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Mutation of the dyslexia-associated gene Dcdc2 impairs LTM and visuo-spatial performance in mice

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Abstract

Developmental reading disorder (RD) affects 5–10% of school aged children (American Psychiatric Association, 2000), with a heritability of approximately 60% (Astrom *et al.*, 2011). Genetic association studies have identified several candidate RD susceptibility genes, including DCDC2, however a direct connection between the function of these genes and cognitive or learning impairments remains unclear (Gabel *et al.*, 2010, Paracchini *et al.*, 2007). Variants in DCDC2, a member of the doublecortin family of genes, have been associated in humans with RD and ADHD and Dcdc2 may play a role in neuronal migration in rats (Burbridge *et al.*, 2008, Meng *et al.*, 2005). In this study, we examined the effect of dcdc2 mutation on cognitive abilities in mice using a visual attention and visuo-spatial learning and memory task. We demonstrate that both heterozygous and homozygous mutations of Dcdc2 result in persistent visuo-spatial memory deficits, as well as visual discrimination and long-term memory deficits. These behavioral deficits occur in the absence of neuronal migration disruption in the mutant mice, and may be comorbid with an anxiety phenotype. These are the first results to suggest a direct relationship between induced mutation in Dcdc2 and changes in behavioral measures. Dcdc2 mutant mice should prove useful in future studies designed to further dissect the underlying neural mechanisms that are impaired following Dcdc2 mutation.

Introduction

Developmental reading disorder (RD or dyslexia) is characterized by an unexplained reading impairment which significantly interferes with academic achievement or daily life (American Psychiatric Association, 2000). Reading impairment can include deficits in reading accuracy as well as fluency, which is observed clinically as extremely slow and effortful reading. A number of theories have emerged concerning the etiology of RD, disruptions in phonological processing (Ramus, 2003), auditory processing (Tallal, 1980), visual processing (Eden *et al.*, 1996, Lovegrove *et al.*, 1980), attention (Hari & Renvall, 2001, Ruffino *et al.*, 2010, Vicari *et al.*, 2003), visuo-spatial (Smith-Spark & Fisk, 2007, Smith-Spark *et al.*, 2003, Vidyasagar & Pammer, 2010), cerebellar function (Nicolson *et al.*, 2001, Nicolson & Fawcett, 2005), neuronal migration deficits in the neocortex, and magnocellular thalamic function (Galaburda & Livingstone, 1993, Livingstone *et al.*, 1991, Stein & Walsh, 1997).

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Twin studies have shown that RD has a genetic and environmental component (Gayán & Olson, 2001, Paracchini *et al.*, 2007). Numerous targeted gene association studies in dyslexic families across Finland, Germany, United Kingdom and the US have identified candidate dyslexia susceptibility genes (CDSG), namely DYX1C1, DCDC2 and KIAA0319, ROBO1, MRPL19, C2ORF3 (Anthoni *et al.*, 2007, Gabel *et al.*, 2010, Paracchini *et al.*, 2007). However the connection between these genes and behavioral impairments associated in individuals with RD is unclear.

Recently knockdown studies using *in utero* RNAi directed against *Dcdc2, Kiaa0319*, and *Dyx1c1* have shown that decreased expression of these genes in migrating neurons in developing rat neocortex cause varying degrees of altered neuronal migration (Burbridge *et al.*, 2008, Meng *et al.*, 2005, Paracchini *et al.*, 2006, Rosen *et al.*, 2007, Wang *et al.*, 2006). The data suggest that these genes may play a functional role in neuronal migration, however there has not been a direct genetic test for the function of these genes in mice. Cognitive impairments following Dyx1c1 knockdown in rats have been reported (Szalkowski *et al.*, 2011, Threlkeld *et al.*, 2007). These studies provide some evidence that altered expression of CDSGs result in neuroanatomical anomalies as well as behavioral impairments which parallel those identified in individuals with RD. It is unclear however, whether in these RNAi studies the impairments are caused by the malformations themselves, or by the loss of gene expression in targeted neurons.

Doublecortin domain containing 2 (DCDC2) is a member of the doublecortin (DCX) family of genes that contain tandem or single dcx domains. DCX domains are thought to be critical for binding and stabilizing microtubules; the doublecortin domain has been shown to bind tubulin and enhance microtubule polymerization (Kim *et al.*, 2003). Mutations in the DCX gene are associated with abnormal neuronal migration leading to lissencephaly and double cortex syndrome (Gleeson *et al.*, 1999). Reduced expression of Dcdc2 in rats leads to altered neuronal migration resulting in the formation of neocortical heterotopia (Burbridge *et al.*, 2008, Meng *et al.*, 2005), however a direct genetic test for the function of Dcdc2 has not been reported. Recently, Wang et al. (2011) reported the first phenotypic analysis of Dcdc2 knockout mice. No significant differences in neurodevelopment, general health or behavior. With respect to behavior tested in this previous study, in an open field test of general motor behavior, and a simple maze task testing visuo-spatial learning and memory, knockout mice were not statistically different from controls (Wang et al. 2011).

