Ketogenic Diet and Dendritic Morphology in a Mouse Model of Repetitive Behavior

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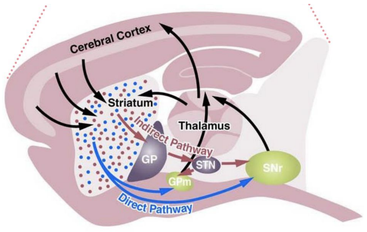
Background

- The ketogenic diet (KD) is a high-fat low-carb intake that has been used to treat epilepsy for years, and more recently has become a popular fad diet.
- KD in mice has been shown to increase social ability and decrease repetitive grooming (Ruskin et al., 2017), potentially making it a useful treatment for Autism Spectrum Disorder (ASD).
- ASD is characterized by stereotypic behaviors (repetitive movements that do not have an apparent function) as well as deficits in social communication and interaction.
- We have previously shown KD can reduce repetitive behaviors, however we do not know by which mechanism it works.
- The basal ganglia is a group of nuclei that includes the striatum and the globus pallidus, which are responsible for motor control and function. The dorsal striatum is one of the primary input areas for the basal ganglia, and fibers from the cerebral cortex, substantia nigra, and thalamus all enter basal ganglia via dorsal striatum.
- Previous research found that basal ganglia circuitry is impared in the development of repetitive behavior (Kim, 2016). We expect the increased dendritic spine density in the dorsal lateral striatum as an explanation of the treatment of stereotypic behavior with KD diet.
- Golgi-Cox staining was used to visualize dendritic branching patterns and dendritic spines in the left and right dorsolateral striatum (see *Figure 1*), which we believe may be a potential mechanism for these effects.



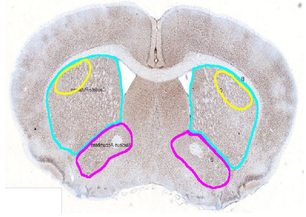


Figure 1. The left image shows the basal ganglia circuitry of rodents. The right image highlights the caudate-putamen (blue), the dorsolateral striatum (yellow), and the nucleus accumbens (pink), which are structures of the striatum.

Methods

Animal Models

- We used FVB/NJ mice (see Figure 2.) that are a popular inbred strain. A spontaneous heritable mutation has resulted in a percentage of mice that show a robust spinning behavior. A greater percentage of females show this spinning behavior, and for this reason our study included females only. Mice were reared in standard hamster cages and categorized as “spinners” and “non-spinners” and fed ad libitum rodent chow or KD.
- Mice were exposed to 60% fat based KD (Bioserv), for 3 weeks during the Fall semester and again for 3 weeks during Spring semester and were sacrificed and put through Golgi-Cox histochemistry.

Golgi-Cox Histochemistry

A silver staining technique that has been used to visualize the neuronal cell body, dendritic spines, and dendritic branching patterns.

Statistical analysis: We used a Repeated Measures ANOVA with Hemisphere as the within-subject factor and DIET and RB status as between-subject factors, and all interactions. Pearson’s correlation analysis was performed for assessing if dendritic spine density in the striatum was predictive of baseline repetitive behavior.

Dendritic Imaging and Analysis

The dendrites were visualized using a Zeiss light microscope at 63x objective magnification. To calculate the dendritic spine density, we measured the length of the dendrites and the number of spines in striatum of each hemisphere by using software Image J. See Figure 4.

Results

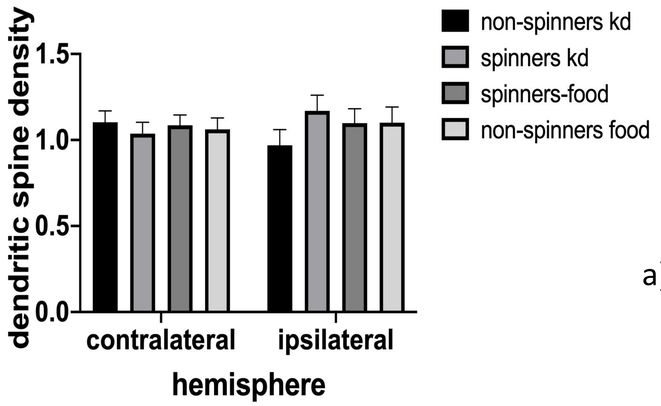


Figure 3. The chart above depicts a RM ANOVA with hemisphere as Within-subject variable and RB and DIET as between-subject variables. There was a non-significant trend for a 3-way interaction between hemisphere, RB status, and DIET ($F(1, 17) = 3.65$), $p = 0.073$). Separating the data by hemisphere and re-running the analysis did not produce any further significant effects.

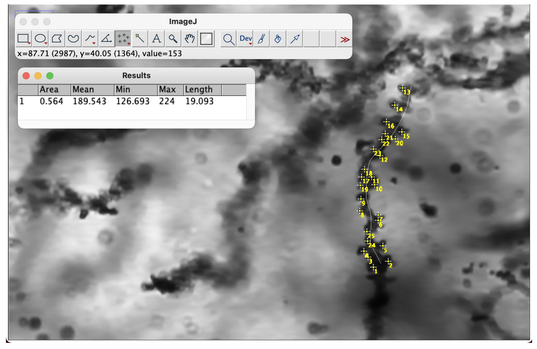
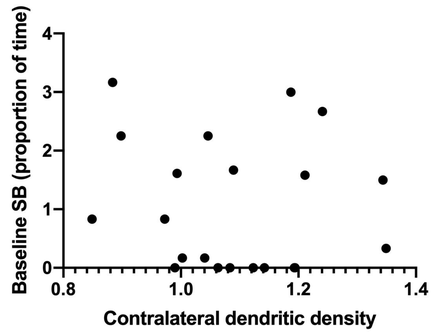


Figure 5. The graphs below show the Pearson correlations for dendritic density in the dorsal lateral striatum of the a) contralateral ($r = -0.101$, $p = .66$) and b) ipsilateral ($r = 10.303$, $p = 0.23$) hemisphere of preferred spinning direction.

a)



b)

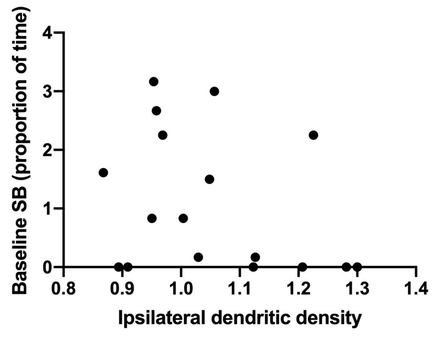


Figure 4. The image to the left depicts the dendritic spines and analyzing of dendritic spine density using Image J. Yellow numbers are counting the number of spines.

Discussion

- Our results show that the correlations between dendritic spine density in the striatum and baseline stereotypic behavior levels were not significant (all p’s > 0.05). This relationship was not significant in either the contralateral or ipsilateral hemisphere.
- We investigated a potential mechanism for the effects of KD on repetitive behavior, however we did not find any evidence that dendritic density in the dorsal lateral striatum predicted baseline repetitive behavior in either hemisphere.
- We would like to repeat this study in males as sex effects in dendritic spine densities have been found previously (Juraska 1988).
- We would also like to assess other basal ganglia structures including the subthalamic nucleus and globus pallidus in order to see if there are differences that could explain the proposed mechanism.

Figure 2. The image to the left depicts an example of a FVB/NJ mouse that was used throughout the study.

Figure 6. The image to the right depicts the dendritic spines within the striatum of a mouse. The image taken on a Zeiss Axiocam ICc3 camera with a 63x objective lens.