

12-20-2015

Learning Delays in a Mouse Model of Autism Spectrum Disorder

Amanda R. Rendall

Department of Psychology, amanda.rendall@uconn.edu

Recommended Citation

Rendall, Amanda R., "Learning Delays in a Mouse Model of Autism Spectrum Disorder" (2015). *Master's Theses*. 865.
https://opencommons.uconn.edu/gs_theses/865

This work is brought to you for free and open access by the University of Connecticut Graduate School at OpenCommons@UConn. It has been accepted for inclusion in Master's Theses by an authorized administrator of OpenCommons@UConn. For more information, please contact opencommons@uconn.edu.

Learning Delays in a Mouse Model of Autism Spectrum Disorder

Amanda Rose Rendall

B.S., Stony Brook University, 2012

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Masters of Arts

at the

University of Connecticut

2016

APPROVAL PAGE

Master of Arts Thesis

Learning Delays in a Mouse Model of Autism Spectrum Disorder

Presented by

Amanda Rendall, B.S.

Major Advisor _____

Dr. R. Holly Fitch

Associate Advisor _____

Dr. Inge-Marie Eigsti

Associate Advisor _____

Dr. John Salamone

Associate Advisor _____

Dr. Etan Markus

Associate Advisor _____

Dr. Heather Read

University of Connecticut

2016

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my graduate advisor, Dr. R. Holly Fitch for her endless support and encouragement. Also, for her valuable and constructive feedback on this study as well as current and past studies. Her guidance has been crucial to my progress in graduate school.

Next, I would like to express my appreciation to all of my committee members, Dr. Read, Dr. Eigsti, Dr. Salamone and Dr. Markus for their time and feedback on this current project.

I would also like to extend my thanks to my lab members, past and present. Michelle, Nhu, Amanda and Hector, thank you so much for your continuous guidance and support. I would like to specifically thank Nhu for her mentorship during my first two years of graduate school.

Finally, I would like to thank my family, Bill, Dina, Jessica, Samantha, Billy and Ryan for their endless love and support while pursuing my PhD.

TABLE OF CONTENTS

Introduction	pg. 5-10
Methods	pg. 10-13
Results.	pg. 13-15
Discussion	pg. 15-16
Figure 1	pg .17
Figure 2	pg. 18
Figure 3	pg. 19
Figure 4	pg. 20
Figure 5	pg. 21
Figure 6	pg. 22
Figure 7	pg. 23
Figure 8	pg. 24
Figure 9	pg. 25
References	pg. 26- 29

INTRODUCTION

Autism Spectrum Disorder (ASD) is a set of neurodevelopmental disorders characterized by a complex behavioral phenotype, encompassing deficits in both social and cognitive domains. Accepted core symptoms are heterogeneous, and range from atypical social interactions and language impairments to repetitive behaviors. Accordingly, individual cases range substantially in severity and presentation of symptoms. Currently, the estimated prevalence in the United States identifies 1 in 68 children as having ASD, and confirms that ASD is consistently more prevalent in boys than girls (1 in 42 boys versus 1 in 189 girls) (Baio, 2010; Elsabbagh et al., 2012). To date, causal mechanisms underlying ASD remain poorly understood, but likely include a complex combination of polygenic and environmental risk factors (Moreno-De-Luca, 2013).

Ongoing ASD research has been focused on investigating the genetic and neurobiological mechanisms of ASD, based on the notion that characterization of the varied neurogenetic features of ASD could provide insight to the diverse behavioral symptoms and variability observed. The genetic contribution in ASD appears to be strong; for example, monozygotic twin studies estimate the concordance rates are as high as 70% - 90% (Bailey et al., 1995; Steffenburg et al., 1989; Rosenberg et al., 2009). Furthermore, the recurrence estimates of infants with at least 1 older sibling with ASD is 18.7% (Orzonoff et al., 2011). Additionally, there are documented familial patterns of inheritance for qualitatively similar phenotypes (albeit with less severe behavioral and cognitive deficits, but falling under the broader autism phenotype) in first-degree relatives of identified probands, further suggesting heritability of ASD (Bolton et al., 1994; Bishop et al., 2004). However, the relative proportion of ASD that can be accounted for by either rare or common genetic variation remains to be determined and no single gene has been identified as a major cause. In fact, over 1000 risk genes have been reported,

indicating a very complex genetic etiology (Rubeis & Buxbaum, 2015). Additionally, no one of these known genetic contributors accounts for more than 1-2% of the phenotypic variance seen in ASD, despite having strong inheritance patterns (Abrahams & Geschwind, 2008). Notably, however, most of the genes identified have been found to play a critical role in neurodevelopment, and in fact converge onto three functional pathways. These include: (1) synaptic function; (2) Wnt signaling during development; and (3) chromatin remodeling (Krumm, 2014). Specifically, Wnt signaling is involved in embryonic development and plays a critical role in cell fate specification, cell proliferation and cell migration; chromatin remodeling is also an important determinant in cell fate and function.

One of these autism susceptibility candidate genes -- contactin-associated-like-protein 2 (*CNTNAP2*) -- was first linked to Specific Language Impairment, and more recently has been linked to ASD as well (Alarcón et al., 2008; Arking et al., 2008). Specifically, in clinically language impaired populations; *CNTNAP2* variants have been associated with difficulties with non-word repetition -- a measure of working memory that critically underlies language and social cognition (Vernes et al., 2008; Peter et al., 2011). *CNTNAP2* is located on chromosome 7, and is responsible for encoding a cell adhesion protein regulating synaptic signal transmission (Alarcón et al., 2008). To better understand the behavioral and biological underlying mechanisms of ASD, a transgenic mouse model was created with a genetic knockout (KO) of the rodent homolog *Cntnap2* (Poliak et al., 2003). Initial behavioral studies of this mouse revealed poor social interactions, perseveration, and reduced pup vocalizations -- all strongly resembling the human symptoms making this a strong fundamental model of ASD (Peñagarikano et al., 2011; Penagarikano & Geschwind 2012). *CNTNAP2*'s role in neurodevelopment has been further studied using this mouse model, revealing that *Cntnap2* KO mice show abnormalities in

myelin formation -- consistent with a hypo-connectivity model of ASD (Poliak et al., 2003). Furthermore, these mice also exhibit abnormal cortical neural synchrony (i.e., enhanced asynchrony), fewer inter-neurons (which are mostly inhibitory), and atypical neuronal migration (Peñagarikano et al., 2011). All of these cellular anomalies can be linked to current biological theories behind the casual mechanisms of ASD. Finally, more recent studies from our lab have revealed that the KO mice exhibit unexpected enhancements in frequency processing, despite impairments on more complex silent gap detection tasks (Truong et al., in press). The latter results have been linked with anomalies at the level of the thalamus, and also could reflect atypical patterns of cortical connectivity as documented in other labs. These sensory findings have been interpreted in light of the atypical auditory enhancements (e.g., higher incidence of perfect pitch) seen among ASD individuals, as well as documented language impairments that concurrently and paradoxically occur in the same subjects (Truong et al., in press).

