THE EFFECT OF DYSLEXIA GENE *DCDC2* KNOCKOUT ON PERFORMANCE DURING A PREDICTION TASK IN RATS

by

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ABSTRACT

Dyslexia is a prevalent developmental disorder characterized by unexpected reading difficulty in children and adults with otherwise normal nonverbal IQ . Dyslexia is a heterogeneous disorder and a variety of deficits are observed in the population, with auditory perception and rapid stimulus processing deficits occurring most frequently, though not always in the same individuals (Ferrer et al., 2009; Peterson & Pennington, 2012, Shaywitz, 1998). Genetic variants are likely related to this heterogeneity. One such gene that has been reliably linked to dyslexia is the neural migration gene *DCDC2* (Galaburda et al., 2006; Neef et al., 2017, Scerri et al., 2011). Suppression of this gene in a rat model dramatically impairs speech-sound discrimination ability from a stream of rapidly-presented auditory stimuli (Centanni et al., 2016), suggesting a potential role for this gene in rapid stimulus processing deficits in humans and supporting a prior study linking this gene to reading speed (Neef et al., 2017). One potential casualty of processing speed impairments is the ability to process unpredictable stimuli. In the current study, we designed a rapid speech sound discrimination and prediction task to evaluate whether the rapid speech sound impairment previously linked with *Dcdc2* also causes deficits on a prediction task. If increased presentation rate impairs the ability to process unpredictable stimuli, then the addition of a stable predictor sound should improve performance. To test this hypothesis, homozygous *Dcdc2*-knockout, heterozygous *Dcdc2*-knockout, and wild type rats were trained to respond to a target sound /dad/ in a stream of rapidly presented distractors in the presence or absence of a predictor sound /bad/, which occurred reliably prior to the target in 40% of trials. In wild type rats, the results indicate the presence of a predictor enhances response to the target /dad/ at low speeds, but as the stimulus presentation rate increased the rats began responding to the predictor

/bad/ rather than the target. I will present these findings as well as pilot data from rats with *Dcdc2* knockout to investigate the role of this gene on the response to a stable predictor.

The Effect of Dyslexia Gene DCDC2 Knockout on Performance During a Prediction Task in Rats

Dyslexia is a developmental disorder characterized by unexpected reading difficulty in children and adults (Shaywitz, 1998). Individuals with dyslexia often have difficulties with accurate word recognition, spelling, and decoding. However, the deficits associated with dyslexia are not due to deficiencies in nonverbal IQ (Ferrer et al., 2009; Peterson & Pennington, 2012). Dyslexia is a heterogeneous condition with multiple etiologies and can be caused by a variety of deficits. There are several plausible core deficits believed to contribute to developmental dyslexia, including but not limited to theories of phonological, low-level auditory, and cerebellar deficits (Valdois et al., 2004).

Phonological processing theory postulates there is a deficit in the representation, storage, or retrieval of speech sounds (Ramus, 2003). A deficit in phonological processing is the most consistent finding in cases of dyslexia, encompassing rapid-automatized naming, phoneme awareness, and verbal short-term memory (Ramus, 2003). These specific difficulties in the representation, storage, and retrieval of speech sounds can translate to decreased reading ability and comprehension in individuals with dyslexia. For example, individuals with dyslexia have been shown to exhibit specific deficits in phoneme-grapheme conversions (Snowling, 1981).

A second perspective postulates that differences in auditory perception in individuals with dyslexia could account for the observed reading difficulty. Individuals with dyslexia make more errors in responding to rapidly presented auditory stimuli than typically developing individuals (Tallal, 1980). Further, individuals with dyslexia exhibit reduced processing ability of auditory stimuli, with no observed difference in stimulus detection, suggesting there is an

error in coding (Stein & McAnally, 1995). This could point to auditory dysfunction as a cause of the reading deficits associated with dyslexia.

