice on KD (n = 7); (3) BTBR mice on SD (n = 7); and (4) BTBR on KD (n = 8).

Mice were anaesthetized with a mixture of ketamine (25 mg/kg) and xylazine (2.5 mg/kg). Further ketamine injections of (10 mg/kg) were given to maintain the proper level of anaesthesia. A craniotomy was then performed to expose the left hemispheric motor cortex. This extended 4 mm anterior and 3 mm posterior from Bregma and 3 mm lateral from the midline. The cisterna magna was then punctured with an 18-gauge needle to reduce swelling while the dura matter was removed. Body temperature silicon liquid was then placed on the exposed cortex. A photograph was taken and magnified 32× using a digital camera connected to a stereomicroscope; this image was displayed on a computer and overlaid with a grid consisting of 500 μm squares using Canvas (Version 11, ACD Systems, Victoria, British Columbia) imaging software.

Electrodes were composed of glass capillary tubes, beveled and filled with 3.5 M sodium chloride and had impedance values of 1.0–1.5 MΩ. Electrode penetration occurred at the corners and middle of the grid squares. There was no electrode insertion if a blood vessel appeared on an intersection of the grid. From the surface of the cortex the electrodes were lowered to a depth of 800 μm which is where layer V of the mouse cortex resides. After insertion, stimulation–consisting of 13 monophasic 200 μs cathodal pulses at 300 Hz–was applied. Mice were maintained in a prone position with their forelimbs supported.

Our ICMS procedures were adapted from Scullion et al. [10]. To determine a threshold for each insertion site, the current was increased from 0 μA towards 60 μA until a movement was observed either in the digits, wrist, elbow, shoulder, neck, jaw, whiskers or hindlimb. The current was then decreased, in 1 μA steps, until the movement stopped. The minimal ICMS current able to induce movement was defined as the movement threshold. If there were multiple movements, the last movement at the minimal current was noted. If there was no movement up to 60 μA, the point was labeled unresponsive. The border of the motor map was defined first, consisting of non-forelimb movements and then filled in with movements of the digits, wrist, elbow and shoulder. Positive response sites were re-visited during surgery to assess for changes in movement threshold as an indicator of anaesthesia levels.

Maps were analyzed with Canvas software (ACD systems), to calculate the extent of the caudal forelimb area (CFA). Forelimb motor map area was calculated across all four groups based on mean group size and compared across strain and diet treatment. Movement thresholds within each group were paired up using a random number generator. The map with the least number of forelimb points was assigned a maximum threshold point of 60 μA for each forelimb point that was unresponsive compared to the larger map. The mean movement threshold for each group was then calculated and compared across strain and diet treatment. SPSS (IBM) statistical software was used for further data analysis. Results were analyzed using a two-way ANOVA for map size, movement type and threshold calculations. Significance level was set at $p < 0.05$.

Motor map size showed a significant ($F(1, 26) = 16.03$, $p < 0.001$) main effect of strain; specifically, B6 mice had significantly smaller maps (3.15 $\mu m^{2)}$ than BTBR mice (4.82 μm^2). However, the main effect of diet was not significant (F (1, 26) = 2.71, $p = 0.11$), indicating that the KD did not alter baseline map areas (3.64 μm^2) compared to SD (4.33 μm^2). Nevertheless, there was a significant interaction between strain and diet ($F(1, 26) = 4.54$, $p = 0.04$). Ketogenic diet treatment decreased motor map area in BTBR mice (4.03 μm^2) as compared to SD (5.61 μm^2) more than B6 mice on the KD (3.25 μm^2) vs. SD (3.05 μm^2). When B6 mice were fed the KD compared to SD, there was no significant change in motor map size ($p = 0.74$). In contrast, when BTBR mice were fed the KD, there was a significant reduction in motor map size compared to SD ($p = 0.02$). In summary, the KD reduced abnormally larger cortical map sizes in BTBR mice compared to that of B6 mice fed SD ($p = 0.09$). Representative motor maps from each treatment group is shown in Fig. 1.

Analysis of proximal (elbow and shoulder) versus distal (digit and wrist) movements categories revealed that the main effect of strain was not significant ($F(1, 26) = 0.02$, $p = 0.90$) indicating that B6 and BTBR mice do not have different specific movements. The main effect of diet was also not significant ($F(1, 26) = 0.52$, $p = 0.48$) indicating that mice on the SD have movement categories similar to mice on the KD. There was a non-significant interaction (F (1, 26) = 0.07, $p = 0.80$). Thus, the KD diet did not affect the organization of movements.

Movement threshold data revealed that the main effect of strain was not significant (F (1, 26) = 2.17, $p = 0.65$) indicating that B6 (31 μA) and BTBR mice (30 μA) do not have different movement thresholds. The main effect of diet was also not significant (F (1, 26) = 2.53, $p = 0.12$) which demonstrates that mice on SD (28 μA) have thresholds similar to mice treated with the KD (32 μA). The interaction between strain and diet was significant ($F(1, 26) = 9.09$, $p = 0.006$). This indicates that the KD increased movement thresholds for BTBR mice (36 μA) as compared to SD (24 μA) significantly more and inversely correlated to that of B6 mice on KD (29 μA) vs. SD (33 μA). From these data, it is clear that B6 mice on SD have