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Behavioral Implications of Knockout for the Dyslexia-Risk Gene Dcdc2 in Mice

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BEHAVIORAL IMPLICATIONS OF KNOCKOUT FOR THE DYSLEXIA-RISK GENE Dcdc2 IN MICE

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ABSTRACT

Several genetic linkage and epidemiological studies have provided strong evidence that DCDC2 is a candidate gene for developmental dyslexia, a disorder that impairs a person's reading ability despite adequate intelligence, education, and socio-economic status. Studies investigating embryonic intra-ventricular RNA interference (RNAi) of Dcdc2, a rat homolog of the DCDC2 gene in humans, indicate disruptions in neuronal migration in the rat cortex during development. Interestingly, these anatomical anomalies are consistent with post mortem histological analysis of human dyslexic patients. Other rodent models of cortical developmental disruption have shown impairment in rapid auditory processing and learning maze tasks in affected subjects.

The current study investigates the rapid auditory processing abilities of mice heterozygous for Dcdc2 (one functioning Dcdc2 allele) and mice with a homozygous knockout of Dcdc2 (no functioning Dcdc2 allele). It is important to note that this genetic model for behavioral assessment is still in the pilot stage. However, preliminary results suggest that mice with a genetic mutation of Dcdc2 have impaired rapid auditory processing, as well as non-spatial maze learning and memory ability, as compared to wildtypes. By genetically knocking out Dcdc2 in mice, behavioral features associated with Dcdc2 can be characterized, along with other neurological abnormalities that may arise due to the loss of the functioning gene.

1. Introduction

Developmental dyslexia is a neurological disorder that impairs a person's learning and reading ability despite adequate intelligence, educational opportunity, and socio-economic status [8]. Those diagnosed with developmental dyslexia exhibit behavioral and cognitive deficits that can include delays in language acquisition, deficits in rapid temporal processing of auditory information, and/or deficits in phonological processing [1,22,23,24,26]. In addition to behavioral and cognitive abnormalities, neurological anomalies concurrent with dyslexia patients consist of developmental cortical malformations such as abnormal neuronal migration, thin corpus callosum, and neuronal ectopias and dysplasias (cellular anomalies due to impaired cortical neuronal migration) seen most frequently in the inferior frontal and superior temporal regions of the brain located on the left hemisphere [3].

Family and twin studies focusing on developmental dyslexia have established a strong genetic component in the etiology of the disorder, and through various genetic linkage analyses, four candidate dyslexia susceptibility genes have emerged over the past decade [2,9,14,19]. Thus far DYX1C1 (on chromosome 15), ROBO1 (on chromosome 3), and DCDC2 and KIAA0319 (both on chromosome 6) have been implicated within various human dyslexic populations for the reading disorder [11,13,15,16,20].

Rodent studies investigating the rodent homologs of the four human candidate dyslexia genes have shown that each gene plays critical a role in neuronal migration [11,15,10,16]. For example, studies using embryonic intra-ventricular RNA interference (RNAi) of Dyx1c1, Kiaa0319, or Dcdc2 (rodent homologs of the DYX1C1, KIAA0319, and DCDC2 genes respectively) in rats have demonstrated that a genetic knockdown of the expression of one of the candidate dyslexia genes disrupts neuronal migration within the developing cortex of the rat brain, leading to cortical malformations similar to those seen in post mortem brains of human dyslexic patients [3,10,11,15,16]. Prior to the discovery of the dyslexia candidate genes, there

was no clear etiological explanation for the cellular anomalies found within the human dyslexic brains. However, with these findings, there now appears to be a direct correlation between abnormal neuronal migration and the candidate genes for dyslexia.

One of the human candidate susceptibility genes for dyslexia, DCDC2, can be further implicated in its role involving neuronal migration. DCDC2, located on chromosome 6, locus 22 on the short arm (6p22), is related to a gene that is well known for its important involvement in neuronal migration: a doublecortin containing gene called DCX [12,27]. Within the genetic sequence of DCDC2, it contains two doublecortin peptide domains that could also be found in DCX [11]. Mutations within the genetic sequence of DCX cause a disease called double cortex syndrome that disrupts microtubule organization within the developing cortex, resulting in impaired neural migration [21]. Such mutations within the doublecortin peptide domains of DCX are also encoded within DCDC2, thus providing an additional possible link connecting the function of DCDC2 to neuronal migration [11].

Rodent models for developmental dyslexia have shown that subjects with both induced and spontaneous cortical anomalies, similar to those found in human dyslexia patients, presented with difficulties in rapid auditory processing, a key behavioral marker of developmental dyslexia in humans [1,3,4,5,6,7,17,18,22,28]. Studies have also established that RNAi of Dcdc2 in rats is associated with developmental cortical malformations [11,30]. However, the role of such cortical developmental anomalies in phonological processing has not been examined. Behavioral assessment has however, been conducted for the Dyx1c1 gene. These studies found that embryonic intra-ventricular RNAi of Dyx1c1 in rats led to later impairments in rapid auditory processing [29]. To further investigate the role of the candidate dyslexia susceptibility genes in behavior, the current study employs a novel genetic model for behavioral assessment, specifically using a genetic knockout model of Dcdc2 in mice. By generating a partial or full

knockout of Dcdc2 in mice (i.e., partially or fully inactivating the expression of Dcdc2), the current study seeks to characterize the behavioral features associated with Dcdc2 anomalies in comparison to mice with fully functioning Dcdc2 genes.

2. Methods

2.1 Pilot

Since behavioral assessment of C57black6J mice has not been performed within our laboratory prior to the experiments described here, an initial pilot study was conducted to examine the viability of using this strain of mice in our behavioral testing paradigms. It was determined from results of the pilot study that C57black6J could be used as appropriate subjects for the behavioral tasks implemented within our laboratory, following several modifications to tasks developed for Wistar rats, specifically making the tasks slightly easier for this species/strain.

2.2 Study 1

2.2.1 Subjects

To generate the Dcdc2 knockout in C57black6J mice, site-specific recombination using the Cre/loxp system was applied to target the Dcdc2 gene. Exon 2 of the Dcdc2 gene sequence was excised from the sequence through a series of selective breedings. Subjects were bred at the University of Connecticut, Department of Physiology and Neurobiology under the supervision of Dr. Joseph LoTurco, and all procedures were approved by the Institutional Care and Use Committee. Litters were a product of a heterozygous knockout (one functioning Dcdc2 allele) and wildtype (two fully functioning Dcdc2 alleles) mating, and were genotyped at birth by "tail-snips" (see below for details). Based on prior evidence that behavioral deficits are greater in males [6] only male subjects (8 wildtype and 5 heterozygous) were selected for behavioral assessment, and these subjects were weaned and transferred to the University of Connecticut,

Department of Psychology, Behavioral Neuroscience Division, on P21.

