CC2D1A Loss-of-Function Mutation Disrupts Cognitive and Social Behaviors, PDE4D Inhibitor Treatment Rescues Learning Deficits

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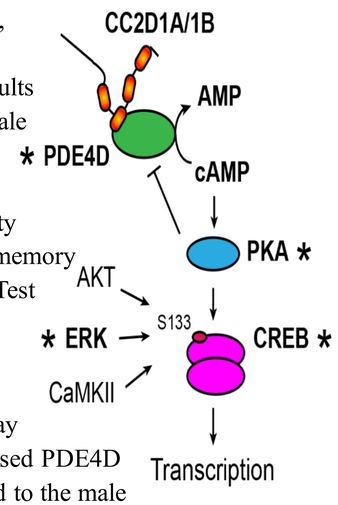
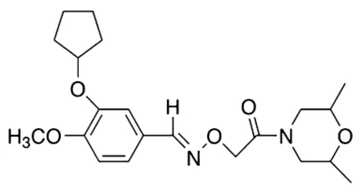
Introduction

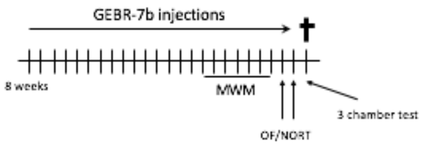
Previous research done by the lab at The George Washington University focused on CC2D1A conditional knockout (cKO) mice, as a model for autism spectrum disorder (ASD). The behavior results showed that the CC2D1A cKO male mice had delayed learning in the Morris Water Maze (MWM), increased anxiety and hyperactivity in the Open Field Test, impaired memory in the Novel Object Recognition Test (NORT), and abnormal social behavior in the 3-Chamber Test. Previous Western Blot results showed a disrupted CREB pathway in the hippocampus, due to increased PDE4D activation in male cKO, compared to the male wildtype (WT) mice. Through binding mechanisms, CC2D1A typically decreases the activation of PDE4D, which is involved in regulating cyclic AMP (cAMP) levels. The increased activation of PDE4D occurs in the hippocampus of the cKO mice, leading to a decrease in cAMP, and further altering the levels of PKA and CREB. It was hypothesized that a PDE4D inhibitor treatment would decrease CREB levels in the hippocampus of CC2D1A cKO mice, as well as rescue their behavioral deficits. The GEBR-7b, a novel PDE4D selective inhibitor. Previous research has shown that at non-emetic doses, GEBR-7b leads to memory improvement in rodents.





Activity (Methods)

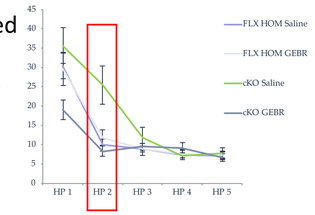
Once the mice were 2 months old, GEBR-7b treatment began for male cKO and WT mice. Saline was used as a control treatment. Five cohorts of mice were treated every day for 14 days before they began behavioral testing. On days 15 through 21 mice were tested on the MWM. During days 22 and 23 the mice were tested on the Open Field Test and NORT. Days 24 and 25 were when the mice were tested on the 3-chamber test. After the mice did the tests each day, they were treated with saline or GEBR-7b.



Outcomes (Results)

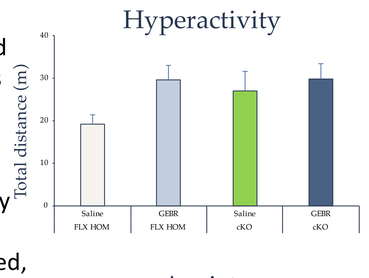
Morris Water Maze

The MWM is administered to test the spatial learning and memory of mice. ASD has been shown to result in learning and memory deficits in humans. By previously testing the performance of the CC2D1A cKO mice on the MWM, it was seen that they had delayed learning when compared to WT mice. Testing the performance of GEBR-7b treated cKO mice will determine if the learning deficit previously seen in male mice will be rescued. The results showed that the learning delay in the CC2D1A cKO male mice was rescued when treated with GEBR-7b. On Hidden Platform Day 2 of the test, is when the learning delay can be seen, with the cKO saline mice taking significantly longer to locate the platform than the WT mice.