Two major biological theories associated with the etiology of ASD (and also related to *CNTNAP2*'s function) include: (1) defective synaptic function, and (2) abnormal brain connectivity (Zoghbi, 2003; Geschwind & Levitt, 2007; Zoghbi & Bear, 2012). ASD is in fact sometimes referred to as a “synaptopathy,” due to the numerous autism candidate susceptibility genes that are associated with synaptic structure, function and regulation. Therefore, disruption of synapses and signal transmission is thought to be a major cause of ASD. In addition to these findings, evidence has shown that connections across cortical regions are often diminished in ASD. This developmental “disconnection” may account for clinical heterogeneity, as well as the frequent late emergence during development (around 2 yrs) seen in ASD (Belmonte et al., 2004). Functional whole-brain connectivity analyses have also revealed that individuals with ASD show subcortical areas that exhibit hyperconnectivity, even though corticocortical areas in the same

subjects are predominantly hypoconnected (Di Martino et al., 2014). It has also been reported that individuals with ASD have difficulty with multisensory integration. These impairments in the integration of sensory information could in turn reflect diminished cross-modal white matter connectivity, as reported in some DTI/MRI studies (Maximo et al., 2013; Travers et al., 2012). The purported hypoconnectivity and multisensory integration issue may be further disrupting higher-order cognitive abilities, such as learning and social communication.

It has been also been suggested that working memory may be specifically disrupted in ASD, also in association with a connectivity deficiency. Indeed, observed impairments in working memory in individuals with ASD seem to be caused by a global disconnection rather than a focused deficit in the prefrontal cortex, as revealed in neuroimaging research (Barendse et al., 2013). Although this topic of working memory deficits in ASD has been understudied, recent work indicates that this deficit in the temporary storage of information may be playing a central role in complex cognitive processes needed to support social interactions and cognition. This is not surprising, since executive function deficits are commonly seen in ASD (although they are not considered a core deficit; Geurts et al., 2009; Ozonoff, Pennington & Rogers, 1991; Hill, 2004; O'Hearn et al., 2008; Robinson et al., 2009; Dawson et al., 2002). These deficits are evident throughout adolescence, and also are present in adulthood. Working memory problems are even more pronounced when the cognitive load of the task is high. Therefore, the type of working memory task conducted is important, and cross-study variation in this regard may explain why some studies do not report working memory deficits in adolescents with ASD, even though many others do (Williams et al., 2005; Landa & Goldberg, 2005; McGonigle-Chalmers, 2008). It is also important to note that most of these working memory impairments are found in the spatial domain but have also been observed in complex verbal working memory tasks

(Schuh & Eigsti, 2012; Steele et al., 2007; Luna et al., 2007; Williams, Goldstein, & Minshew, 2005; Willims et al., 2005).

With regards specifically to the spatial domain, evidence has shown impairments of spatial navigation in individuals with ASD (Lind et al., 2013). Spatial navigation refers to the ability to maintain a sense of direction and location while moving around the environment, and can be supported by external representations that are initially translated into sensory experiences and then further encoded (Wolber & Hegarty, 2010). It is thought that impairments in spatial navigation among ASD individuals could stem in part from anomalies in relevant sensory processing and integration. This follows from the fact that effective navigation relies on sufficient sensory input and integration to be able to form and remember a cognitive map. Thus anomalies in sensory input may limit opportunities to practice generating detailed cognitive maps (Lind et al., 2013). As such, the difficulty of sensory integration may be influencing these impairments in spatial navigation overall.

The current study was designed to further assess the intermediate behavioral phenotype of the *Cntnap2* KO mouse model, with a focus on putative anomalies in spatial learning and memory. Previous studies found similar learning curves on the Morris Water maze task for *Cntnap2* KO versus WT controls suggesting a *lack* of spatial learning and memory impairments (Peñagarikano et al., 2011). However, when these animals were presented with a classic Morris Water Maze reversal task, *Cntnap2* KOs *did* show significant impairments in learning the new platform location (as indicated by longer latencies to find the platform, as well as performance on the probe task). Thus as seen in the clinical population, difficulty of task may play a role in these inconsistent findings. Our goal was to further assess *Cntnap2* KOs spatial memory ability utilizing a 4/8 arm radial water maze task. This task allows for the analysis of both reference and working memory

abilities, while also introducing a higher cognitive load on the subjects as compared to the Morris Water maze task. Finally, this task generates a much longer learning curve, allowing us to adequately evaluate acquisition and retention periods of learning this task.

MATERIALS AND METHODS

Subjects

10 *Cntnap2* KO mice (B6.129(Cg)-*Cntnap2*^{tm1^{Pele}}/J; stock number 017482) and 11 wild type (WT) controls (C57BL/6J; stock number 000664) were obtained from The Jackson Laboratory (Bar Harbor, ME). Subjects were delivered to the University of Connecticut, Department of Psychology arriving at 7 weeks of age. Upon arrival, all subjects were single housed in standard plexiglass laboratory cages (12:12 light/dark cycle) with food and water available *ad lib*. Only male subjects were used for testing, based on evidence of a higher incidence of ASD and developmental language impairments in males as compared to females (Baio, 2012). Maze testing began when the animals were around 24 weeks of age, and occurred during the subjects' light cycle. All procedures were performed blind to subject genotype and were conducted in compliance with the National Institutes of Health and approved by the University of Connecticut's Institutional Animal Care and Use Committee (IACUC).

Water maze assessment – Visible platform and 4/8 radial water maze

Subjects were initially tested on a visible platform control task (also known as “water escape”) prior to the 4/8 radial water maze task, to evaluate if there were any underlying impairments that might confound further maze testing (i.e., deficits in motivation, swimming, or visual acuity). Subjects were placed in the far end of an oval tub (103 cm x 55.5 cm) filled with room temperature water, and were given 45 seconds to swim to a visible escape platform (8.5 cm

in diameter; 1 cm above water surface) located at the opposite end of the tub. Latencies to the visual platform were recorded for assessment. None of the subjects displayed any impairments, and there were no observed differences between genotypes on this task. We therefore proceeded to testing on the water version of the 4/8 radial arm maze (adapted from Hyde, Hoplight & Denenberg, 1998).

The 4/8 radial arm water maze assesses spatial reference and working memory abilities simultaneously, using a standard 8 arm radial maze with 4 arms baited (i.e., containing submerged goal platform), and 4 arms open but never baited with a platform (Fig. 1). Configuration of goal arms were counterbalanced between subjects but remained fixed for each subject across all test sessions. Additionally, high contrast extra maze cues were present in the room, and the locations of these remained static for the entire experiment.

The day prior to testing (Day 1), subjects were given a training session where all arms that would not contain a platform were blocked, forcing the animals to only enter arms containing a platform. Subjects were placed in the middle of the maze and were given 120 seconds to locate the platform. Every subject completed 4 training trials. Each time they found the platform, the recently located platform was removed, and the entrance to that arm was blocked. This ensured that the subject could no longer enter this arm for the remainder of the training session. If the subject failed to find a platform in this time-period, they were guided to the nearest available goal. Once on the platform, subjects remained on the platform for 20 seconds and then were removed from the maze to their home cage (30 second inter-trial interval; ITI).

Testing began on Day 2 and continued for an additional 14 consecutive days. The testing session followed training procedures, except instead of blocking the goal arm of the most

recently located platform, the platform was simply removed during the 30 second ITI. This arm remained open and unbaited for the remainder of the test session. Test sessions were recorded using a Sony camera, integrated with the SMART video-tracking program (Panlab, Barcelona, Spain). An arm entry was counted for a subject when all four paws entered an arm. Three types of errors were quantified for analysis: 1) Working memory errors (the number of initial and repeat entries into arms from which a platform had been removed during a testing session on a given day); 2) Initial reference memory errors (the total number of first entries into arms that never contained a goal platform) and; 3) Repeat reference memory incorrect errors (the total number of repeat entries (following the initial entry) into arms that never contained escape platforms). Total errors per test session in each category were tabulated, averaged within Genotype, and used for analysis across days of testing.