For genotyping purposes, no more than 2 mm of tail tissue were obtained from each subject at P10. Tail tips were placed in a centrifuge tube filled with 106 μL of a DNA lysate buffer/proteinase K solution (100 μL/6 μL). Samples were incubated in a dry bath set at 55°C for 2 hours and then 95°C for 5 minutes. To perform the PCR, 1 μL of sample of the previously incubated DNA solution was added to 12 μL of the PCR master solution, 0.5 μL each for the respective forward and reverse primer, and 10.5 μL of ddH_2O. Samples in PCR solution were then placed into a PCR machine and allowed to amplify for 35 cycles. After amplification, DNA samples were placed in an ethidium bromide containing agarose gel to separate the DNA.

Subjects were weaned and pair housed on P21, and were single housed on P65 in a 12 h light/dark cycle with food and water available *ad lib*. Behavioral testing began P36 and continued through P141. Following behavioral testing, all subjects were weighed, anesthetized, and transcardially perfused.

2.2.2 Auditory Testing

Startle Reduction Paradigm

The startle reduction paradigm measures the acoustic startle reflex (ASR), which is a large amplitude, involuntary, motor response as a result of a startle eliciting stimulus (SES). When a pre-stimulus is detected prior to an SES, the ASR response should attenuate--a phenomenon also known as pre-pulse inhibition (PPI). Thus an uncued SES should elicit a greater ASR response in comparison to a cued SES. Based on this expected ratio, a reduction in startle was used as a measure for acoustic discrimination. This attenuation was measured using an “attenuation score”, which was calculated as (cued trial/uncued trial)*100. An attenuation score of 100% indicates a chance response (no difference in the startle reflex for cued and uncued trials). A score below 100% suggests a reduction in startle response during cued trials,

indicating that an acoustic discrimination was made for the pre-stimulus cue. In this study, all SES were 105 dB, 50 ms white noise bursts.

Equipment

During auditory processing tasks, each subject was placed on individual load-cell platforms (MED Associates, Georgia, VT). The output from each platform was amplified (linear amp PHM-250-60 MED Associates) into a Biopac MP100WS Acquisition system connected to a Macintosh computer that recorded the amplitude of the startle reflex for each trial. Specifically, the amplitude for of each subject's ASR was recorded in mV after the presentation of the SES by taking the maximum peak value from the 150 ms signal period following the onset of the SES. These values were coded for cued and uncued trials, and displayed the subject's absolute response amplitude for each trial. Auditory stimuli were produced using a Dell Pentium IV PC with custom programmed software and a Tucker Davis Technologies real time processor, and sound files were created and played using a custom program and delivered via powered Cambridge Sound Works speakers located approximately 30 cm above each load cell platform.

Normal single tone

A normal single tone test session consisted of 104 cued/uncued trials presented in a pseudo-random order. Uncued trials consisted of a silent background with a 105 dB, 50 ms SES presented at a variable interval (16-22 seconds). Cued trials followed the same procedure, but 50 ms before the SES, a 75 dB, 2300 Hz tone was presented for 7 ms. Results were used to calculate a mean pre-pulse inhibition attenuation baseline score for each subject. These scores were used to determine whether the subject had any deficiencies (e.g. motor or hearing) that would prevent further participation in auditory tasks.

Silent gap

A silent gap test session consisted of 300 variable length cued/uncued trials presented in a pseudo-random order. A total of 5 sessions measuring moderate length silent gap detection (0-100 ms) were given to each subject (ages P41 through P45). Uncued trials consisted of a constant broad band white noise background (75 dB) followed by a 105 dB, 50 ms SES. Cued trials consisted of the same background stimulus, however 100 ms before the SES, a silent gap of variable duration (2, 5, 10, 20, 30, 40, 50, 75, and 100 ms) cued the upcoming SES. A total of 10 sessions measuring short silent gap detection (0-10 ms) were also given to each subject (ages P48 through P52 and P76 through P80). Procedure for the short silent gap task was identical to that of the moderate silent gap task, however, for the cued trials, the silent gap before the SES differed (2, 3, 4, 5, 6, 7, 8, 9, and 10 ms). Additional silent gap detection tasks (0-200 ms and 0-300 ms) were used (P132 through P141) following a similar procedure described above, but including longer gap durations.

Complex oddball

A complex oddball test session consisted of 104 cued/uncued trials presented in a pseudo-random order. A total of 5 test sessions were given to each subject (P83 through P87). A complex oddball procedure consisted of the repeated presentation of a sequence of two (high/low) 75 dB tone pips, separated by a within stimulus interstimulus interval (ISI) of variable length per individual test session (325, 275, 225, 175, and 125 ms respectively). Each repeating two tone pair (background) was separated by a between sequence ISI of 200 ms greater than the variable within stimulus length. On uncued trials, a 105 dB, 50 ms SES occurred 100 ms after the last two tone pair. In cued trials, the subject was presented with a reversal (low/high) of the two tone pair 100 ms prior to the 105 dB, 50 ms SES.

FM sweep procedure

An FM sweep test session consisted of 104 cued/ uncued trials presented in a pseudo-random order. A total of 5 sessions were given to each subject (ages P90 through P94). The FM sweep consisted of the repetition of a 75 dB downward FM sweep (2300-1900 Hz) with a random 105 dB, 50 ms SES as the uncued trials and an upward FM sweep (1900-2300 Hz) presented as the cue before the SES. Each sweep was of a variable length (325, 275, 225, 175, and 125 ms respectively), with only one sweep duration used per test session. The ISI between repeating sweeps was always 200 ms longer than the sweep length.

2.2.3 Water Escape, Morris Water Maze, and Non-spatial Water Maze

Water Escape

Prior to any water maze task, all subjects underwent a water escape task to ensure that subjects did not have a motor or visual impairment that would prevent them from effectively performing the tasks. Subjects were placed in one end of an oval tub (40.5 in. x 21.5 in.) filled with room temperature water (8 in.), and had to swim to a visible platform (3.5 in. in diameter) on the other end of the tub opposite to where they were released. Time latency to switch to the visible platform was recorded.

Morris Water Maze

The following day, subjects began Morris water maze (MWM) testing. Over a period of 5 testing days, subjects had to find a submerged platform (3.5 in. in diameter) 2 cm. below the surface of the water that was placed in a fixed location (southeast quadrant) within a round black tub (48 in. in diameter). All locations of extra maze cues (varying shapes painted on testing room wall, location of experimenter, door, etc.) were fixed throughout the 5 testing sessions such that escape from the maze required use of extra-maze spatial cues to determine the location of the submerged platform. Each day, subjects underwent 4 trials, and in each trial, they started from a random compass point (north, south, east, west). On day 1 of MWM testing prior to the

first trial, subjects were placed on the submerged platform for 10 seconds, removed from the platform, and then placed back into the water at one of the compass locations. Latency to reach the platform on each of the 4 trials was recorded for all subjects on all days.

