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Research report

Learning delays in a mouse model of Autism Spectrum Disorder

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HIGHLIGHTS

- *Cntnap2* KOs displayed impairments on a 4/8 radial arm water maze.
- *Cntnap2* KOs exhibited significant deficits in reference and working memory.
- These impairments were specific to the acquisition period of testing.
- These findings suggest *Cntnap2* KOs displayed delayed learning.

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ABSTRACT

Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder with core symptoms of atypical social interactions and repetitive behaviors. It has also been reported that individuals with ASD have difficulty with multisensory integration, and this may disrupt higher-order cognitive abilities such as learning and social communication. Impairments in the integration of sensory information could in turn reflect diminished cross-modal white matter connectivity. Moreover, the genetic contribution in ASD appears to be strong, with heritability estimates as high as 90%. However, no single gene has been identified, and over 1000 risk genes have been reported. One of these genes – contactin-associated-like-protein 2 (*CNTNAP2*) – was first associated with Specific Language Impairment, and more recently has been linked to ASD. *CNTNAP2* encodes a cell adhesion protein regulating synaptic signal transmission. 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*. Initial studies on this mouse revealed poor social interactions, behavioral perseveration, and reduced vocalizations—all strongly resembling human ASD symptoms. *Cntnap2* KO mice also show abnormalities in myelin formation, consistent with a hypo-connectivity model of ASD. The current study was designed to further assess the behavioral phenotype of this mouse model, with a focus on learning and memory. *Cntnap2* KO and wild-type mice were tested on a 4/8 radial arm water maze for 14 consecutive days. Error scores (total, working memory, reference memory, initial and repeated reference memory), latency and average turn angle were independently assessed using a 2×14 repeated measures ANOVA. Results showed that *Cntnap2* KO mice exhibited significant deficits in working and reference memory during the acquisition period of the task. During the retention period (i.e., after asymptote in errors), *Cntnap2* KO mice performed comparably to wild-type mice. These findings suggest that *CNTNAP2* may influence the development of neural systems important to learning and cross-modal integration, and that disruption of this function could be associated with delayed learning in ASD.

1. Background

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 ranging from atypical social interactions and language impairments to repetitive behaviors. Accordingly, individual cases vary substantially in severity and presentation of symptoms. The current estimated prevalence for ASD in the United States is 1 in 68, and is consistently more prevalent in boys than girls (1 in 42 boys versus 1 in 189 girls) [5,9]. To date, causal mechanisms underlying ASD remain poorly under-

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stood, but likely include a complex combination of polygenic and environmental risk factors [15].

Ongoing ASD research has focused on 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. The genetic contribution in ASD appears to be strong; for example, monozygotic twin studies estimate the concordance rates are as high as 70–90% [4,26,23]. 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, pointing to a very complex genetic etiology [7].

One of the 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 [1,2]. *CNTNAP2* has also been linked to other complex neurological disorders such as schizophrenia, dyslexia and depression in genome-wide association studies [28,16,20,12]. Thus *CNTNAP2* mutations could underlie similar endophenotypes across various disorders. 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 [28,20]. Further studies have highlighted significant association between specific SNPs in *CNTNAP2* and language endophenotypes of ASD including age at first word [1] and age at first phrase [3].

CNTNAP2 is located on chromosome 7, and is responsible for encoding a cell adhesion protein regulating synaptic signal transmission [1]. 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* [21]. Initial behavioral studies of this mouse revealed poor social interactions, perseveration, and reduced pup vocalizations – all strongly resembling human ASD symptoms [18,19]. *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 [21]. These mice also exhibit abnormal cortical neural synchrony (i.e., enhanced asynchrony), fewer inter-neurons (which are mostly inhibitory), and atypical neuronal migration [18]. All of these cellular anomalies can be linked to current biological theories for mechanisms of ASD. More recent studies from our lab revealed that the KO mice exhibit unexpected enhancements in acoustic frequency processing, despite impairments on more complex silent gap detection tasks [27]. The latter results have been linked with anomalies at the level of the thalamus, and also could also reflect atypical patterns of cortical connectivity.

The current study was designed to further assess the behavioral phenotype of the *Cntnap2* KO mouse model, with a focus on putative anomalies in spatial learning and memory. Specifically, impairments in working memory have been noted in individuals with ASD, and these deficits are more pronounced when the task load is high [6]. It is also important to note that although most of these working memory impairments in ASD are found in the spatial domain (e.g. [13], they have also been observed in complex verbal working memory tasks [24,25,14,30,29]. Previous studies investigating the *Cntnap2* KO mice, however, found similar learning rates on the Morris Water maze task for KOs versus WT controls. This result suggests a *lack* of spatial learning and memory impairments [18]. However, when presented with a maze reversal task, *Cntnap2* KOs *did* show significant impairments in learning the new platform location [18]. These results reinforce the notion that difficulty of task may play a role in inconsistent findings for memory deficits associated with ASD. Our goal was to further assess *Cntnap2* KOs spatial memory ability utilizing a more difficult 4/8 arm radial water maze task. This task also allows for the analysis of *both* reference and working memory, while introducing a higher cognitive load (compared to Morris Water maze, with only one platform). Finally, this task generates a more extended learning curve, allowing us to adequately evaluate performance during acquisition and retention periods separately.

2. Materials and methods

2.1. Subjects

10 *Cntnap2* KO mice (B6.129(Cg)-*Cntnap2*^{tm1Pele}/J; stock number 017482) and 11 wild type (WT) controls (C57BL/6J; stock number 000664) were obtained from The Jackson Laboratory (Bar Harbor, ME)[1]. [1]Subjects were delivered to the University of Connecticut, Department of Psychology at 7 weeks of age. Upon arrival, 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 [31]. 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).

2.2. 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 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 × 55.5 cm) filled with room temperature water, and given 45 s 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 [11].

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 containing a submerged goal (escape) platform, and 4 open arms that never contain 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 never 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 s to locate a platform. Every subject completed 4 training trials. Each time they found a 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

[1] Jax guarantees "rigorous genetic quality control and mutant gene genotyping programs" for mouse strains with identified molecular mutations (see Terms of Sale).