Finally, in order to determine whether subjects utilized a spatial or chaining (swimming to successive adjacent arms) strategy to solve the water maze, angles of arm choices were derived and analyzed. Specifically, video tracking data obtained from the SMART system was reviewed, and turn angle entry was calculated to determine the average turn angle utilized across sessions. Lower turn-angle averages (closer to 45°) suggest that subjects preferred adjacent arm choices to solve the maze. Alternatively, higher averages (around 90° and greater) suggest a preference for more spatial strategies to solve the maze.

Statistical Analysis

An univariate ANOVA was conducted to compare latency to platform for the water escape task as a function of Genotype. Average total, working memory, total reference memory, initial reference memory, repeated reference memory errors and average turn angle on the 4/8 radial arm maze were independently assessed using a 2 x 14 repeated measures ANOVA, with

Genotype (2 levels: WT and *Cntnap2* KO) as the between measure, and Days (14 levels) as the within measure. Some analyses also were performed as a function of test periods, as defined by Acquisition (days 1-7) and Retention (days 8-14) portions of the learning curve (as observed).

RESULTS

Water Escape

Prior to spatial water maze testing, all subjects completed a water escape control task to assure there were no underlying impairments that could confound the results of the water maze performance (anomalous visual acuity, swimming ability or motivation). An univariate ANOVA on latencies to platform found no main effect of Genotype [$F(1,19)=.915$, N.S.]. Thus no subjects showed any impairment on this task, and all 10 *Cntnap2* KO and 11 WT mice advanced to the testing sessions (Fig. 2).

Total errors

The 4/8 radial arm water maze was used to simultaneously measure spatial working and reference memory performance. Analysis of the average number of total errors (working memory, initial reference, and repeated reference memory errors) revealed a significant difference between WT and *Cntnap2* KO groups [$F(1,19)=4.791$, $p<0.05$] via repeated measures ANOVA, with *Cntnap2* KOs making significantly more errors than WT. A main effect of Day [$F(13,247)=4.036$, $p<.001$] also was observed, confirming that both groups reduced errors across days (i.e., showed learning). Within test session analysis of total errors across days revealed a Day \times Genotype interaction [$F(13,247)=1.886$, $p<0.05$], with *Cntnap2* KOs making significantly more errors during the Acquisition period of testing (days 1-7 of testing) [$F(1,19)=5.332$, $p<.05$], but performing comparably to WT during the Retention period (days 8 – 14 of testing) [$F(1,19)=1.846$, N.S.] (Fig. 3).

Reference Memory

We examined the group differences for four different performance error types including working memory, initial reference memory, repeated reference memory and total repeated reference memories (METHODS, Fig. 1). A repeated measures ANOVA on total reference memory errors (across Days) revealed that *Cntnap2* KOs did in fact make significantly more errors than WT subjects [$F(1,19) = 4.514, p < 0.05$]. As seen with total errors, there was also a Day x Genotype interaction [$F(1,19) = 4.514, p < 0.05$], wherein the *Cntnap2* KOs made significantly more errors during the Acquisition period [$F(1,19) = 3.305, p < 0.01$], but performed comparably to the WTs during the Retention period [$F(1,19) = 2.902, N.S$] (Fig. 4). Further analysis of reference memory error type also revealed that *Cntnap2* KOs made significantly more initial reference memory errors [$F(1,19) = 5.522, p < 0.05$] (Fig. 5). *Cntnap2* KOs also trended to make more repeated reference memory errors across the 14 days of testing, but there was no significant main effect of Genotype [$F(1,19) = 3.040, N.S$] (Fig. 6).

Working Memory

A repeated measures ANOVA on working memory errors revealed that *Cntnap2* KOs made significantly more working memory errors, and specifically so during the Acquisition period [$F(1,19) = 4.560, p < 0.05$]. However, they performed comparably to WTs during the Retention period of the task [$F(1,19) = .257, N.S$] (Fig. 7). There was no main effect of Day [$F(13,247) = 1.277, N.S$].

Latency

Total latency across the 4 trials was computed, and a repeated measures ANOVA was performed to analyze Genotype and Day differences (as above). This revealed no significant difference of total latency to the platform during testing sessions, when comparing *Cntnap2* KOs

and WT_s [$F(1,19) = 2.842$, N.S.]. There was, however, a main effect of Day, indicating both groups were completing the task more quickly as testing progressed (Fig. 8).

Average Turn angle

Average turn angle per testing session was recorded and analyzed to assess possible strategies used to complete the task. A repeated measures ANOVA revealed no main effect of Genotype [$F(1,19) = .343$, N.S.], but did reveal a significant Day effect [$F(13,246) = 2.856$, $p < .01$]. Overall, subjects used shorter turn angles during the beginning of testing, but as testing continued, subjects used wider turn angles (Fig. 9).

DISCUSSION

Cntnap2 KO and wild-type mice were tested on a 4/8 radial arm water maze for 14 consecutive days. Results showed that *Cntnap2* KO mice exhibited significant deficits in spatial working and reference memory, specifically during the acquisition period of the task. However, during the retention period (i.e., after an asymptote in errors), *Cntnap2* KO mice performed comparably to wild-type mice. These findings indicate that these animals are able to learn, but have delayed learning -- resulting in a different learning curve. It is important to note that differences between *Cntnap2* KOs and WT_s are particularly robust on days 5 through 8. This seems to be due to that fact that *Cntnap2* KOs do not show as rapid learning of the platform location which is indicated by the number of reference memory errors they make. However, these subjects do display some improvement, but as they are learning the platform locations they are making more working memory errors during this time period. This would suggest that once the *Cntnap2* KOs begin to learn the platform location they are perseverating on these locations. Furthermore, *Cntnap2* KO mice and WT mice displayed similar turn angles throughout testing, suggesting they used similar strategies to complete the maze. That is, as testing proceeded, wider

turn angles were noted, indicating subjects used more of a spatial strategy and less chaining to find a platform. These findings were likely due to the difficulty of the task used in this study, based on prior findings that failed to show a *Cntnap2* deficit when compared to WT on a simple MWM learning task (Peñagarikano et al., 2011).

These findings are consistent with the notion that there are deficits in executive learning as has also been demonstrated in ASD. Moreover, our findings may further explain the dyad of core symptoms, given the central role of executive processing in both higher and lower levels of processing. That is, the global connectivity deficiency seen in ASD could contribute to the spatial working memory and learning impairments observed. This pattern has been seen in neuroimaging studies with high functioning ASD participants (Di Martino et al., 2014). This disconnection may result in problems with sensory integration and therefore disrupt learning, which could explain why *Cntnap2* KOs require more experience to effectively learn the maze as compared to their WT controls. This is also consistent with the clinical ASD literature.

The impairments observed in the current study also may be explained by the abnormal myelin formation seen in this transgenic mouse model. This would be consistent with the hypo-connectivity theory of the neurobiology of ASD, as well as consistent with the spatial learning deficits seen in ASD. Future studies are planned to look into neuroanatomical differences in white matter tracks spanning cortical regions, and correlate these measures to the cognitive differences seen here (using anatomy from these same subjects). Overall, these behavioral findings suggest that *CNTNAP2* definitely plays an underlying role in the development of neural systems important to learning and cross-modal integration, and disruption of this function could be associated with delayed learning observed in individuals with ASD.