Non-spatial Water Maze

Non-spatial water maze (NSWM) testing followed 2 days after the last MWM test session and lasted for a period of 5 days. Like the MWM, subjects had to find a submerged platform (3.5 in. in diameter) within a round tub (48 in. in diameter). However, unlike the MWM, the round tub contained a black, metal, rotating insert with various intramaze cues (vertical black and white stripes, horizontal black and white stripes, black polka dots on a white background, and white polka dots on a black background) painted on it. For this task, the location of the submerged platform was not fixed, but instead was paired with the vertical black and white striped intramaze cue. Escape from the maze required subjects to form and recall an association between the vertical striped intramaze cue and the platform, regardless of extra maze spatial cues, to correctly determine the location of the platform. For all 4 trials during the testing session, the subject was placed in the same compass location (north). However, during each trial, the spatial location of the intramaze cue and platform pair within the testing room was rotated randomly into one of the four quadrants (southwest, southeast, northwest, northeast). On day 1 of NSWM testing prior to the first trial, subjects were placed on the submerged platform for 10 seconds, removed from the platform, and then placed back into the water at the north compass point. Latency to reach the platform on each trial (different spatial location of cue/platform pair) was recorded for all subjects on all days.

2.3 Study 2

2.3.1 Subjects

Generation and genotyping of mice with a partial or full knockout of Dcdc2 was similar to the procedure used in Study 1, however for Study 2, litters were a product of homozygous knockout and heterozygous knockout matings. Subjects were weaned and transferred to the University of Connecticut, Department of Psychology, Behavioral Neuroscience Division from the University of Connecticut, Department of Physiology and Neurobiology on P21. Wildtype controls of C57black6J mice were ordered from Charles River Laboratories, Wilmington, MA, and arrived at the University of Connecticut, Department of Psychology, Behavioral Neuroscience Division on P21. Again, only male subjects (9 wildtype, 6 heterozygous knockout, and 2 homozygous knockout) were used for Study 2.

Subjects were weaned and pair housed on P21, and were single housed on P77 in a 12 h light/dark cycle with food and water available *ad lib*. Behavioral testing began P35 and continued through P108. Following behavioral testing, all subjects were weighed, anesthetized, and transcardially perfused.

2.3.2 Auditory Testing

Subjects were tested using the same startle reduction paradigm and equipment as discussed in Study 1.

Normal Single Tone

Subjects were administered the same Normal Single Tone procedure discussed in Study 1 on both P37 and P83.

Silent Gap

The general testing procedure for the Silent Gap detection task was identical to the one used in Study 1. A total of 9 testing sessions measuring long silent gap detection (0-300 ms.) were given to each subject (P40 through P44 and P84 through P87). Subjects were also

administered 10 testing sessions measuring 0-100 ms. silent gap detections (P47 through P51 and P90 through P94)

2.3.3 Water Escape, Morris Water Maze, and Non-spatial Water Maze

Procedures for these tasks were identical to those described in Study 1.

3. Results

3.1 Study 1

3.1.1 Auditory Testing: Normal Single Tone

Comparison of the mean acoustic startle response of cued and uncued trials using a paired samples t-test indicated that all groups could significantly detect the pre-stimulus cue ($p < 0.05$). Analyzing attenuation scores, there were no significant differences between the Dcdc2 wildtype and Dcdc2 heterozygous knockout treatment groups ($p > 0.05$). These results indicated that a partial knockout of Dcdc2 in mice did not alter baseline PPI and basic auditory processing abilities (see Figure 1).

3.1.2 Auditory Testing: Silent Gap

A paired samples t-test comparing the mean acoustic startle response indicated that all groups could significantly detect silent gaps over 30 ms on the 0-100 ms task (although scores were quite high compared to prior studies, indicating poor performance). Analysis of attenuation scores using a repeated measures ANOVA with Treatment (2 levels) x Day (5 levels) x Gap (9 levels) as fixed factors indicated no significant Treatment effects between wildtype and heterozygous groups on the 0-100 ms silent gap task ($F(1,11) = 1.106$, $p > 0.05$). This suggests that both groups performed similarly on the task, possibly reflecting overall task difficulty (basement effects).

For the 0-10 ms silent gap task during both juvenile and adult testing periods, comparison of the mean acoustic startle response using a paired samples t-test showed that there was no

significant discrimination between the silent gaps within the 0-10 ms range and the uncued response across all groups. These results indicate that the subjects could not effectively perform the task at the 0-10 ms level.

A repeated measures ANOVA was used to analyze attenuation scores between the wildtype and heterozygous treatment groups for the 0-200 ms silent gap task using Treatment (2 levels) x Day (4 levels) x Gap (9 levels) as fixed factors. Results show a near significant main Treatment effect between wildtype and heterozygous groups [$F(1,11) = 3.798$, $p = 0.077$], suggesting that mice heterozygous for Dcdc2 performed worse than wildtype mice on this task (see Figure 2).

Data for the 0-300 ms silent gap task were pooled with Study 2 (described below).

3.1.3 Auditory Testing: Complex Oddball and FM sweep

For both the complex oddball and FM sweep tasks, comparison of the mean acoustic startle response using a paired samples t-test for both assessments showed that there was no significant discrimination between the cued and uncued responses across all groups. These results indicated that the subjects could not effectively perform the complex oddball and FM sweep tasks.

3.1.4 Water Maze Testing

Visual Platform (Control Task)

A univariate ANOVA comparing mean latency to target platform between the wildtype and heterozygous groups showed no significant difference in performing the task ($p > 0.05$), indicating that a partial knockout of Dcdc2 did not impair the subject's motor or visual capabilities to accomplish the task.

Morris Water Maze (Spatial)

For the MWM, a repeated measures ANOVA with Treatment (2 levels) x Day (5 levels) was used to analyze the mean latency to reach the platform across 4 trials. Despite a pattern of results suggesting worse performance by heterozygous mice, analysis showed no significant Treatment effect between the wildtype and heterozygous groups [$F(1,11) = 2.49$, $p > 0.05$] (see Figure 3).

Non-spatial Water Maze

A Treatment (2 levels) x Day (5 levels) repeated measures ANOVA was used to analyze the mean latency to target across 4 trials for the NSWM. There was a significant main Treatment effect between the wildtype and heterozygous groups [$F(1,11) = 8.046$, $p < 0.05$], indicating that mice heterozygous for Dcdc2 performed worse (longer latencies) on the NSWM task in comparison to the mice that were wildtype for Dcdc2 (see Figure 4).

3.2 Study 2: Statistical Considerations

Data collected for Study 2 revealed aberrant poor performance by the Dcdc2 wildtypes (n=9). Because wildtype controls for this study were obtained from an outside supplier, and as a result had different parental lineages (as well as different early experiences) as compared to all other groups in Studies 1 and 2 (which were bred in-house), it was determined that they may provide a poor control group. An analysis on adult 0-300 ms silent gap data focusing on the 175-250 ms range (i.e., the range that showed significant detection of the silent gap cue) was performed to show that Dcdc2 wildtype mice from Study 2 performed significantly worse than the Dcdc2 wildtype mice used in Study 1, and were thus dropped from further analysis.

