Neurobiological Changes Associated with a Ketogenic Diet in a Mouse Model of Autism Spectrum Disorder

Abigail Grzeskowiak, Abigail Schwartzman, Madison Forcier, Gabrielle Skiba & Allison R. Bechard

State University of New York at Geneseo, Departments of Psychology and Neuroscience

Background

- The three key criteria for Autism Spectrum Disorder (ASD) include repetitive behavior, impaired communication skills, and impaired social interaction (Lordan et. al., 2021).
- The Ketogenic Diet (KD) is a high-fat and low carb diet. It is noninvasive and has been proven to lessen many neurological issues in humans and reduce repetitive behavior in the mouse model (Ruskin et al., 2013, Brady et al. 2022).
- Current research has found that KD increases sociability and helps improve other symptoms of ASD (Ruskin et al., 2017).
- Mouse models are useful for studying ASD behaviors and associated neurobiology as mice develop quickly and demonstrate visible symptoms at a young age.
- We hypothesized that 3 weeks of KD would improve social abilities and motor performance in a mouse model of ASD.
- The goal of this study is to investigate the mechanisms by which KD is playing a role in sociability and locomotion.
- Immunohistochemistry (IHC) for dopamine D2 receptor (D2DR) gene expression in the striatum (a nuclei important in movement) tested a role for KD on the dopamine system, and GFAP tested for immune response.

Methods

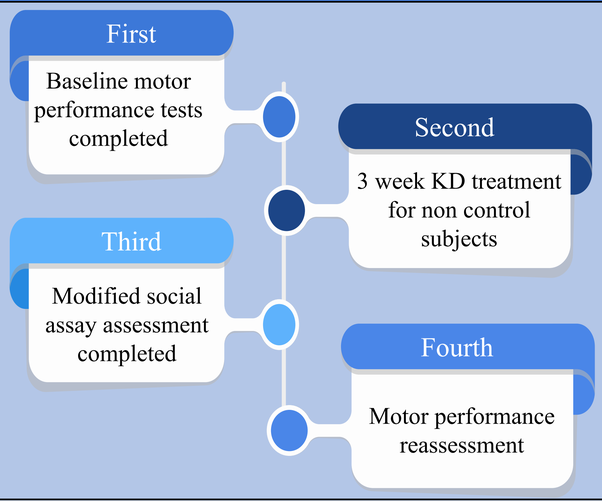
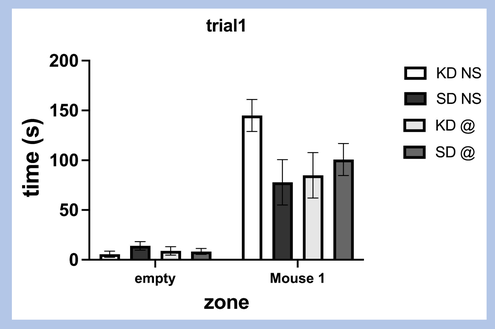


Figure 1: Timeline for social and motor tests

Subjects: Twelve FVB males and two B6 females as stranger mice.
Social test: Each of the subjects completed a social task where two B6 stranger mice (Mouse 1 and New mouse) represented a familiar or novel social buddy. Trial 1: Empty vs Mouse 1; 5-min. Trial 2: Mouse 1 vs New mouse placed; 5 min.
Locomotor test: One hour locomotor tests performed before and after diet intervention. Photobeams are "broken" when subject passes through them. These broken beams show locomotor activity.
Immunohistochemistry (IHC): We used two different approaches to staining the tissue. A slow (indirect) HRP detection method, and a fast (direct) HRP detection method. The fast D2DR stain included a counterstain for nissl bodies (cresyl violet). Imaging was performed using a light microscope at 10x objective and expression quantified using Image J.

Results



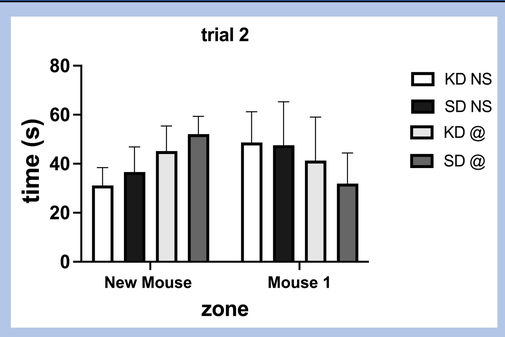


Figure 2: Social data (Trial 1 and Trial 2) for males. All mice preferred mouse 1 over an empty chamber ($F(1,8)=68.7$, $p<0.001$). There was a trend for stereotypy to interact with diet ($F(1,8) == 4.2$, $p=0.068$) showing that non spinners on KD spent more time with mouse 1. There were no differences across groups in the preference for a new mouse over mouse 1.