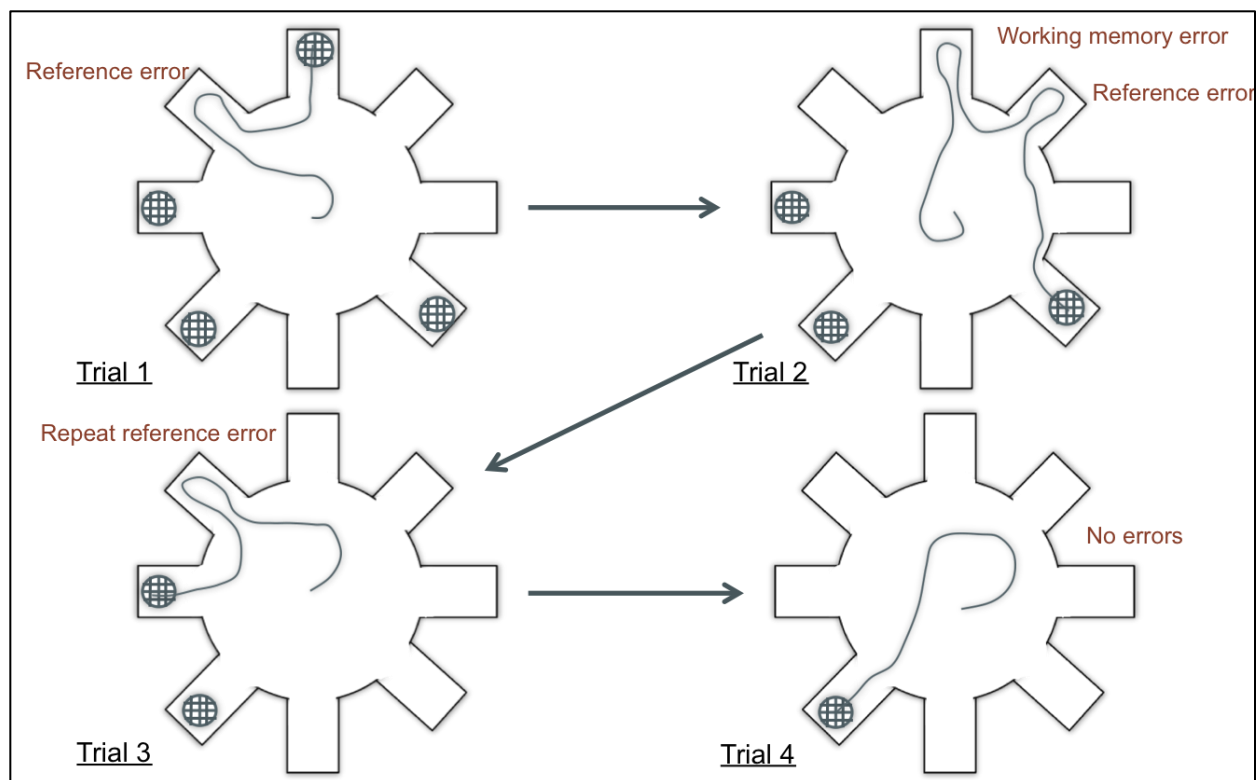


Figure 1: A schematic of the 4/8 radial arm maze and the categorization of memory errors used to evaluate all subjects.

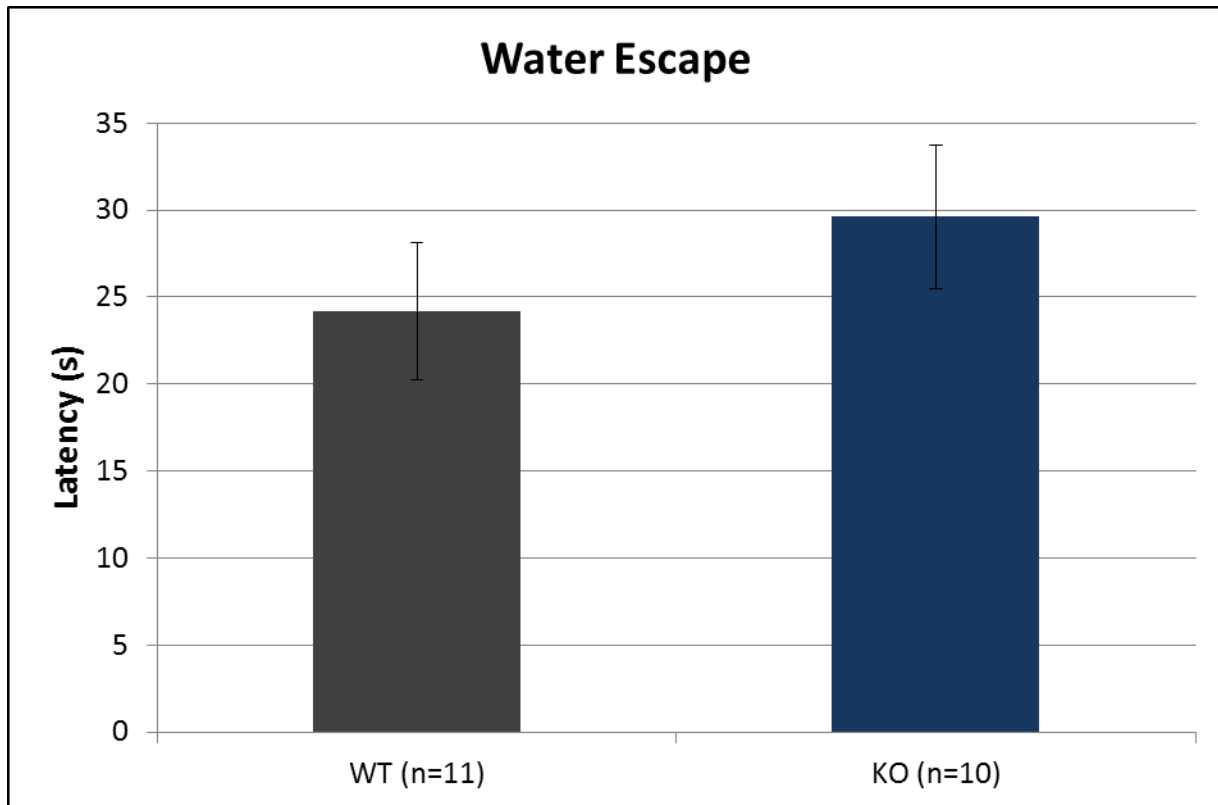


Figure 2: Latency to platform in the water escape task.