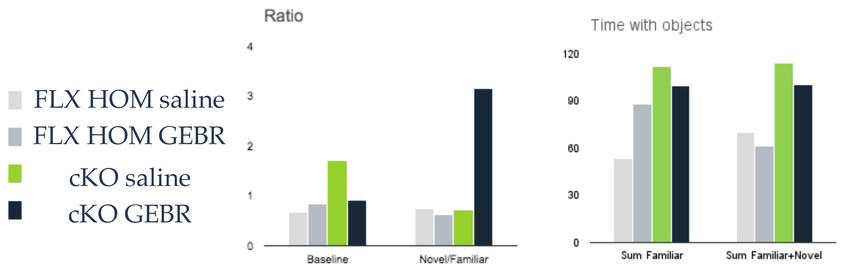
Open Field Test

The Open Field Test is used to test locomotor activity levels and anxiety in mice. Hyperactivity and anxiety can be seen in ASD individuals and previous results showed CC2D1A cKO male mice had Increased hyperactivity and anxiety. Hyperactivity is measured by the distance traveled, with higher distances relating to increased hyperactivity. Anxiety is measured by the time spent in the center, with lower times related to increased anxiety. As expected, hyperactivity was higher in saline cKO mice, compared to saline WT mice. The GEBR-7b cKO mice had similar hyperactivity levels as saline cKO. The GEBR-7b was not able to rescue hyperactivity. As for anxiety, saline cKO showed a decrease in time spent in the center, compared to saline WT. The GEBR-7b treated cKO mice showed equal time in the center as saline cKO, showing GEBR-7b did not rescue the increased anxiety.



Novel Object Recognition Test

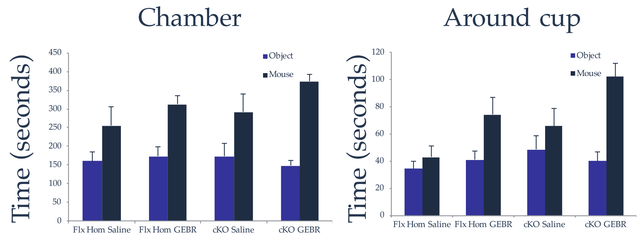
The NORT is used to assess the mouse's recognition memory. Previous results showed CC2D1A cKO mice had impaired memory, meaning they will spend equal time with the familiar and novel objects, compared to WT mice who will spend more time with the novel object. cKO saline mice did not spend equal time with the two familiar objects at baseline, unlike WT mice, yet spent equal time between the familiar and novel objects. GEBR-7b cKO mice showed an increased preference for the novel object.



Outcomes (continued)

3-Chamber Test

The 3-chamber test is administered to test the social interaction of mice. It was expected that cKO mice would have a social interaction deficit, and would prefer to spend more time with the empty cup than with a stranger mouse. No deficit was seen in cKO mice compared to WT. The GEBR-7b cKO showed an increase in time spent with the stranger mouse, compared to WT mice and saline-treated cKO mice.



Reflection (conclusions)

From these first five cohorts of male mice, it was able to be seen that GEBR-7b cKO treated mice were able to rescue their learning deficit seen in cKO mice during The Morris Water Maze. This rescue was not seen in other behavioral tests. These results have provided insight into the genetic involvement of autism and by understanding this, will allow for the improvement in treatment.

During my six months at The George Washington University, I learned amazing research techniques and skills that I would not have otherwise been exposed to. I was able to learn how to work with mice, overall mice husbandry, IP injections, Western Blotting, and behavioral testing of mice. This experience has laid the foundation for me to build more of my skills to further my ability to contribute significantly to the field of autism research.

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