In this study we examined the effect of Dcdc2 mutation on cognitive abilities in mice using a visual discrimination and long-term memory task (novel object recognition), as well as visuo-spatial learning and memory (Hebb-Williams maze) task. The Hebb-Williams maze has been used to test visuo-spatial abilities in both humans and rodents. Experiments using a virtual version of the maze, showed similar learning curves in humans and rodents, thus making it possible to translate the results across species (Macleod *et al.*, 2010, Shore *et al.*, 2001). The NOR task is a non-spatial task of recognition memory utilizing the natural tendency of mice to explore novel environments. Visual long term memory can be tested by manipulating the time between introduction of the familiar and novel objects. In this study we examine the effects of Dcdc2 knockout on cognitive abilities in mice. If Dcdc2 plays a role in visual attention, visual long-term memory and visuo-spatial learning and memory abilities, we would expect that targeted mutation of Dcdc2 would result in decreased performance on these tasks. Interestingly, we found that despite a lack of neuroanatomical defect (Wang et al. 2011), Dcdc2 mutation impairs visual long-term memory, and performance efficiency on a visuo-spatial task without interfering with the ability of the mice to learn the task.

Methods

Subjects

Dcdc2 mutants carried a dcdc2 allele with a deletion of Exon 2 on Chromosome 6p22 (del2) in a 129SJ × C57BL/6J hybrid background. A total of 35 male mice (72 – 83 days postnatal at the start of testing) were used during behavioral testing, including 12 wildtype (dcdc2$^{wt/wt}$), 12 heterozygous (dcdc2$^{wt/del2}$), and 11 homozygous (dcdc2$^{del2/del2}$). All mice were generated by matings between heterozygous (dcdc2$^{wt/del2}$) pairs. These heterozygous pairs were from F8-F10 mice generated as described below. All generations were produced by dcdc2$^{wt/del2}$ matings. The three genotypes were recovered in the expected mendelian ratio of 1:2:1. The estimated percentage of C57BL/6J and 129S6 founder strain in these hybrid mice is 75% to 25% respectively. Mice were singly housed with free-access to food and water, unless otherwise indicated. Animals were kept on a 12 hour light-dark cycle, with lights on at 8 am. All procedures were approved by the Institutional Animal Care and Use Committee.

Gene targeting and genotyping

In order to generate the mice used in this study, first a colony of mice carrying a loxp-exon2-loxp conditional allele of *Dcdc2* (*Dcdc2*^{flox2}) were generated by the University of Connecticut Health Center (UCHC) Gene Targeting and Transgenic Facility. Embryonic stem cells harboring a floxed allele of exon two of *Dcdc2* were produced by electroporating mouse embryonic stem (ES) cells (129S6/SvEvTac) with a homologous recombination targeting construct containing an engineered floxed-exon2 and drug selection cassette flanked by Flippase Recognition Target (FRT) recombination sites. ES clones were drug selected with positive and negative selection and screened by PCR for correctly targeted ES cell clones. A single positive clone was expanded and used for embryo re-aggregation to produce five chimeric mice. Three of these mice were shown to germline transmit the targeted allele to offspring in a cross with C57BL6/J mice. The PGK-neo drug selection cassette in the targeting construct was then removed by crossing these mice with 129S4/SvJaeSor-*Gt(ROSA)26Sor*$^{tm1(FLP1)Dym}$/J mice (Jackson Laboratory, Bar Harbor, ME, USA). These offspring were used to produce a colony of homozygous *Dcdc2*$^{flox2/flox2}$ mice. In order to generate *Dcdc2*$^{del2/del2}$ mice with a deletion of exon 2 we crossed *Dcdc2*$^{flox2/flox2}$ mice with Hrpt-Cre mice, C57Bl6-*Hprt*$^{tm1(cre)Mnn}$/J mice (UCHC, 129S1/Sv-Hprt$^{tm1(cre)Mnn}$/J backcrossed to C57BL6 for 10 generations). Four *Dcdc2*$^{del2/wt}$ offspring were used to start a separate colony of *Dcdc2*$^{wt/del2}$ mice. All *Dcdc2*$^{wt/del2}$; *Dcdc2*$^{del2/del2}$, and *Dcdc2*$^{wt/wt}$ mice used in this study are from crosses between *Dcdc2*$^{wt/del2}$ mice from within this colony. Genotyping for the two Dcdc2 alleles within this colony was performed by PCR using two pairs of primers (loxP F: 5′-agtggatctgcagggttaac, loxP R: 5′-cttcggttgttacacgagcaat; Exon2 F: 5′-gagtggatctgcagggttaacat; Exon2 R: 5′-aagccgaggcaagcagatcttta).