A second analysis on adult 0-300 ms silent gap data was performed to show that Dcdc2 heterozygous knockouts from Study 2 (who were bred comparably in-house) were equivalent in

performance to heterozygous subjects from Study 1 (no significant difference), and thus these subjects were pooled for further analysis.

However, Dcdc2 heterozygous subjects from Study 1 and 2 did show significant differences on maze tasks. Therefore, only adult data from silent gap 0-300 ms was reanalyzed using data pooled across Studies 1 and 2. Maze data from Study 2 was unable to be analyzed due to the loss of the control group.

3.3 Reanalysis of Silent Gap 0-300 ms: Study 1 and 2 Pooled

Silent gap 0-300 ms data during the adult period was reanalyzed using the following groups: Dcdc2 wildtypes (n=8); Dcdc2 heterozygous knockouts (n=11); and Dcdc2 homozygous knockouts (n=2). Given the small number of Dcdc2 homozygous knockouts, they were combined with Dcdc2 heterozygous knockouts to form a larger treatment group termed the "Dcdc2 genetically mutant" group. A repeated measures ANOVA with the parameters Treatment (2 levels) x Day (3 levels) x Gap (9 levels) was used to analyze the attenuation scores of the new pooled data from Studies 1 and 2. Results showed no significant Treatment effect between the wildtype and Dcdc2 genetically mutant group ($p > 0.05$) when all 3 days of testing were analyzed together, indicating that both groups performed similarly (see Figure 5). However, it appeared that mice only showed consistent discrimination between 175-250 ms. Moreover, prior evidence shows that performance increases with experience, and thus days 1-3 were examined separately. That is, since prior research from this laboratory has shown that Treatment effects can be masked on a task that is difficult for shams, and has also shown that progressive experience improves performance [25], we examined the effects of Treatment (2 levels) and gap (9 levels) at each of the three days separately.

Results showed no overall significant Treatment effects on each of the 3 days of testing on the silent gap 0-300 ms task. However, given evidence that subjects were discriminating cues only in the 175-250 ms range, we examined performance at these gaps more directly. Results showed a significant Treatment effect at both the 175 and 250 ms silent gap intervals ($p = 0.035$ and 0.027 respectively) on day 3 of silent gap 0-300 ms testing, with wildtypes performing significantly better than the genetically mutant group (see Figure 6a-c). Examination of the PPI abilities of the Dcdc2 wildtype controls over the 3 days of testing showed improvement in performance on the task with ongoing testing, whereas Dcdc2 genetically mutant mice showed minimal improvement with progressive experience.

4. Discussion

Prior research using embryonically RNAi transfected rats targeting Dcdc2 have shown that the gene plays a role in neuronal migration within the rat neocortex, with disruption resulting in the development of neuroanatomical anomalies consistent with those seen in human dyslexia patients [3,11,30]. Studies of a fellow dyslexia candidate gene, DYX1C1, have also revealed a role in neuronal migration [10,16]. Moreover, behavioral effects of this gene have been further studied, and demonstrate that RNAi transfected rats targeting Dyx1c1 exhibit impairments in detecting rapid acoustic stimuli in comparison to shams [29]. The current set of studies assessing a genetic mutation of Dcdc2 through either a partial or full knockout in mice are suggestive of similar behavioral deficits in these subjects, although experimental difficulties limit the conclusions that can be drawn. .

4.1 Study 1: Auditory processing impairments in mice with a partial knockout of Dcdc2

Although it appeared that both groups performed similarly throughout most of the auditory processing tasks, closer analysis of the data displayed that there was either no significant discrimination of the task (silent gap 0-10, FM, and oddball) indicating that the task

could not be performed, or the task was simply too difficult for the subjects thus reflecting possible basement effects (silent gap 0-100). However, near significant Treatment effects between the wildtype and heterozygous Dcdc2 groups overall for the four days of testing do suggest rapid auditory processing impairments in mice heterozygous for Dcdc2. At the longer end of the silent gap spectrum for the 0-200 ms task, a separation between rapid auditory processing capabilities of the two groups becomes evident at 175 ms, with a significant Treatment main effect that can be seen at the 200 ms gap. This high threshold supports the interpretation that the previous auditory processing tasks the mice were exposed to (< 100 ms gaps) were too difficult for C57black6J subjects. However, when the cognitive demand of the task was reduced by lengthening the silent gap interval, we did see that the wildtype mice were able to perform the task effectively, thus allowing for evidence of auditory processing impairments in the heterozygous mice to emerge. Results from the silent gap 0-200 ms task suggest a rapid auditory processing impairment in mice with a partial knockout of Dcdc2.

4.2 Study 1: Water maze learning impairments in Dcdc2 heterozygous mice

Maze data indicated that there was no significant difference in Morris spatial maze learning ability between the wildtype and heterozygous Dcdc2 groups. However significant deficits were seen for heterozygous subjects on the non-spatial maze, indicating some form of learning impairment. In the RNAi study of Dyx1c1, rats transfected with RNAi of Dyx1c1 also showed deficits in water maze learning ability [29]. However, subjects in that study displayed deficits in the Morris spatial maze and not the nonspatial maze.

4.3 Study 2: Reanalysis of Silent Gap 0-300 ms using a combined Dcdc2 genetically mutant group show auditory processing impairment

As discussed earlier, studies have shown that behavioral tasks too difficult for the control group can mask Treatment effects, but with progressive experience, performance can improve,

thus allowing possible Treatment effects to emerge [25]. This can be seen in the successful use of silent gap 0-300 ms during the adult period. Daily testing showed improvements in the detection of silent gaps 0-300 ms across each of the three days, specifically in wildtype controls. In fact, on the third day of testing, the wildtype control group showed significant improvement on the task as compared to Day 1, while the Dcdc2 genetically mutant group did not perform better on Day 3 versus Day 1. Thus significant Treatment effects did appear to emerge over time in the gap range that could be successfully detected by wildtypes (175-250 ms range). This suggests that a rapid auditory processing impairment may in fact be present in Dcdc2 genetically mutant mice, consistent with evidence implicating DCDC2 as a dyslexia risk gene.

5. Conclusion: Dcdc2 in mice and its behavioral implications in developmental dyslexia

These series of studies sought to characterize the behavioral features associated with a mutation of the dyslexia risk gene DCDC2, through the use of a novel animal model that partially or fully knocked out the Dcdc2 gene in mice. By creating a link between a key behavioral marker of developmental dyslexia in humans (impaired rapid auditory processing), and the function of the Dcdc2 gene in mice, it would help to solidify DCDC2's position as a candidate gene for dyslexia in humans. Moreover, such studies could pave the way for future studies using a genetic knockout model to further assess the neurobehavioral aspects of developmental dyslexia. Our current results suggest rapid auditory processing and maze learning impairments within subjects with a genetic mutation in either one or both of its Dcdc2 alleles. However, additional research using less demanding auditory processing tasks, as well as examining correlations between behavioral performance and presence of neuroanatomical malformations, will be needed in order to provide an improved understanding of the neurobehavioral effects of Dcdc2 in mice and its relation to developmental dyslexia.