The cerebellar theory of dyslexia posits that reading deficits associated with dyslexia are a symptom of a larger deficit in the cerebellum (Argyropoulos, 2016; Nicolson & Fawcett, 1990; Rae et al., 2002). The cerebellum has been demonstrated to play a role in cognition and can impact lexical retrieval and semantic prediction during language processing (D'Mello et al., 2017; Marien et al., 2001). Further, some individuals with dyslexia exhibit gray matter asymmetry of the cerebellum, with differences from typically developing individuals occurring in the right cerebellum (Eckert et al., 2003; Pernet et al., 2009; Rae et al., 2002). Since the cerebellum is contralateral, this suggests it plays a role in reading, and asymmetry of the cerebellum may cause deficits associated with dyslexia. The cerebellum generates predictions during language processing, and the reading difficulties symptomatic of dyslexia could be due to an inability to accurately predict and automatize language (Argyropoulos, 2016; Nicholson et al., 2001). Thus, it is possible that a deficit in cerebellar functioning could lead to an inability to make accurate predictions while reading, contributing to the symptoms of dyslexia.

There are several genes associated with dyslexia which may account for its observed heterogeneity, including *DCDC2*, *KIAA0319*, *DYX1C1*, and *ROBO1* (Galaburda et al., 2006). Of these, *KIAA0319* and *DCDC2* have been the most studied. *KIAA0319* has a demonstrated positive association with neural variability in individuals with dyslexia (Centanni et al., 2018; Cope et al., 2005; Neef et al., 2017). Further, knockdown of *Kiaa0319* in rats has been shown to reduce corpus callosum size, which could imply reductions in temporal processing contributing to deficits associated with dyslexia (Szalkowski et al., 2012). In contrast, *DCDC2* is involved in neural migration and has been linked to auditory and language processing deficits (Neef et al.,

2017, Scerri et al., 2011). Suppression of *Dcdc2* in rats has been shown to cause deficits in discrimination of rapidly-presented speech sounds (Centanni et al., 2016). This suggests that *DCDC2* plays a role in auditory processing, and changes in *DCDC2* may contribute to deficits in speech-sound discrimination. It is possible that reading deficits in some individuals with dyslexia are due to cerebellar abnormalities caused by genetic variation in *DCDC2*. The current study used speech sound discrimination and prediction tasks to explore the effect of *Dcdc2* knockout on prediction ability in a rat model. The study was designed to test the hypothesis that *Dcdc2* knockout contributes to cerebellar abnormalities which cause deficits in language prediction symptomatic of developmental dyslexia.

Methods

Animals

CRISPR/Cas9 was used to target the rat homolog of *DCDC2* (*Dcdc2*) in Sprague-Dawley rats, produced by GenOway (https://www.genoway.com). The animals ranged in age from 3 to 6 months at the time of study. The subjects included 3 wild type rats, serving as a control. The genetically modified group consisted of 2 heterozygotes and 1 homozygous knockout. Throughout the course of their training, subjects were food-deprived, but maintained a body weight above 85% of their pre-deprivation weight. All researchers were blind to the genotype of the subjects throughout data collection.

Behavioral Paradigm

Rats were trained on a 25-stage behavioral paradigm created to assess prediction ability in the context of rapidly presented speech sounds. Training occurred in soundproof booths, with each subject training in the same booth for one-hour sessions twice per day. Rats were presented with a sequence of English consonant-vowel-consonant (CVC) speech sounds and were trained

to respond only to the target sound /dad/. Trials were initiated when the rat entered an infrared-activated nose poke. Animals indicated the presence of the target sound by removal of the nose. Correct responses were rewarded with a 45 mg sugar pellet. Incorrect responses triggered a light-out period where the program was paused for approximately 6 seconds.