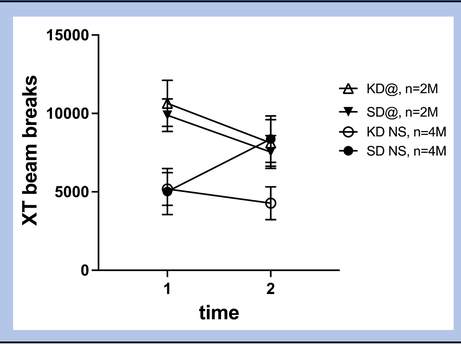


Figure 3: Locomotor activity at baseline (time 1) and after treatment (time 2) was greater in stereotypic mice (@) compared to non-stereotypic mice (NS) ($F(1,8) = 18.6$, $p = 0.003$)

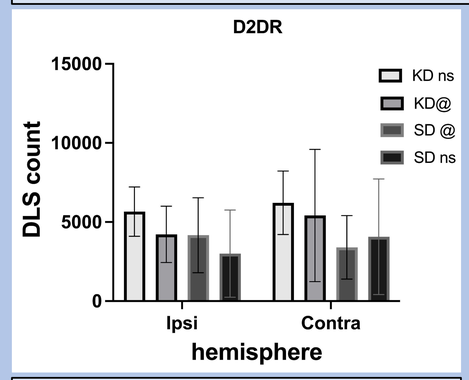


Figure 4: D2DR + cresyl stain data for males $p > 0.05$.

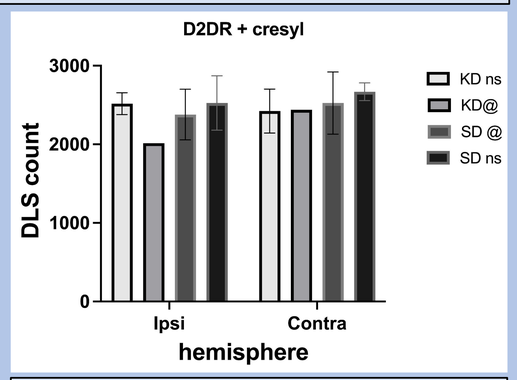


Figure 5: D2DR + cresyl data in dorsolateral striatum. No significance.

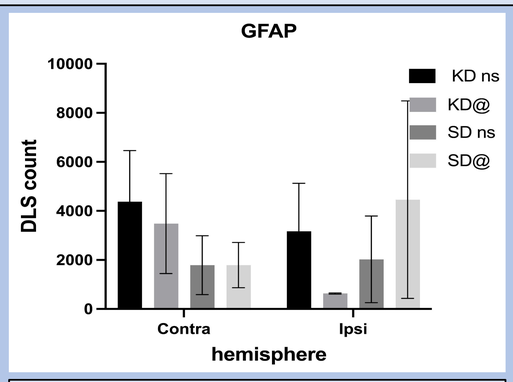


Figure 6: GFAP data in dorsolateral striatum. No significance.

Figure 7: The red arrow points to dopamine (brown dots). The blue arrow points to cell bodies (cresyl violet). Images show staining at 10x magnification.

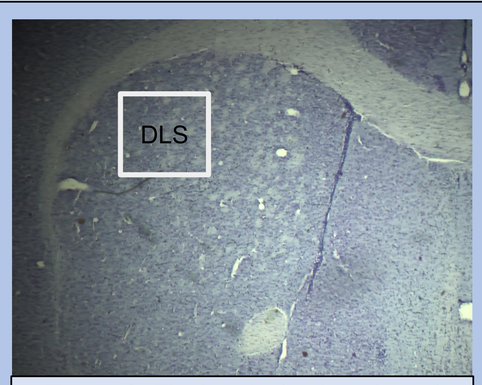


Figure 8: The box is the dorsolateral striatum (DLS) at 2.5x magnification.

Discussion & Future Direction

- We are beginning to investigate the neurobiological changes associated with KD.
- We did not find a strong effect of KD on social behavior. However, a trend showed that non spinners on KD spent more time with mouse 1 (figure 2).
- Locomotor activity was greater in stereotypic mice compared to non-stereotypic mice (figure 3) but unaffected by KD. This does not match previous findings (Brady et al. 2022), however subjects were old isolated males.
- Neurobiological results were not significant in this region of the striatum. We have other regions to assess including the hippocampus and motor cortex.
- Future directions include exploring how intermittent fasting correlates with ketosis and decreasing neuroinflammation markers.
- Another future direction would be looking at the neurobiology of KD effects in females

References

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