References

1. A. Benasich and P. Tallal, Infant discrimination of rapid auditory cues predicts later language impairment, *Behav. Brain Res.* **136** (2002), pp. 31–49.

2. A.M. Galaburda, J. LoTurco, F. Ramus, R.H. Fitch, G.D. Rosen. From genes to behavior in developmental dyslexia. *Nat. Neurosci.* **9** (2006), pp. 1213-1217.

3. A.M. Galaburda, G.F. Sherman, G.D. Rosen, F. Aboitiz, N. Geschwind. Developmental dyslexia: four consecutive cases with cortical anomalies. *Ann. Neurol.* **18** (1985), pp. 222–233.

4. A.M. Peiffer, C.K. Dunleavy, M. Frenkel, L.A. Gabel, J.J. LoTurco, G.D. Rosen, and R.H. Fitch. Impaired detection of variable duration embedded tone in ectopic NZB/BINJ mice. *Neuroreport*, **12** (2001), pp. 2875-2879.

5. A.M. Peiffer, G.D. Rosen and R.H. Fitch, Sex differences in rapid auditory processing deficits in ectopic BXSB/MpJ mice. *Neuroreport* **13** (2002), pp. 2277–2280.

6. A.M. Peiffer, G.D. Rosen, and R.H. Fitch. Sex differences in rapid auditory processing deficits in microgyric rats. *Developmental Brain Research.* **148** (2004), pp 53-57.

7. A.M. Peiffer, J.T. Friedman, G.D. Rosen and R.H. Fitch, Impaired gap detection in juvenile microgyric rats. *Brain Res. Dev. Brain Res.* **152** (2004), pp. 93–98.

8. B.A. Shaywitz, J.M. Fletcher, J.M. Holahan, S.E. Shaywitz. Discrepancy compared to low achievement definitions of reading disability: results from the Connecticut longitudinal study. J. Learn. Disabil. 25 (1992), pp. 639-648.

9. B. Hallgren. Specific dyslexia ('congenital word blindness'): a clinical and genetic study. *Acta Psychiatr. Neurol*. **65** (1950), pp. 1–287

10. G.D. Rosen, J. Bai, Y. Wang, C.G. Fiondella, S.W. Threlkeld, J.J. LoTurco, A.M. Galaburda. Disruption of neuronal migration by RNAi of Dyx1c1 results in neocortical and hippocampal malformations. *Cereb. Cortex.* **17** (2007), pp. 2562-2572.

11. H. Meng, S. Smith, K. Hager, M. Held, J. Liu, R. Olson, B. Pennington, J. DeFries, J. Gelernter, T. O'Reilly-Pol, S. Somlo, P. Skudlarski, S. Shaywitz, B. Shaywitz, K. Marchione, Y. Wang, M. Paramasivam, J. LoTurco, G. Page and J. Gruen, DCDC2 is associated with reading disability and modulates neuronal development in the brain, *Proc. Natl. Acad. Sci. U.S.A.* **102** (2005), pp. 17053–17058.

12. J. Bai, R. Ramos, J. Ackman, A. Thomas, R. Lee and J. LoTurco, RNAi reveals doublecortin is required for redial migration in rat neocortex, *Nat. Neurosci.* **6** (2003), pp. 1277–1283.

13. J. Shumacher, H. Anthoni, F. Dahdouh, I.R. Konig, A.M. Hillmer, N. Kluck, M. Manthey, E. Plume, A. Warnke, H. Remschmidt, J. Hulsmann, S. Cichon, C.M. Lindgren, P. Propping,

M. Zucchelli, A. Ziegler, M. Peyrard-Janvid, G. Schulte-Korne, M.M. Nothen and J. Kere, Strong genetic evidence of DCDC2 as a susceptibility gene for dyslexia, *Am. J. Hum. Genet.* **78** (2006), pp. 52–62.

14. J.C. DeFries, S.M. Singer, T.T. Foch, F.I. Lewitter**.** Familial nature of reading disability. *Br. J. Psychiatry*. **132** (1978), pp. 361-367.

15. K. Hannula-Jouppi, N. Kaminen-Ahola, M. Taipale, R. Eklund, J. Nolpola-Hemmi, H. Kaariainen and J. Kere. The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. *PLoS Genet.* **1** (2005), pp. e50.

16. M. Taipale, N. Kaminen, J. Nopola-Hemmi, T. Haltia, B. Myllyluoma, H. Lyytinen, K. Muller, M. Kaaranen, P. Lindsberg, K. Hannula-Jouppi and J. Kere. A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. *Proc. Natl. Acad. Sci. U.S.A.* **100** (2003), pp. 11553–11558.

17. M.E. Farmer and R.M. Klein, The evidence for a temporal processing deficit linked to dyslexia: a review. *Psychon. Bull. Rev.* **2** (1995), pp. 460–493

18. M.G. Clark, G.D. Rosen, P. Tallal and R.H. Fitch, Impaired processing of complex auditory stimuli in rats with induced cerebrocortical microgyria: an animal model of developmental language disabilities. *J. Cogn. Neurosci.* **12** (2000), pp. 828–839.

19. N. Choudhury & A.A. Benasich. A family aggregation study: the influence of family history and other risk factors on language development. *J. Speech, Lang, Hear, Res*. **46** (2003), pp. 261-272.

20. N. Cope, D. Harold, G. Hill, V. Moskvina, J. Stevenson, P. Holmans, M.J. Owen, M.C. O'Donovan, and J. Williams. Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. *Am. J. Hum. Genet.* **76** (2005), pp. 581-591.

21. O. Reiner, F.M. Coquelle, B. Peter, T. Levy, A. Kaplan, T. Sapir, I. Orr, N. Barkai, G. Eichele, S. Bergmann. The evolving doublecortin (DCX) superfamily. *BMC Genomics*. **7** (2006), pp. 1-16.

22. P. Tallal and A. Benasich. Developmental language learning impairments. *Dev. Psychopathol.* **14** (2002), pp. 559–579.

23. R. Hari & H. Renvall. Impaired processing of rapid stimulus sequences in dyslexia. *Trends Cogn. Sci*. **5** (2001), pp. 525–532.

24. R.H. Fitch and P. Tallal, Neural mechanisms of language-based learning impairments: insights from human populations and animal models, *Behav. Cogn. Neurosci. Rev.* **2** (2003), pp. 155–178.

25. R.H. Fitch, S.W. Threlkeld, M.M. McClure, and A.M. Peiffer. Use of a modified prepulse inhibition paradigm to assess complex auditory discrimination in rodents. *Brain Res. Bull.* **76** (2008), pp. 1-7.

26. R.K. Wagner & J.K. Torgesen. The nature of phonological processing and its causal role in the acquisition of reading skills. *Psychol. Bull.* **101** (1987), pp. 192–212.