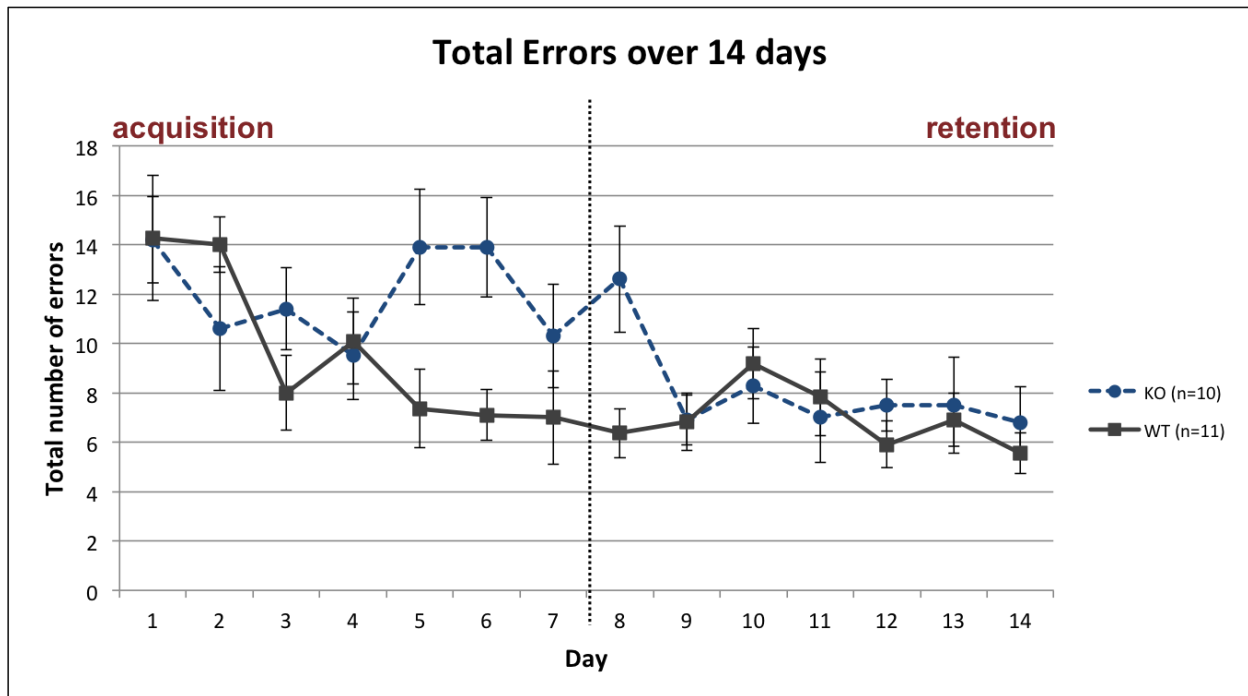


Figure 3: Total number of errors in the 4/8 arm radial water maze task over 14 days of testing.

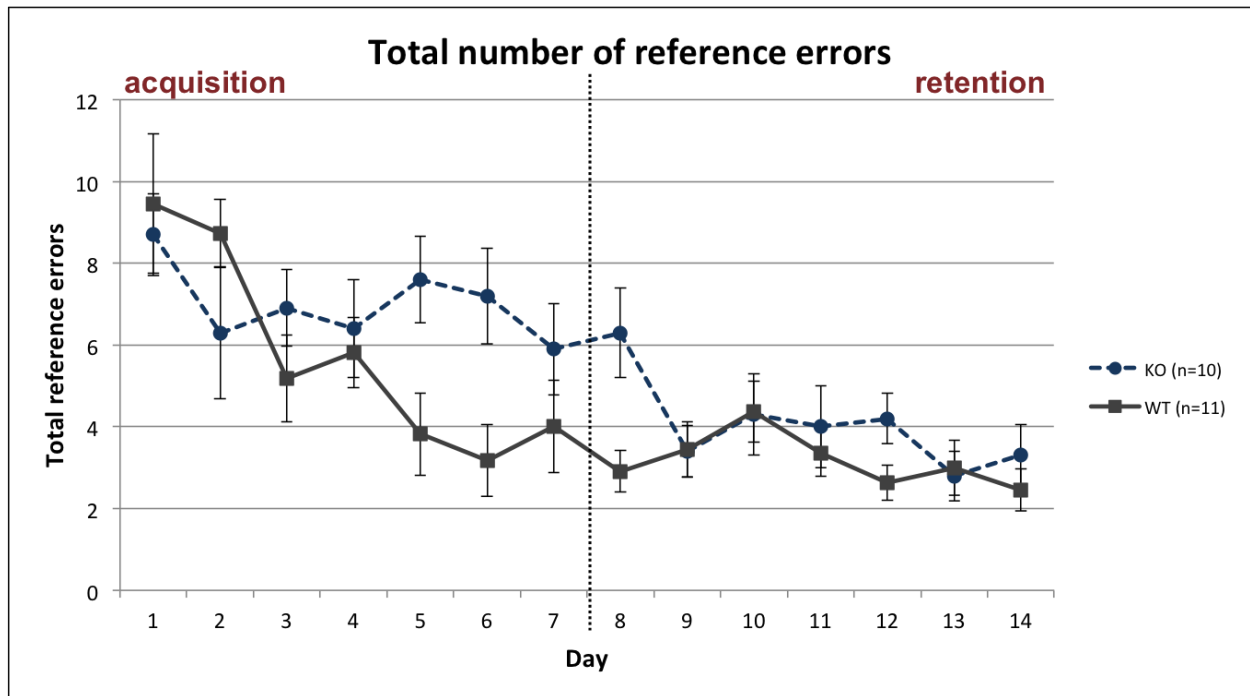


Figure 4: Total number of reference memory errors in the 4/8 arm radial water maze task over 14 days of testing

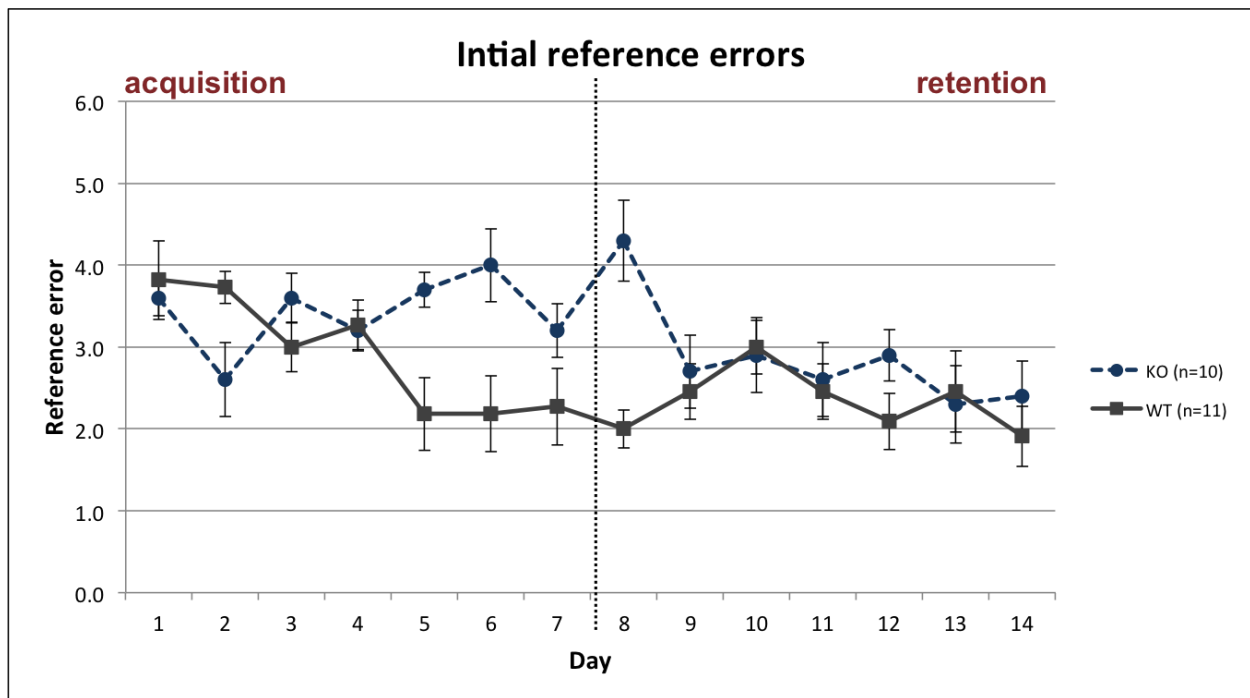


Figure 5: Total number of initial reference memory errors in the 4/8 arm radial water maze task over 14 days of testing.