Subjects were first trained under a shaping protocol to learn to associate the infrared-activated nose poke with the target sound /dad/ and the sugar pellet reward. Animals advanced to the next stage of training when they were able to earn 100 or more sugar pellets in a one-hour training session. Shaping was followed by hold training, in which animals were required to remain stationary in the nose poke until they heard the target sound /dad/, with the delay between trial start and target onset progressively increasing. When subjects achieved a d' of 1.5 or greater for 10 sessions they advanced to the next stage of training. The animals were next trained in a series of stages to learn to respond to /dad/ and ignore several distractor sounds: /sad/, /tad/, /gad/, and /bad/ (Centanni et al., 2014). Rats were rewarded for a correct response within 500 ms of the target onset and were punished with a 6s pause session for false alarms. Stages 3-6 consisted of the target sound and one distractor, with each stage including a different distractor sound. Stage 7 consisted of the target sound randomized with all distractors. Stages 8-11 consisted of all distractors and strings of 2 distractors presented with the target sound. Stages 12-17 consisted of all distractors and varying compressions. After completing stage 17, subjects moved from the speech discrimination protocol into the prediction protocol. Criteria for advancement through the stages of the speech discrimination paradigm are shown in Table 1.

In the prediction paradigm, the predictor sound /bad/ was always followed by the target sound /dad/. The target and predictor were presented in succession surrounding by a randomized stream of distractors: /gad/, /sad/, /tad/. Rats were only presented with the sound while their nose

was in the poke and were rewarded for correct responses within 500ms of the target sound presentation (Centanni et al, 2016). Subjects first completed a stage in which they were exposed to the predictor /bad/ preceding the target sound /dad/ to establish the sound /bad/ as a reliable predictor. Next, rats completed a ten-session assessment stage in which they were exposed to the target sound /dad/ in the presence and absence of the predictor /bad/. The predictor sound /bad/ was presented prior to the target approximately 40% of the time. The ten-session assessment included all distractors and all compressions. Criteria for advancement through the stages of the prediction paradigm are shown in Table 2.

Table 1

Speech Discrimination Criteria

Stage	d'	Number
1	any	any
2	1.5	10
3-11	1.5	2
12	1.5	8
13-16	any	4
17	any	10

Table 1. Criteria required for rats to advance through stages of the speech discrimination paradigm.

Table 2

Prediction Criteria

Stage	d'	Number
19	1.5	2
25	any	10

Table 2. Criteria required for rats to advance through stages of the prediction paradigm.

Statistics

Analyses for training and prediction testing data were conducted using repeated-measures analysis of variance (ANOVAs). To determine the accuracy of the groups in responding to the target /dad/, the sum of all false alarms was subtracted from the total number of hits. Response accuracy was determined by comparing this value to the total number of responses. Response rates were determined at each compression and compared within group. Heterozygous *Dcdc2*-knockout rats and homozygous *Dcdc2*-knockout rats were grouped together for statistical analysis to determine the effect of variation in *Dcdc2*. Speech discrimination training data were analyzed using a 2x2 ANOVA for genotype (wild type x *Dcdc2*-knockout) and sex (male x female). Prediction data was analyzed using a 2x2x2 ANOVA to account for genotype (wild type x *Dcdc2*-knockout), sex (male x female), and condition (predictor present x predictor absent). Response accuracy was examined at each compression and compared within group to determine the effects of the introduction of the predictor. Significant main effects and interactions were then evaluated further using post-hoc t-tests.

Results

Of the 6 rats beginning the training paradigm, one heterozygous *Dcdc2*-knockout rat was a statistical outlier, unable to complete the study before exceeding the 6-month age parameter, and therefore excluded from the data set.

There were no significant differences between groups in response to the target /dad/ in the presence or absence of the predictor (Figure 1). However, homozygous knockouts responded more to the distractors than did wild type or heterozygous rats, further supporting previous findings that *Dcdc2* knockout impairs speech sound discrimination of rapidly-presented stimuli (Centanni et al., 2016). As presentation rate increased, all genotypes began to respond to the

predictor /bad/ rather than the target /dad/ (Figure 1). This appears to be due to anticipation of the stimulus rather than stimulus generalization, as the effect is only observed for the predictor /bad/ and not observed for all distractors.