27. S.E. Fisher & C. Francks. Genes, cognition and dyslexia: learning to read the genome. *Trends Cogn. Sci.* **10** (2006), pp. 250-257.

28. S.W. Threlkeld, M.M. McClure, G.D. Rosen, and R.H. Fitch. Developmental time frames for induction of microgyria and rapid auditory processing deficits in the rat. *Brain Res.* **1109** (2006), pp. 22-31.

29. S.W. Threlkeld, M.M. McClure, J. Bai, Y. Wang, J.J. LoTurco, G.D. Rosen, R.H. Fitch. Developmental disruptions and behavioral impairments in rats following in utero RNAi of Dyx1c1. *Brain Res. Bull.* **71** (2007), pp. 508-514.

30. T.J. Burbridge, Y. Wang, A.J. Volz, V.J. Peschnsky, L. Lisann, A.M. Galaburda, J.J. LoTurco, and G.D. Rosen. Postnatal analysis of the effect of embryonic knockdown and overexpression of candidate dyslexia susceptibility gene homolog Dcdc2 in the rat. *Neuroscience*, **152** (2008), pp.723-733.

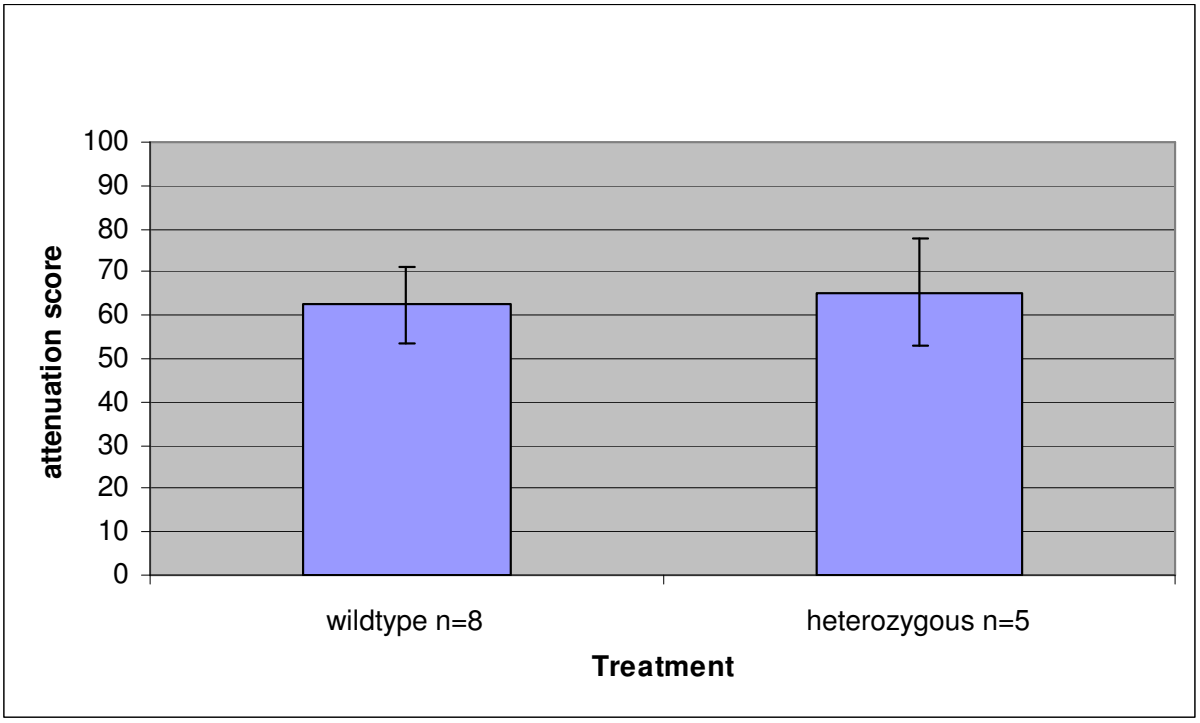

Figure 1. Attenuation scores for Study 1, Normal Single Tone. This procedure is used to determine deficiencies (e.g. motor or hearing) that would prevent further participation in auditory tasks. There were no significant differences between the Dcdc2 wildtype and Dcdc2 heterozygous knockout Treatment groups ($p>0.05$); (100% = chance, lower scores = better performance).

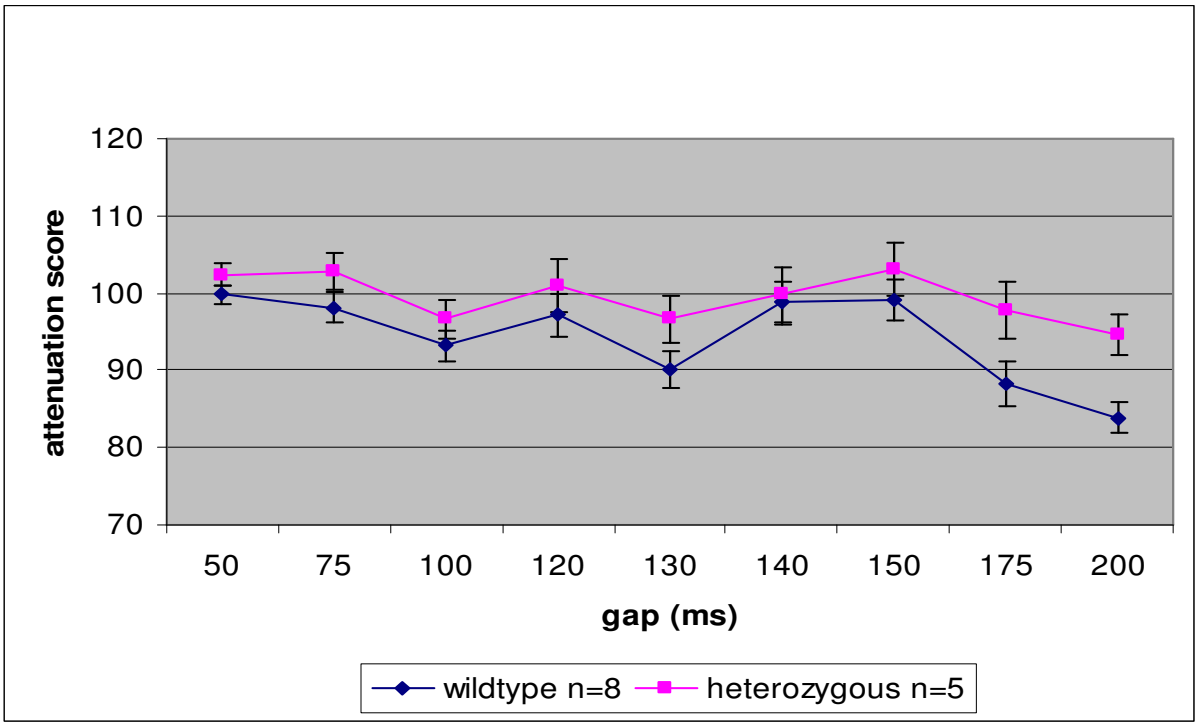

Figure 2. Attenuated scores for Study 1, Silent Gap 0-200 ms (4 days, averaged). Results show a near significant Treatment effect for wildtype and heterozygous groups [$F(1,11) = 3.798$, $p = 0.077$], indicating impaired rapid auditory processing ability in Dcdc2 heterozygous knockout mice.