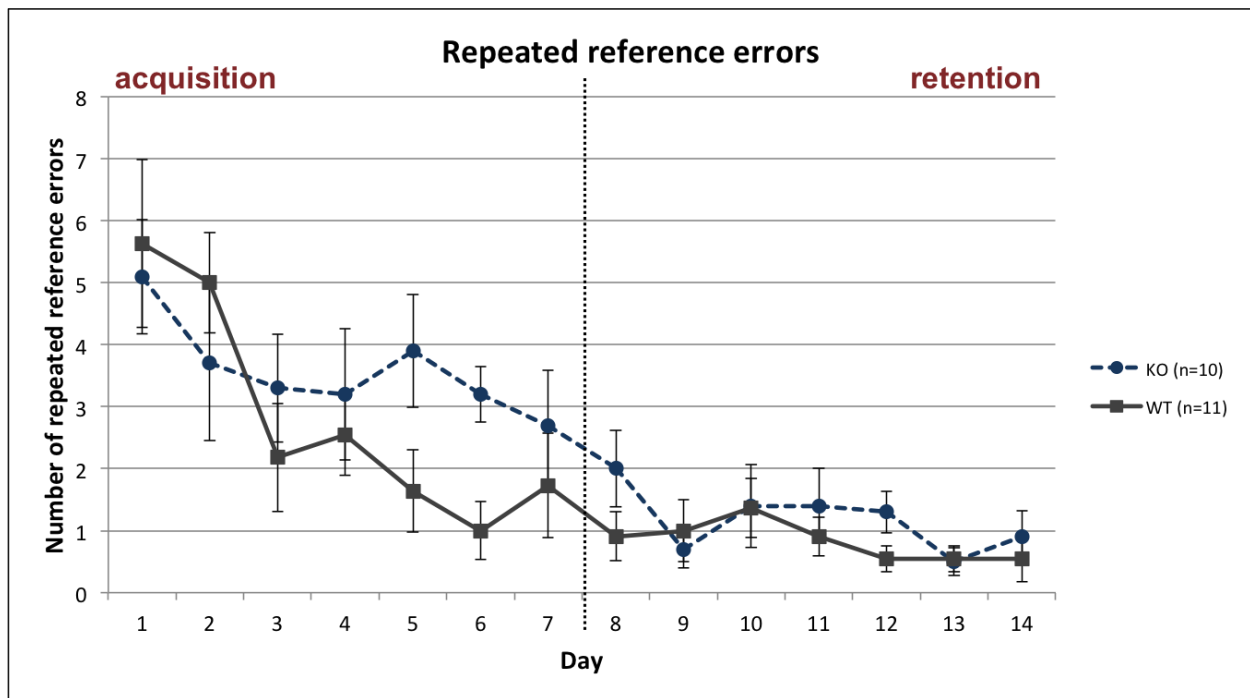


Figure 6: Total number of repeated reference memory errors in the 4/8 arm radial water maze task over 14 days of testing.

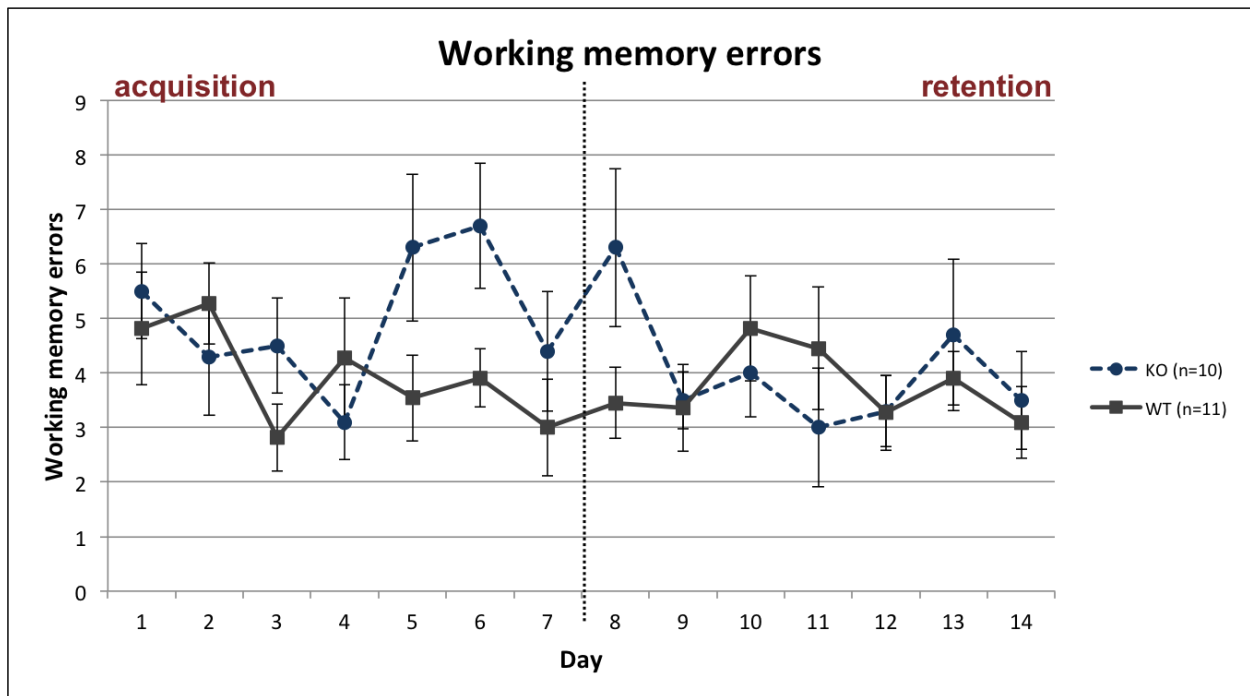


Figure 7: Total number of working memory errors in the 4/8 arm radial water maze task over 14 days of testing