Hebb-Williams maze

A 60 × 60 cm Hebb-Williams maze, with attached start and goal box, was constructed using black acrylic plastic; the removable floor and top were made with clear Plexiglass (Figure 1). Removable interior walls (made of black acrylic plastic) enabled the assembly of multiple maze configurations. One week prior to testing mice were given free access to water and restricted to 2 g of chocolate-flavored food pellets per day (Bio-Serv, Frenchtown, NJ, USA). Mice (dcdc2$^{wt/wt}$ n= 12; dcdc2$^{wt/del2}$ n= 12; dcdc2$^{del2/}$del2 n= 11) were weighed daily and maintained at 85% of their normal body weight. During habituation, mice were allowed to explore the maze without interior walls for a maximum of 10 trials of 120 s duration. Once the subject left the start box a black plastic guillotine door was closed to prohibit reentry. The trial ended once the mouse ate one chocolate-flavored food pellet

located in the goal box, or 120s was reached. If the subject did not reach the goal box in 120s he was gently guided to the goal box, the door to the goal box was closed, and the mouse remained until one food pellet was consumed. Habituation ended when all 10 trials were completed, or the subject completed three consecutive trials in less than 30s. The next day mice were habituated to the interior walls using the Maze 1 configuration (Meunier *et al.*, 1986). The mice completed 6 trials in a maximum time of 120s per trial. Testing consisted of one maze configuration per day, 6 trials per maze, for a maximum of 120s per trial. Mazes 5, 6, 11, and 12 were utilized for testing (Figure1).

Errors, trial duration, distance traveled, mean speed and performance efficiency were measured. Errors were calculated when the center of mass of the subject crossed into an error zone (Figure 1). Mean speed is calculated by the distance traveled divided by the time to complete the maze. Performance efficiency of mice is based on a calculation described by Shore et al. (2001), which calculates the average of the standardized duration and error scores to provide a compound measure of performance on the Hebb-Williams maze. More specifically, performance efficiency is measured by computing the z score (using the overall grand means and standard deviations from all subjects) for both error and time to complete the task. The average z score is calculated for each animal, with error and time weighted equally. This provides a composite measure where large positive numbers reflect a relatively poor performance.

Novel Object Recognition (NOR)

A 60 × 60 cm open field arena was utilized for the NOR task. Mice (dcdc2$^{wt/wt}$ n= 10; dcdc2$^{wt/del2}$ n= 10; dcdc2$^{del2/del2}$ n= 10) were habituated to the arena for 5 min., on the day of testing, with no objects present. During the training session mice were exposed to two identical objects for a period of 3 minutes. The objects consisted of either glass votive holders or lego configurations of equal width and height; objects were heavy enough not to be displaced by the subjects. The familiar and novel objects were counterbalanced across subjects. Objects were positioned equidistant from the subject at the opposite end of the arena, approximately 15 cm from the sides of the arena to allow exploration of all sides of the object. Following the 3 minute session the mouse was returned to his home cage for 3 minutes at which time the arena and the objects were cleaned, and one familiar object was replaced with a novel object (discrimination trial). The position of the novel object within the arena was counterbalanced across subjects. A retention trial took place 3 hours following the discrimination session. During the retention trial the familiar object was paired with a novel object. The position of the novel object was counterbalanced across trials.

The number of contacts with each object was recorded. A contact is operationally defined as the subject having its nose < 2mm from the object or touching it with the nose. During the training session the proportion of contacts made with any one of the familiar objects was calculated (*number of contact with one of the familiar objects divided by the total contacts made with both objects*). A discrimination index was used to calculate the proportion of contacts made with the novel object during the discrimination and retention trials (*number of contacts with the novel object divided by the total contacts made with both objects*). Exploration behavior was analyzed by calculating the total number of contacts made with the objects during each trial.

Elevated Plus Maze (EPM)

The EPM (Stoelting, Wood Dale, IL, USA) consisted of two open arms (35×5 cm), and two enclosed arms (35×5×15 cm) that extended from a central platform (5×5 cm). The maze was elevated 40 cm from the ground. Subjects (dcdc2$^{wt/wt}$ n= 5; dcdc2$^{wt/del2}$ n= 6; dcdc2$^{del2/del2}$ n= 6) activity was recorded for 5 minutes. The number of entries into, and time spent in the

closed and open arms, as well as the center, of the EPM were recorded. Percentage of time spent in the open EPM arms (anxiety measure), total distance travelled (locomotor activity) during testing were calculated. Entries into an arm were recorded when the center of mass of the subject crossed into an arm.