Figure 1

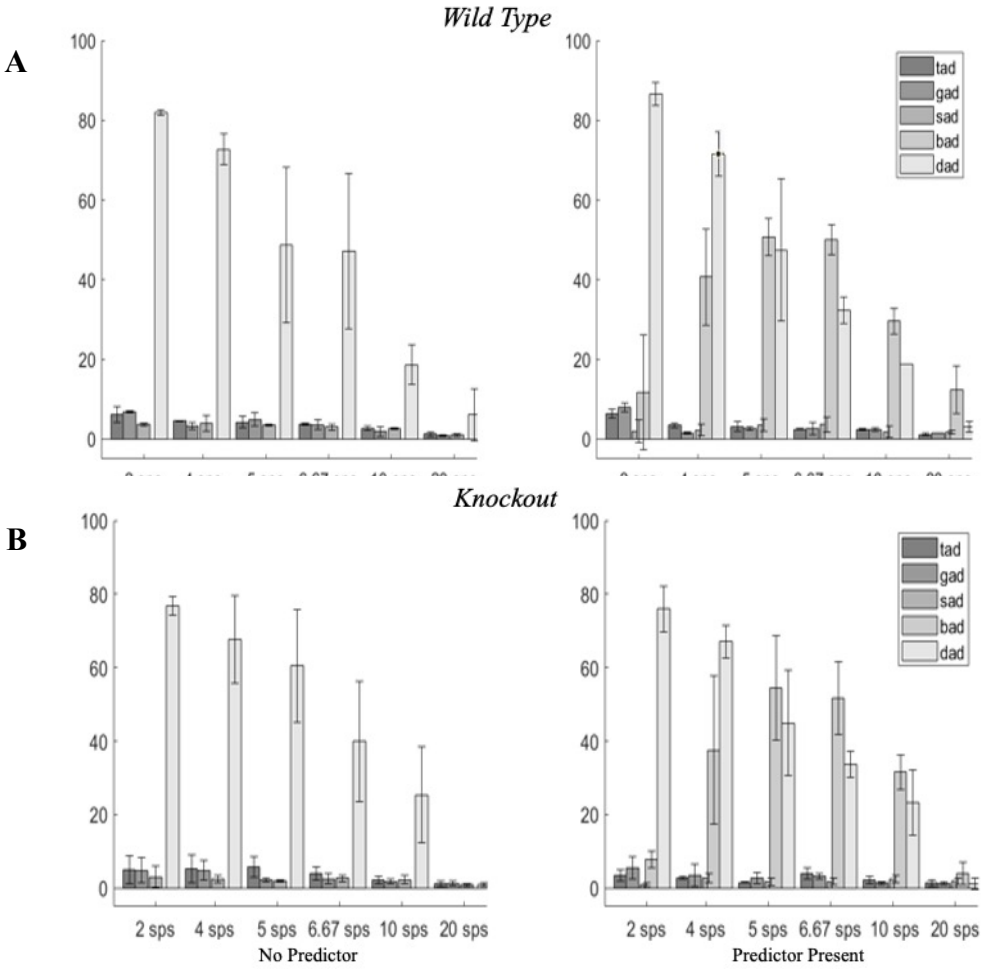

Figure 1. Response rates of rats to target /dad/ and all distractors (/tad/, /gad/, /sad/, /bad/) in the presence and absence of the predictor /bad/. **A.** Response rates to all stimuli of wild type rats in the presence and absence of the predictor /bad/. **B.** Response rates to all stimuli of *Dcdc2*-knockout (both homozygous and heterozygous) rats in the presence and absence of the predictor /bad/.

There were no significant differences between wild type and *Dcdc2*-knockout rats when the predictor was not present (2-tailed unpaired t-test, t (df) = tstat, $p > 0.253$). There were also no significant differences between wild type and *Dcdc2*-variant rats when the predictor /bad/ was present (2-tailed unpaired t-test, t (df) = tstat, $p > 0.122$). The *Dcdc2*-knockout rats performed worse in the presence of the predictor /bad/ than in its absence (Figure 2). Notably, the *Dcdc2*-knockout rats were highly variable in response in the presence of the predictor.