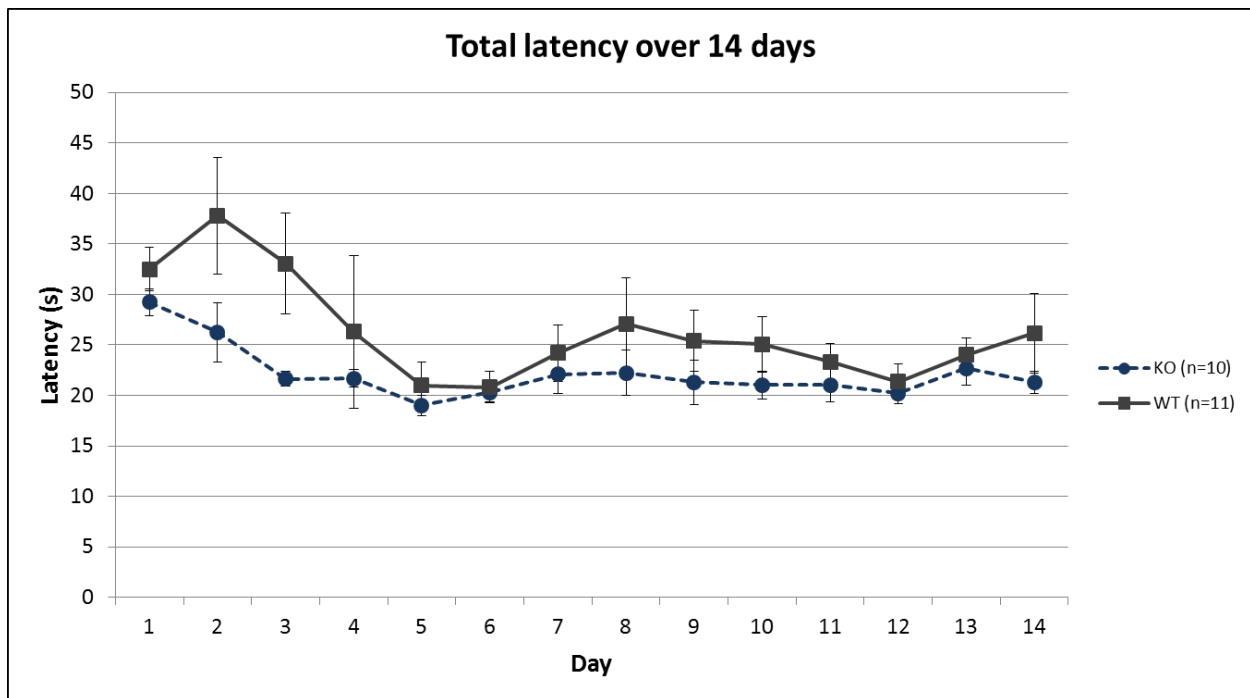


Figure 8: Total latency over testing sessions in the 4/8 arm radial water maze task over 14 days of testing.

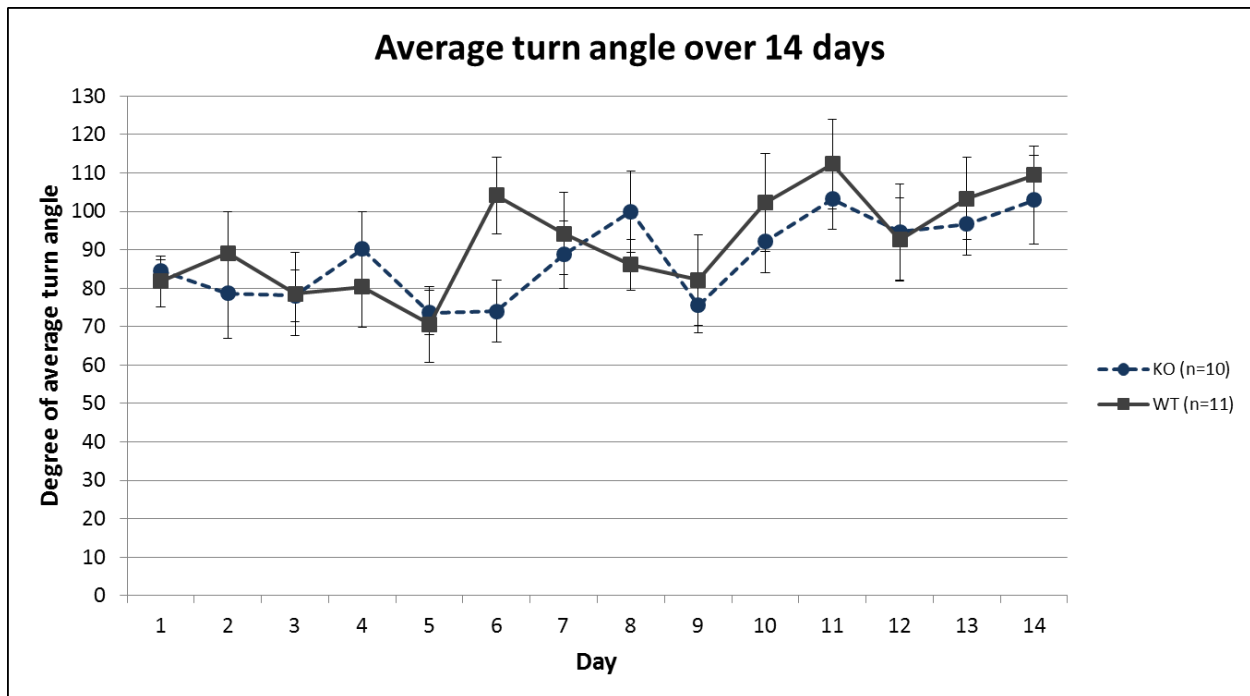


Figure 9: Average turn angle over testing sessions in the 4/8 arm radial water maze task over 14 days of testing.

References

- Abrahams, Brett S., and Daniel H. Geschwind. "Advances in autism genetics: on the threshold of a new neurobiology." *Nature Reviews Genetics* 9.5 (2008): 341-355.
- Alarcón M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, Sebat J, Wigler M, Martin CL, Ledbetter DH, Nelson SF, Cantor RM, Geschwind DH. 2008. Linkage, association, and gene- expression analyses identify CNTNAP2 as an autism-susceptibility gene. *Am J Hum Genet.*, 82, 150-9.
- Arking DE, Cutler DJ, Brune CW, Teslovich TM, West K, Ikeda M, Rea A, Guy M, Lin S, Cook EH, Chakravarti A. 2008. A common genetic variant in the neurexin superfamily member CNTNAP2 increases familial risk of autism. *Am J Hum Genet.*, 82, 160-4.
- Bailey A, Le Couteur A, Gottesman I et al (1995) Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med* 25(1):63–77
- Baio, J. (2010). Prevalence of Autism Spectrum Disorders: Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008. Morbidity and Mortality Weekly Report. Surveillance Summaries. Volume 61, Number 3. *Centers for Disease Control and Prevention*.
- Belmonte, M. K., Allen, G., Beckel-Mitchener, A., Boulanger, L. M., Carper, R. A., & Webb, S. J. (2004). Autism and abnormal development of brain connectivity. *The Journal of Neuroscience*, 24(42), 9228-9231.
- Bishop DV, Maybery M, Maley A, Wong D, Hill W, Hallmayer J (2004) Using self-report to identify the broad phenotype in parents of children with autistic spectrum disorders: a study using the autism-spectrum quotient. *J Child Psychol Psychiatry* 45(8):1431–1436
- Bolton, P., Macdonald, H., Pickles, A., Rios, P., Goode, S., Crowson, M., ... & Rutter, M. (1994). A case-control family history study of autism. *Journal of Child Psychology and Psychiatry*, 35(5), 877-900.
- Barendse, E. M., Hendriks, M. P., Jansen, J. F., Backes, W. H., Hofman, P. A., Thoonen, G., ... & Aldenkamp, A. P. (2013). Working memory deficits in high-functioning adolescents with autism spectrum disorders: neuropsychological and neuroimaging correlates. *Journal of neurodevelopmental disorders*, 5(1), 14.
- Dawson, G., Webb, S., Schellenberg, G. D., Dager, S., Friedman, S., Aylward, E., & Richards, T. (2002). Defining the broader phenotype of autism: Genetic, brain, and behavioral perspectives. *Development and psychopathology*, 14(03), 581-611.
- Di Martino, A., Yan, C. G., Li, Q., Denio, E., Castellanos, F. X., Alaerts, K., ... & Milham, M. P. (2014). The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. *Molecular psychiatry*, 19(6), 659-667.