Data Acquisition and Statistical Analysis

ANY-maze tracking software (Stoelting) and a web camera (Logitech Quick Cam, Fremont, CA, USA) were used to record and analyze behavior. If the automated tracking system was unable to accurately track the subject, an offline analysis the behavior was performed by a blind observer. To determine the distance travelled offline, the subject's movement was tracked using Adobe Photoshop CS5.1. Briefly, a replica of the maze was made to scale, a line of known distance was drawn with the pencil tool (scale bar) and the pixel count of the scale bar was determined. The trajectory of the mouse was drawn using the pencil tool, and the pixel count of the path was determined. The distance travelled was then extrapolated from these data (i.e. distance travelled (m) = scale bar (m) multiplied by the trajectory (pixels) divided by the scale bar (pixels). Once we determined we could accurately and reliably detect the distance travelled, by comparing the extrapolated value to the value generated by ANY-maze, we were able to incorporate these data into the analysis. Statistical tests were performed using PASW 17 software (IBM corporation, Somers, NY, USA), or Microsoft Excel. It is important to note that during behavioral testing we randomized the order in which the mice were tested and counterbalanced the groups across trials in a given experiment. Furthermore, we counterbalanced the order in which the mice were exposed to the behavioral tasks. A 4 × 6 × 3 mixed factorial ANOVA was performed, with maze (Maze 5, 6, 11, and 12) and trial (trials 1–6) as the within subjects factors and genotype (dcdc2$^{wt/wt}$, dcdc2$^{wt/del2}$, dcdc2$^{del2/del2}$) as the between subjects factor, to analyze errors, trial duration, mean speed, performance efficiency and distance traveled on the Hebb-Williams maze. A 3 × 3 mixed factorial ANOVA, with trial as the within subjects factor and genotype as the between subjects factor, was performed to analyze proportion of contacts with object A2 and B on the NOR task. For repeated measures ANOVAs, if Mauchly's *W* was significant; therefore the sphericity assumption was not met, a Huynh-Feldt correction was applied. An independent samples t-test was performed to examine anxiety level and locomotor activity between wildtype (dcdc2$^{wt/wt}$) and dcdc2 mutant (dcdc2$^{wt/del2}$, dcdc2$^{del2/del2}$) mice. All values are reported as Means ± SEM, unless otherwise indicated.

Results

Dcdc2 knockout impairs visual discrimination and long-term working memory

A total of 30 subjects (10 dcdc2$^{wt/wt}$, 10 dcdc2$^{wt/del2}$, 10 dcdc2$^{del2/del2}$) were examined on a two trial novel object recognition task. If genotype expression influences visual memory then there should be a significant interaction between genotype and trial on the proportion of contacts made the objects. Mice should explore the novel object during trial 2 with greater frequency than the familiar object. A 3 × 3 Mixed factorial ANOVA, with trial as the within subjects factor, was performed to determine if altered Dcdc2 expression effected visual memory. There was a significant interaction between genotype and trial, $F_{(4,56)} = 2.61$, $p < .05$ (figure 2). Following a planned comparison analysis using a one-sample t-test versus chance occurrence with a Bonferonni correction ($\alpha = 0.013$) we demonstrated that the dcdc2$^{wt/wt}$ mice are able to discriminate between the novel and familiar object and retain this memory following a three hour latency, whereas the dcdc2$^{wt/del2}$ discriminate between the two objects, but do not retain the memory of the familiar object. The dcdc2$^{del2/del2}$ mice are unable to discriminate between the novel and familiar objects even after the short latency between training session and presentation of the novel object (discrimination trial) three minutes later. There was a significant main effect of trial, $F_{(2,56)} = 12.55$, $p < 0.001$.

Following a one-sample t-test versus chance occurrence with a Bonferonni correction ($\alpha = 0.017$) indicating mice made proportionally more contacts with the novel object in the discrimination trial and retention trial compared to chance occurrence with no difference during training. There was no main effect of genotype, $F_{(2, 27)} = 0.73$, *ns*. Examination of exploration behavior demonstrates that there is no significant interaction between trial and genotype ($F_{(4, 54)} = 0.90$, ns), nor a main effect of genotype ($F_{(2,27)} = 0.07$, ns). There is a significant main effect of trial ($F_{(2,54)} = 7.26$, $p < 0.01$) suggesting that the mice made fewer total contacts with the objects across trials.