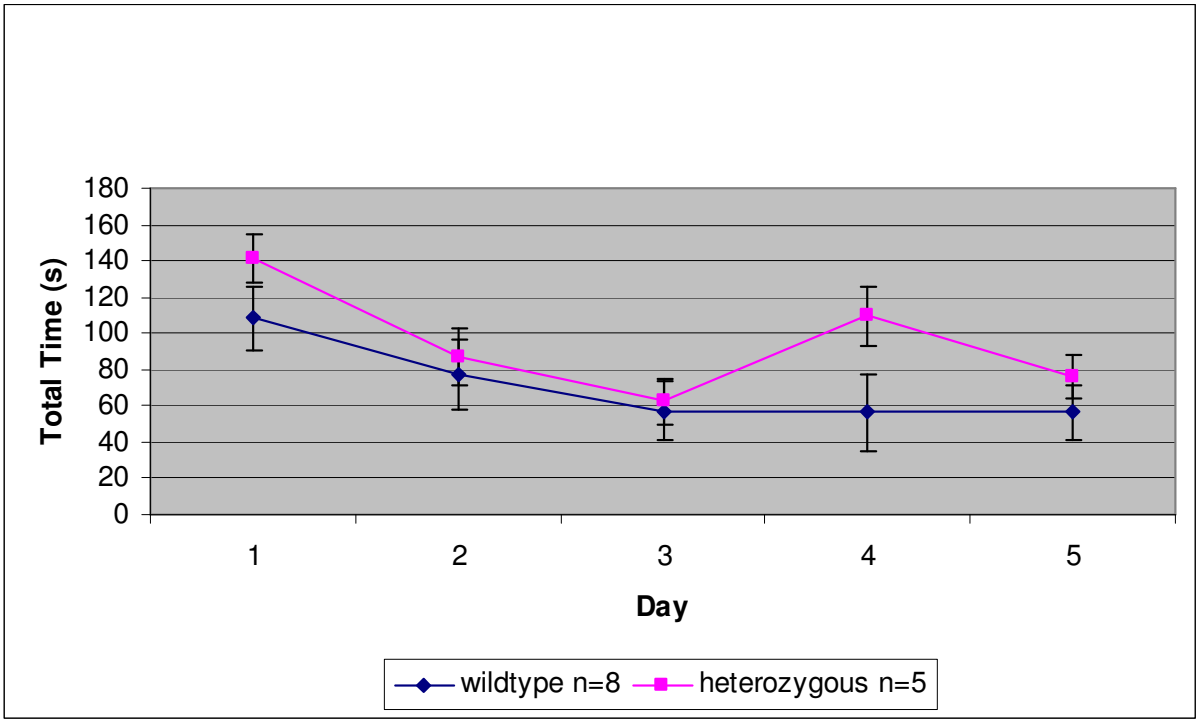

Figure 3: Latency scores (in seconds) for Study 1, Morris Spatial Water Maze (5 days, average latency). Data shows no significant Treatment effect between the wildtype and heterozygous groups [$F(1,11) = 2.49$, $p > 0.05$].

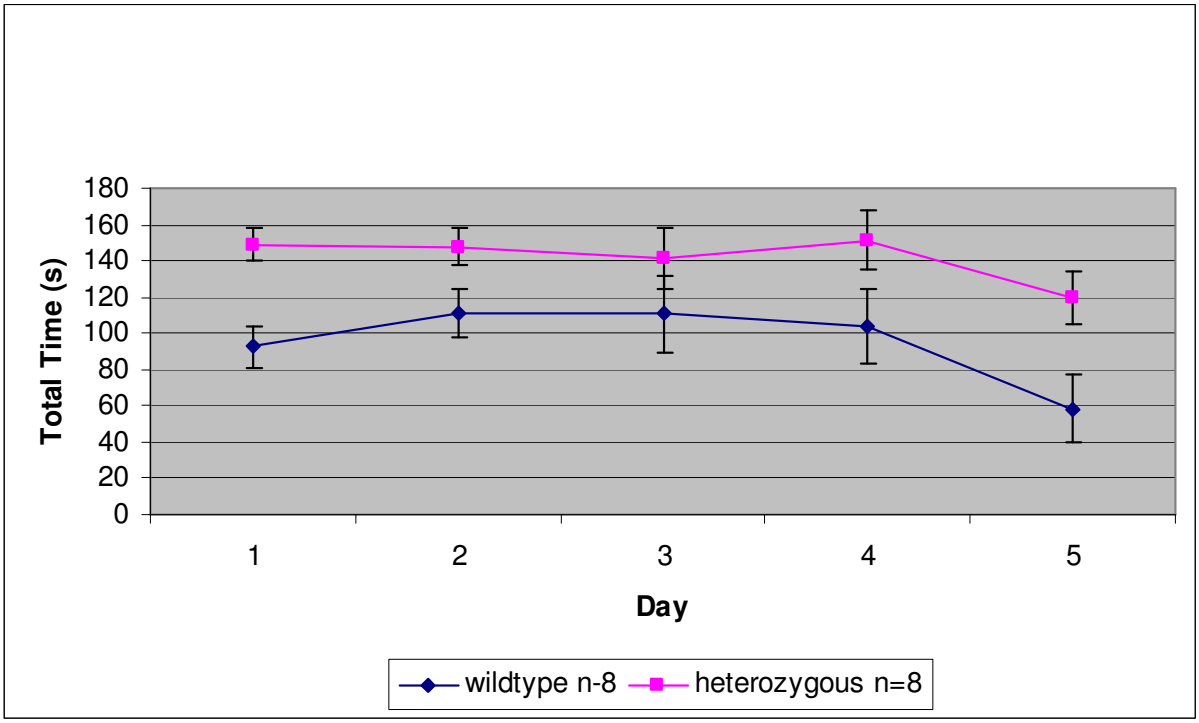

Figure 4: Latency scores (in seconds) for Study 1, Non-spatial Water Maze (5 days, average latency). Results show a significant main Treatment effect between the wildtype and heterozygous groups [$F(1,11) = 8.046$, $p < 0.05$], indicating that mice heterozygous for Dcdc2 performed worse (longer latencies) on the NSWM task.