- Elsabbagh, M., Divan, G., Koh, Y. J., Kim, Y. S., Kauchali, S., Marcín, C., ... & Fombonne, E. (2012). Global prevalence of autism and other pervasive developmental disorders. *Autism Research*, 5(3), 160-179.
- Geschwind, D.H. & Levitt, P. 2007. Autism spectrum disorders: developmental disconnection syndromes. *Current Opinion in Neurobiology*, 17, 103 - 111.
- Geurts, H. M., Verté, S., Oosterlaan, J., Roeyers, H., & Sergeant, J. A. (2004). How specific are executive functioning deficits in attention deficit hyperactivity disorder and autism?. *Journal of child psychology and psychiatry*, 45(4), 836-854.
- Hill, E. L. (2004). Executive dysfunction in autism. *Trends in cognitive sciences*, 8(1), 26-32.
- Hyde, L. A., Hoplight, B. J., & Denenberg, V. H. (1998). Water version of the radial-arm maze: learning in three inbred strains of mice. *Brain research*, 785(2), 236-244.
- Jorde LB, Hasstedt SJ, Ritvo ER et al (1991) Complex segregation analysis of autism. *Am J Hum Genet* 49(5):932–938
- Krumm, Niklas, et al. A de novo convergence of autism genetics and molecular neuroscience. *Trends in neurosciences* 37.2 (2014): 95-105.
- Lind, S. E., Williams, D. M., Raber, J., Peel, A., & Bowler, D. M. (2013). Spatial navigation impairments among intellectually high-functioning adults with autism spectrum disorder: Exploring relations with theory of mind, episodic memory, and episodic future thinking. *Journal of abnormal psychology*, 122(4), 1189.
- Luna, B., Doll, S. K., Hegedus, S. J., Minshew, N. J., & Sweeney, J. A. (2007). Maturation of executive function in autism. *Biological psychiatry*, 61(4), 474-481.
- Maximo JO, Keown CL, Nair A, Müller RA. 2013. Approaches to local connectivity in autism using resting state functional connectivity MRI. *Front Hum Neurosci*. 7, 605.
- Moreno-De-Luca, A., Myers, S. M., Challman, T. D., Moreno-De-Luca, D., Evans, D. W., & Ledbetter, D. H. (2013). Developmental brain dysfunction: revival and expansion of old concepts based on new genetic evidence. *The Lancet Neurology*, 12(4), 406-414.
- O'Hearn, K., Asato, M., Ordaz, S., & Luna, B. (2008). Neurodevelopment and executive function in autism. *Development and psychopathology*, 20(04), 1103-1132.
- Ozonoff, S., Pennington, B. F., & Rogers, S. J. (1991). Executive function deficits in high-functioning autistic individuals: relationship to theory of mind. *Journal of child Psychology and Psychiatry*, 32(7), 1081-1105.

- Ozonoff, S., Young, G. S., Carter, A., Messinger, D., Yirmiya, N., Zwaigenbaum, L., ... & Stone, W. L. (2011). Recurrence risk for autism spectrum disorders: a Baby Siblings Research Consortium study. *Pediatrics*, 128(3), e488-e495.
- Penagarikano, O., Abrahams, B.S., Herman, E.I., Winden, K.D., Gdalyahu, A., Dong, H., Sonnenblick, L.I., Gruver, R., Almajano, J., Bragin, A., Golshani, P., Trachtenberg, J.T., Peles, E. & Geschwind, D.H. 2011. Absence of CNTNAP2 Leads to Epilepsy, Neuronal Migration Abnormalities, and Core Autism-Related Deficits. *Cell*, 147, 235 – 246.
- Penagarikano, O. & Geschwind, D. 2012. What does CNTNAP2 reveal about autism spectrum disorder? *Trend Mol Med*, 18, 156 – 163.
- Peter, B., Raskind, W. H., Matsushita, M., Lisowski, M., Vu, T., Berninger, V. W., ... Brkanac, Z. (2011). Replication of *CNTNAP2* association with nonword repetition and support for *FOXP2* association with timed reading and motor activities in a dyslexia family sample. *Journal of Neurodevelopmental Disorders*, 3(1), 39–49. doi:10.1007/s11689-010-9065-0
- Poliak, S., Salomon, D., Elanhany, H., Sabanay, H., Kiernan, B., Pevny, L., Stewart, C.L., Xu, X., Shrager, P., Furley, A.J. & Peles, E. 2003. Juxtaparanodal clustering of Shaker-like K⁺ channels in myelinated axons depends on Caspr2 and TAG-1. *J Cell Biol*, 162, 1149 – 60.
- Robinson, S., Goddard, L., Dritschel, B., Wisley, M., & Howlin, P. (2009). Executive functions in children with autism spectrum disorders. *Brain and cognition*, 71(3), 362-368.
- Rosenberg, R. E., Law, J. K., Yenokyan, G., McGready, J., Kaufmann, W. E., & Law, P. A. (2009). Characteristics and concordance of autism spectrum disorders among 277 twin pairs. *Archives of pediatrics & adolescent medicine*, 163(10), 907-914.
- De Rubeis, S., & Buxbaum, J. D. (2015). Genetics and genomics of autism spectrum disorder: embracing complexity. *Human molecular genetics*, ddv273.
- Schuh, J. M., & Eigsti, I. M. (2012). Working memory, language skills, and autism symptomatology. *Behavioral Sciences*, 2(4), 207-218.
- Steele, S. D., Minshew, N. J., Luna, B., & Sweeney, J. A. (2007). Spatial working memory deficits in autism. *Journal of autism and developmental disorders*, 37(4), 605-612.
- Steffenburg S, Gillberg C, Hellgren L et al (1989) A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. *J Child Psychol Psychiatry* 30(3):405–416
- Travers, B. G., Adluru, N., Ennis, C., Tromp do, P. M., Destiche, D., Doran, S., et al. (2012). Diffusion tensor imaging in autism spectrum disorder: a review. *Autism Res*. 5, 289–313.

- Vernes, S. C., Newbury, D. F., Abrahams, B. S., Winchester, L., Nicod, J., Groszer, M., ... Fisher, S. E. (2008). A Functional Genetic Link between Distinct Developmental Language Disorders. *The New England Journal of Medicine*, 359(22), 2337–2345. doi:10.1056/NEJMoa0802828
- Williams, D. L., Goldstein, G., Carpenter, P. A., & Minshew, N. J. (2005). Verbal and spatial working memory in autism. *Journal of autism and developmental disorders*, 35(6), 747-756.
- Williams, D. L., Goldstein, G., & Minshew, N. J. (2006). The profile of memory function in children with autism. *Neuropsychology*, 20(1), 21.
- Wolbers, T., & Hegarty, M. (2010). What determines our navigational abilities?. *Trends in cognitive sciences*, 14(3), 138-146.
- Zoghbi, H. Y. (2003). Postnatal neurodevelopmental disorders: meeting at the synapse?. *Science*, 302(5646), 826-830.
- Zoghbi, H. Y., & Bear, M. F. (2012). Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. *Cold Spring Harbor perspectives in biology*, 4(3), a009886.