Visuo-spatial learning and memory is altered in Dcdc2 mutants

A total of 35 subjects (12 dcdc2$^{wt/wt}$, 12 dcdc2$^{wt/del2}$, 11 dcdc2$^{del2/del2}$) were tested to examine the effect of altered Dcdc2 expression on visuo-spatial working memory. Mice were tested on four different Hebb-Williams maze configurations across six trials per maze; errors, duration to complete the maze and distance traveled were measured. The configurations were rated as moderate (Maze 5 & 6) or difficult (Maze 11 & 12) based on difficulty score calculated by the number of errors committed by C57BL6 mice divided by the total number of error zones (Meunier *et al.*, 1986). There was no significant interaction between maze, trial and genotype for errors (Huynh-Feldt, $F_{(21.43, 342.90)} = 0.86$, *ns*), or time to complete the maze, $F_{(30,480)} = 1.02$, *ns*. The interaction between maze and genotype was not significant for errors ($F_{(5.41, 86.63)} = 1.32$, *ns*), or duration ($F_{(6,96)} = 0.55$, *ns*), however there was a significant interaction between trial and genotype for the amount of time to complete the maze ($F_{(10, 160)} = 2.21$, $p < 0.05$), but not for errors (figure 3A &B). A simple main effects post-hoc analysis on the learning curves demonstrates that the latency to complete the maze significantly decreases over time for dcdc2$^{wt/wt}$ ($F_{(1,11)} = 12.53$, $p<0.01$) whereas there is not statistically difference change in duration across trials for dcdc2$^{wt/del2}$ ($F_{(1,11)} = 0.58$, *ns*) or dcdc2$^{del2/del2}$ ($F_{(1,10)} = 0.25$, *ns*) mice. Significant main effects of trial ($F_{(4.36, 139.37)} = 20.98$, $p < 0.001$) and maze ($F_{(2.71, 86.63)} = 24.69$, $p < 0.001$) were found for errors, but only a significant main effect of maze ($F_{(3,96)} = 3.58$, $p< 0.05$) was found for duration, with no main effect of genotype for either dependent variable.

Based on these results we examined whether performance efficiency, a compound measurement equally weighting standardized number of errors and latency to complete the maze across subjects, distance traveled, or mean speed during the completion of the task was affected by Dcdc2 knockout. As expected, a statistically significant linear trial by genotype interaction for performance efficiency was also evidence ($F_{(2,32)} = 4.25$, $p<0.05$) demonstrating that the performance efficiency of wildtype mice improves over successive trials but the performance of mutant mice does not change (figure 3C). The distance traveled during testing was tracked and analyzed for each maze; across trials. No significant interactions were found for distance traveled during the testing phase (figure 4A & B). There was a significant main effect of trial (Huynh-Feldt, $F_{(4.61,147.66)} = 14.31$, $p < 0.001$) demonstrating that distance traveled decreased over trials. Figure 4A provides representative traces, comparing genotype, for the path taken to the goal box across trials for maze 5. However, there was a linear trial by genotype interaction ($F_{(2,32)} = 3.40$, $p<0.05$) for the mean speed of the subjects during testing (figure 4C). These data suggest that, similar to the latency to complete the maze, the speed of the mutant mice does not change across trials whereas the wildtype mice show an increase in mean speed across trials, as would be expected as the mice learn the task.

Dcdc2 mutants display increased anxiety phenotype

Based on the performance of Dcdc2 mutant mice on the Hebb-Williams maze we wanted to determine whether knockout of the Dcdc2 gene resulted in an increased anxiety phenotype compared to wildtype mice. If Dcdc2 mutant mice exhibit an increased anxiety phenotype

then we would predict that dcdc2 dcdc2$^{wt/del2}$ and dcdc2$^{del2/del2}$ mice would spend proportionately less time on the open arms of an EPM than closed arms. A one-tailed, independent samples t-test comparing dcdc2$^{wt/wt}$ mice to dcdc2$^{del2/del2}$ mice demonstrated that knockout mice exhibit an increased anxiety phenotype, spending proportionately less time in the open arms (13.16 ± 3.38%) than dcdc2$^{wt/wt}$ mice (39.08 ± 13.18%), t(9)= 2.08, $p<0.05$; however dcdc2$^{wt/del2}$mice did not spend proportionately less time in the open arms (27.92 ± 8.62%) compared to dcdc2$^{wt/wt}$ mice (t (9) = 0.73, n.s.; Figure 5A). There was no statistical difference between dcdc2$^{wt/wt}$ and dcdc2$^{del2/del2}$ mice (t(9) = −1.41, *ns*) or dcdc2$^{wt/wt}$ and dcdc2$^{wt/del2}$ mice (t(9) = 0.60, *ns*) on the total distance travelled during the 5 minute testing period (Figure 5B).

Discussion

Assessment of visual attention and visuo-spatial learning and memory abilities in Dcdc2 knockout mice demonstrated that Dcdc2 deletion impairs visual discrimination and decreased performance efficiency to complete a visuo-spatial learning and memory task without affecting the ability to learn. Visual discrimination and long term visual memory is differentially affected. More specifically, mice with at least one copy of the Dcdc2 gene were able to discriminate between a novel and a familiar object, but dcdc2$^{wt/del2}$ dcdc2$^{del2/del2}$ mice were unable to hold on to the visual information for a prolonged period of time (i.e. 3 hours). The dcdc2$^{del2/del2}$ mice showed the greatest impairment being unable to discriminate between a novel and familiar object following a brief three-minute delay (figure 2). Menghini et al 2010 reported a similar finding in individuals diagnosed with developmental dyslexia (DD). They found that individuals with DD were equivalent to controls on their ability to visually discriminate between similar objects, but showed a marked impairment over successive trials (Menghini *et al.*, 2010). It is important to note that a deficit in discrimination and long term memory on the novel object recognition task does not preclude a deficit in sensory modalities other than visual; therefore additional examination of these mice for additional sensory deficits, such as auditory processing deficits reported in animal models of DD, and individuals with RD, is needed.