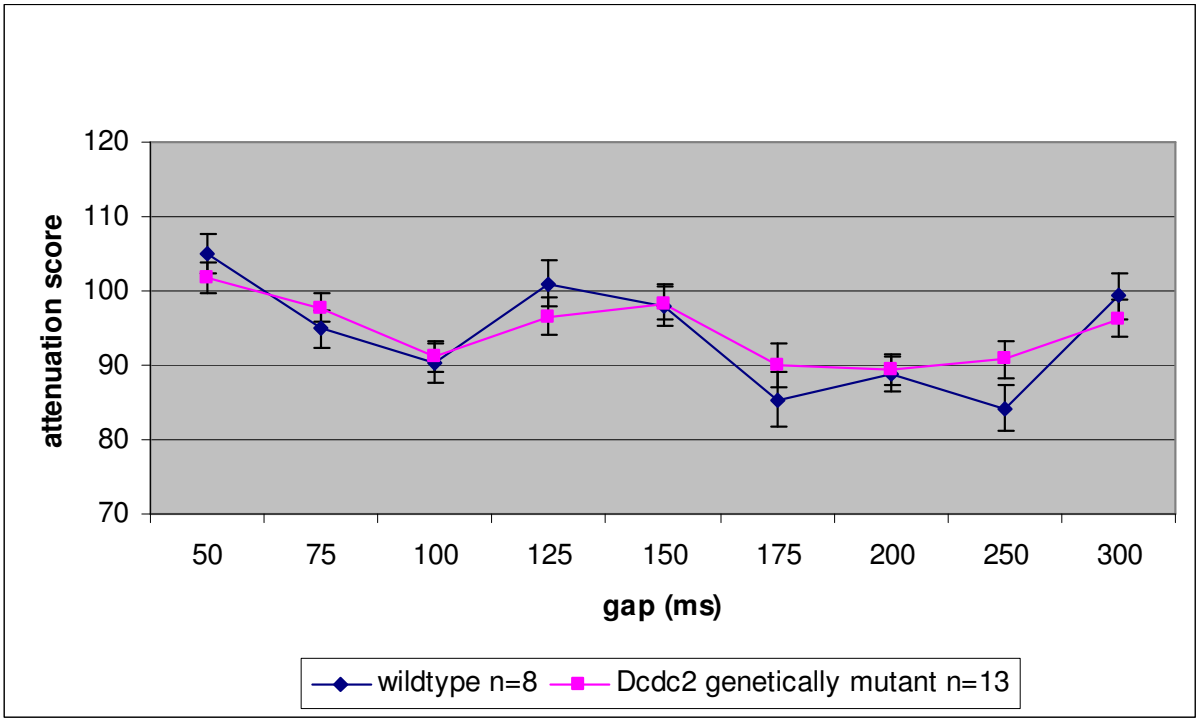

Figure 5: Attenuated scores for Studies 1 and 2 (pooled), Silent Gap 0-300 ms (3 days, averaged). Results indicate no overall significant Treatment effects between wildtype and Dcdc2 genetically mutant groups ($p>0.05$).

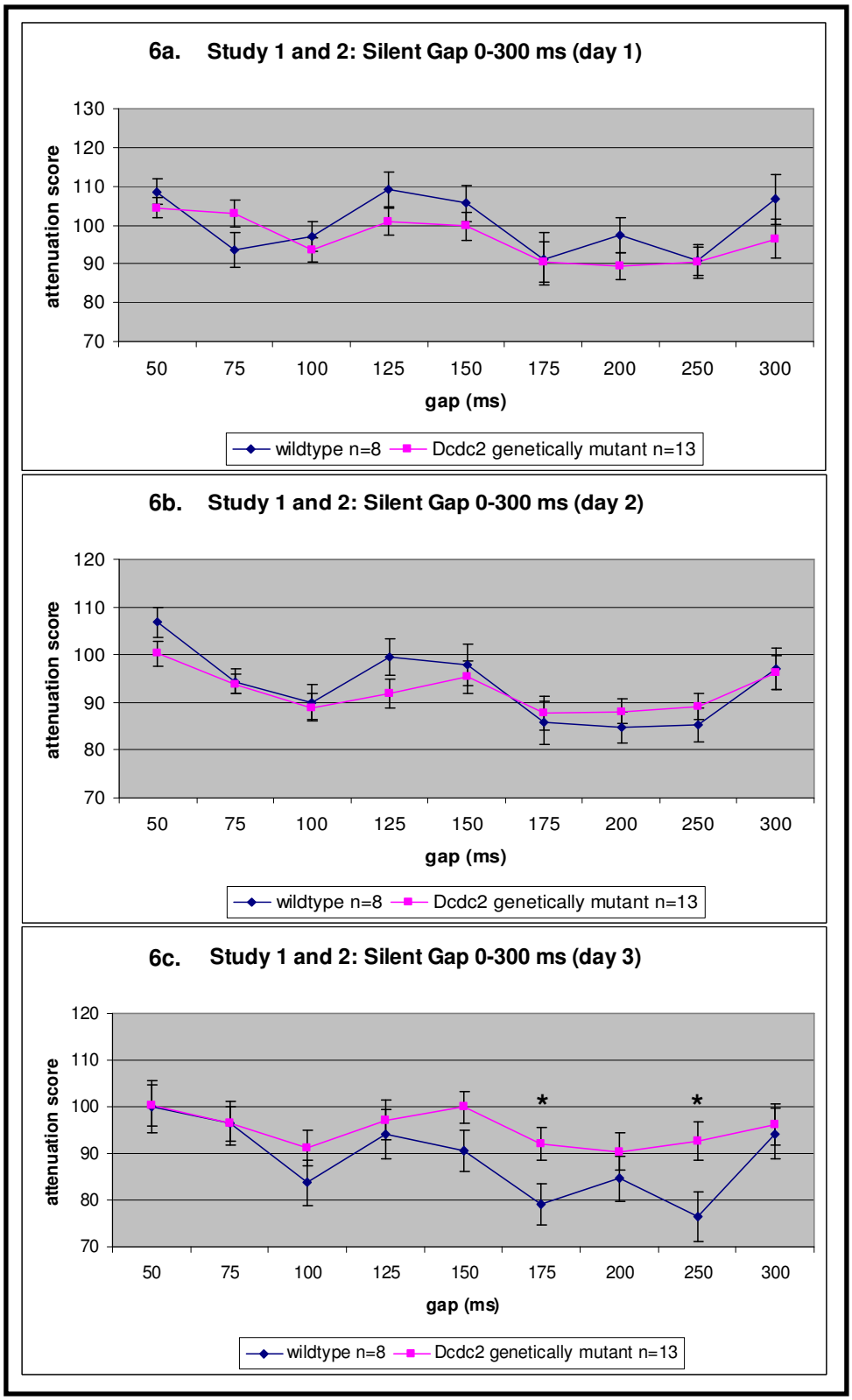

Figure 6a-c:

Separate examination of Silent Gap 0-300 ms testing from Days 1-3. There were no overall significant Treatment effects for each day ($p>0.05$). However, we found significant Treatment effects for both the 175 and 250 ms silent gap intervals ($p = 0.035$ and 0.027 respectively) on Day 3 of testing (wildtype controls performing significantly better than the Dcdc2 genetically mutant group). Wildtype controls also show significant improvement in performance from Day 1 to Day 3, whereas the Dcdc2 genetically mutant group show minimal improvement.