On the visuo-spatial learning and memory task, mice with only one copy (dcdc2$^{wt/del2}$) or no copy (dcdc2$^{del2/del2}$) of the Dcdc2 gene show a persistent deficit in the time to complete the Hebb-Williams mazes (figure 3A). This deficit was not due to an inability to learn how to successfully navigate the mazes (figure 3B), since there was no deficit in the number of errors made during testing, and no difference across genotypes in the total distance traveled (figure 4). Persistent deficits in mean speed and performance efficiency across trials suggest that the mice fail to improve their performance over time (figure 3C). Based on the configurations of the Hebb-Williams maze, studies have examined the effects of maze difficulty and type of memory on behavior (Hoplight *et al.*, 2001, Meunier *et al.*, 1986), however there were no significant interactions found between maze and genotype on behavior so these factors did not affect the outcome of this study. We cannot rule out that altered Dcdc2 expression does not affect working and/or, reference memory, since all mazes require some amount of reference and working memory ability to navigate through the mazes. Therefore additional studies should be performed which will directly measure these two types of memory. It is important to note that this deficit was not evident when utilizing an easier version of the Hebb-Williams maze (Wang *et al.*, 2011), providing indirect evidence that the impaired performance of Dcdc2 knockout mice may be the result increased cognitive load, rather than a motivational problem. Observations during testing indicated that anxiety may have influenced the behavior of the subjects during the visuo-spatial task. Evidence from our laboratory suggests that an anxiety phenotype is present in the mice with a homozygous Dcdc2 mutation, demonstrating that increased anxiety may, at least in part, contribute to the deficit in performance on this task.

It is important to note that it is possible alleles derived from the 129S6 line will flank the mutated gene; whereas alleles derived from the C57BL6 line will flank the wildtype allele, which may influence the behavioral phenotype (Crusio, 2004). A recent study examining visuo-spatial behavior of these two parental strains, as well as the F2 129 × C57BL6 hybrid strain, using a Morris Water Maze (MWM) task suggests that the hybrid strain is superior to either parental strain and the parental strains do not differ from each other on this type of task (Bruin *et al.*, 2006). In our study the dcdc2$^{del2/del2}$ and dcdc2$^{wt/del2}$ mice show a persistent deficit in learning to navigate the maze; this phenomenon was not reported in either parental line or the hybrid stain in the Bruin *et al.* (2006) study which demonstrated that all mice, regardless of genetic background, learned the task. Although the type of motivation utilized in these two visuo-spatial tasks are different, these data suggest that the deficit we see is likely due to the Dcdc2 mutation.

Postmortem analyses of brains of individuals with dyslexia have demonstrated the presence of subtle cortical anomalies located in language areas of the brain (Galaburda & Kemper, 1979, Galaburda *et al.*, 1985). Interestingly, linkage association studies have identified several c candidate dyslexia susceptibility genes all of which play a role in cortical development. It has been suggested that the presence of these malformations may disrupt normal cortical circuitry leading to the cognitive deficits identified in individuals with RD (Galaburda *et al.*, 2006). *DCDC2* contains two DCX domains; DCX encodes for a protein that is involved in the regulation of neuronal migration. Disruptions in DCX result in lissencephaly and double cortex syndrome. Recent studies have demonstrated that genetic knockdown of *Dcdc2* using *in utero* RNAi have resulted in neuronal migration abnormalities in the cortex and hippocampal formation (Burbridge *et al.*, 2008, Meng *et al.*, 2005). Knockdown of another CDSG, *Dyx1c1*, also revealed early disruption of cortical development which was associated with memory impairments (Szalkowski *et al.*, 2011, Threlkeld *et al.*, 2007). In contrast, we did not identify structural abnormalities within the cortex or hippocampus in *Dcdc2* knockout mice (Wang et al. 2011). More specifically, periventricular heterotopias and molecular layer ectopia are absent in mice with a germline mutation in Dcdc2. Therefore, these behavioral deficits are independent of the neuronal migration abnormalities reported in genetic knockdown studies. These data may suggest that neuroanatomical anomalies may only be coincident with RD but not necessarily a direct cause of the cognitive deficits in all cases.

Studies examining cognitive abilities in children and adolescence with RD have provided conflicting evidence for the types of deficits which may underlie this learning disorder. This has led to multiple theories of RD, with varying degrees of support. For example, studies examining the visual-spatial theory of RD have reported a deficit in visual–spatial short term memory (Menghini *et al.*, 2011, Smith-Spark *et al.*, 2003), whereas others have not (Jeffries & Everatt, 2004, Kibby, 2009, Kibby & Cohen, 2008). Some studies have indicated that the results generated could be sample specific, indicating different characteristics of an individual sample from the broader population of individuals with RD, leads to potentially different outcomes (Paracchini *et al.*, 2007, Ramus *et al.*, 2003). Furthermore, multiple genes have been identified through replicated linkage studies indicating a number of potential genetic factors contributing to RD. To take this idea a step further, it is possible that different epigenetic factors, lead to different cognitive impairments, all of which underlie RD. In this study, we demonstrate that altered Dcdc2 expression impairs cognitive performance on a visuo-spatial working memory task in terms of the time it takes to complete the task, but not their ability to learn the task. Furthermore this deficit persists over time and occurs in the absence of gross neuroanatomical disruptions. This deficit is coincident with a visual discrimination and long term memory deficit. Future studies should be directed at determining if there are unique behavioral phenotypes associated with the different CDSGs which may account for the myriad of supported theories surrounding RD.

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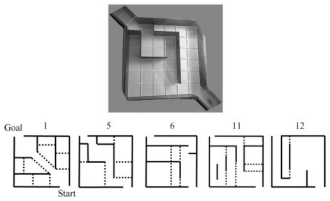

Figure 1.
Hebb-Williams maze configurations. The Hebb-Williams maze was constructed of black acrylic plastic, with removable internal walls in order to allow for construction of multiple mazes (photograph of Maze 5 configuration). Schematic diagrams depict the training maze (Maze 1) and test mazes (Mazes 5, 6, 11 and 12) utilized in this study. Solid lines represent walls; dashed lines indicate error zones within each maze configuration. An error was scored when both forepaws crossed into an error zone.

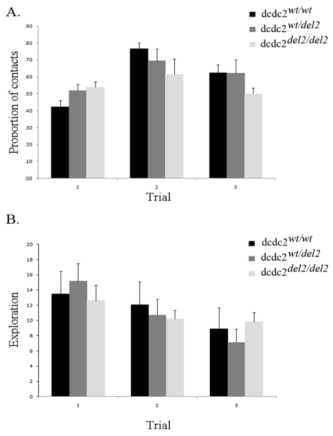

Figure 2.

Deletion of *Dcdc2* alters visual discrimination and long term visual memory. (A) The proportion of contacts made with an familiar or novel object over the total number of contacts made with either object is displayed across trials for wildtype (dcdc2$^{wt/wt}$), heterozygous (dcdc2$^{wt/del2}$), and homozygous (dcdc2$^{del2/del2}$) knockout mice. The training trial contained two identical objects, the discrimination trial, which took place after a three minute inter-trial interval, contained one familiar object from the training trial and one novel object, and the retention trial contained one familiar object and one novel object presented after a three hour inter-trial interval. (B) Exploration behavior is presented as the total number of contacts made with both objects across training, discrimination and retention trials. All data are represented by mean ± SEM.

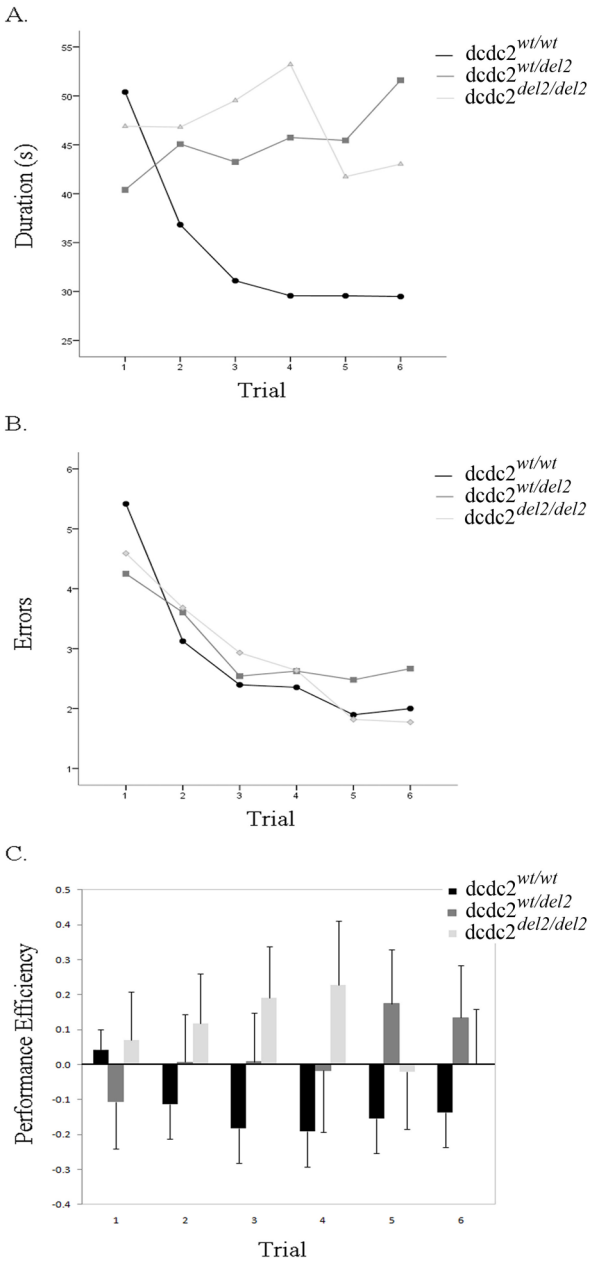

Figure 3.

Deletion of *Dcdc2* impairs visuo-spatial learning and memory. (A) Plot of the duration (sec) to complete the Hebb-Williams mazes by wildtype (dcdc2$^{wt/wt}$), heterozygous (dcdc2$^{wt/del2}$) and homozygous Dcdc2 knockout (dcdc2$^{del2/del2}$) mice across six trials. (B) Plot of the mean errors (i.e. crossing an error zone calculated when the center of mass of the subject crossed into an error zone) committed by dcdc2$^{wt/wt}$, dcdc2$^{wt/del2}$ and dcdc2$^{del2/del2}$ mice, across six trials collapsed across mazes. (C) Performance efficiency was plotted for genotype across six trials collapsed across mazes. Performance efficiency is calculated based on an equally weighted average of the errors committed and duration to complete the task. All data are represented by mean ± SEM.

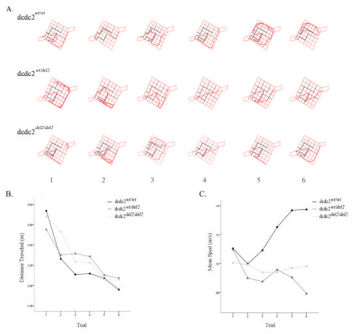

Figure 4.

Visuo-spatial deficits are not the result of altered motor behavior in traversing the mazes. (A) Representative traces, comparing wildtype (dcdc2$^{wt/wt}$), heterozygous (dcdc2$^{wt/del2}$) and homozygous Dcdc2 knockout (dcdc2$^{del2/del2}$) mice, for the path taken to the goal box across trials for maze 5. (B) The distance traveled (m) by dcdc2$^{wt/wt}$, dcdc2$^{wt/del2}$ and dcdc2$^{del2/del2}$ mice during the completion of the mazes across six trials. (C) The mean speed (m/s) of wildtype and Dcdc2 knockout mice during the completion of the mazes across six trials. Data are represented by mean ± SEM.

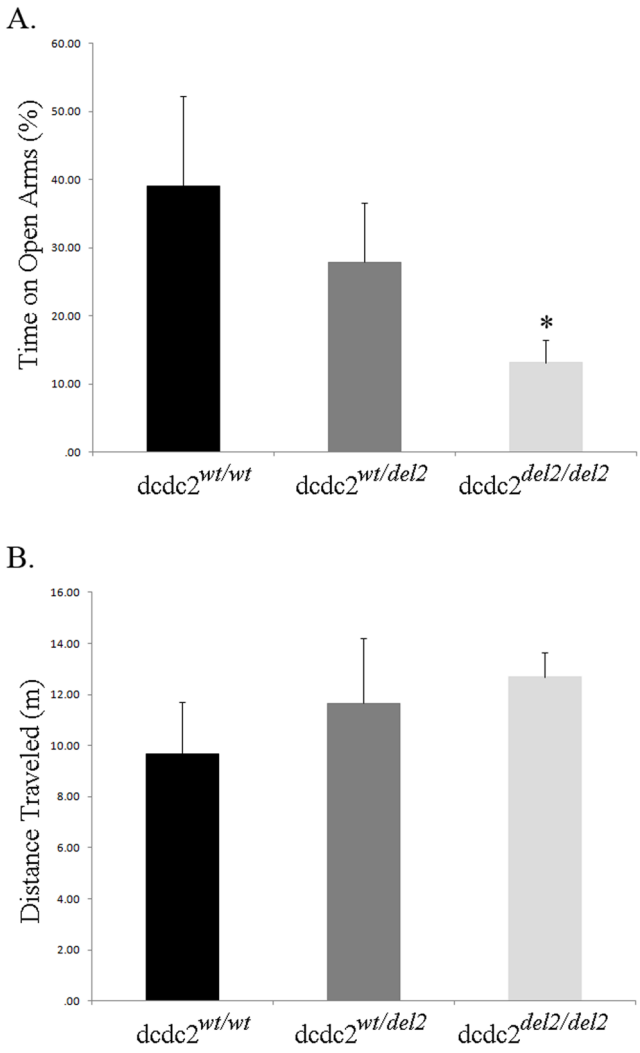

Figure 5.

Deletion of Dcdc2 confers an increased anxiety phenotype. (A) Percentage of time spent in the open arms of an elevated plus maze by wildtype (dcdc2$^{wt/wt}$), heterozygous (dcdc2$^{wt/del2}$) and homozygous Dcdc2 knockout (dcdc2$^{del2/del2}$) mice during a five minute testing session. (B) Distance traveled (m) on the elevated plus maze during the five minute testing is compared across genotype.