Figure 2

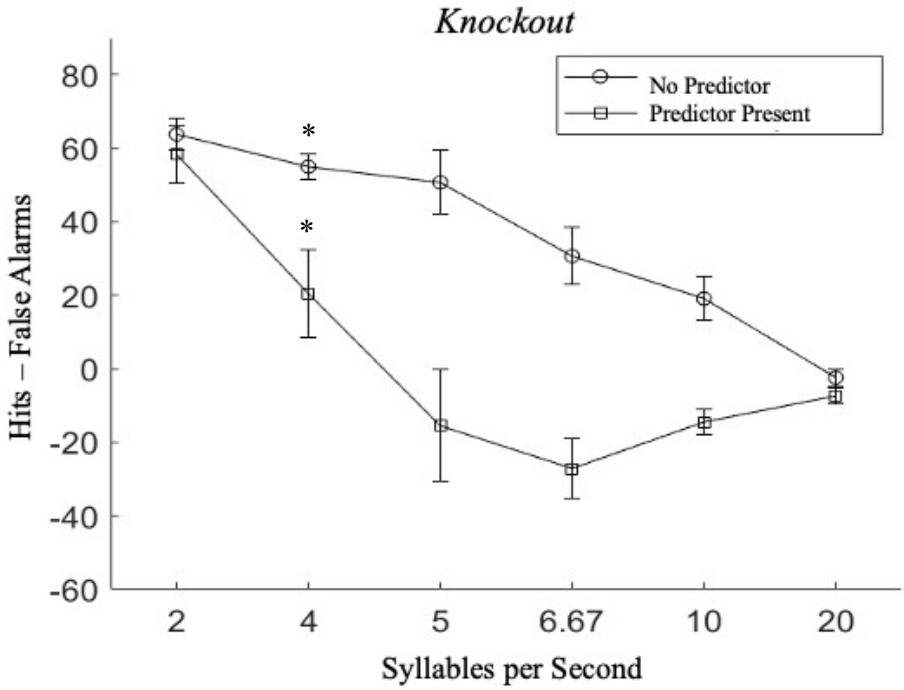

Figure 2. Results of prediction study in wild type and *Dcdc2*-knockout rats. *Dcdc2*-knockout rats' response to the target was significantly impaired in the presence of the predictor.

Discussion

Suppression of neural migration gene *Dcdc2* in a rat model has been shown to significantly impair speech sound discrimination in a stream of rapidly-presented auditory stimuli (Centanni et al., 2016). In the current study, we designed a rapid speech sound discrimination and prediction task to determine whether previously observed deficits in speech sound discrimination also caused deficits in prediction ability.

During the speech sound discrimination task, *Dcdc2*-variant rats responded to distractors /bad/, /gad/, /sad/, and /tad/, more than wild type. This further supports previous findings that *Dcdc2*-suppression causes severe deficits in speech sound discrimination in a stream of rapidly-presented auditory stimuli (Centanni et al., 2016). However, there were no significant differences observed between wild type and Dcdc2-variant groups in the presence or absence of the predictor. This could be due to small sample size (n=5), or could indicate that variation in *Dcdc2* does not cause prediction deficit. While *Dcdc2* has a demonstrated role in auditory processing (Centanni et al., 2016; Neef et al., 2017, Scerri et al., 2011), *Dcdc2* variation may not significantly affect the cerebellum to cause deficits in prediction.

However, rats of all genotypes performed significantly worse in the presence of the predictor /bad/ than in its absence. As stimulus presentation rate increased, rats' performance decreased, regardless of genotype. As speed increased, rats in all groups began to respond to the predictor /bad/ rather than the target /dad/. Dyslexia has been shown decrease the speed of stimulus classification (Nicolson & Fawcett, 1994), however, since this effect is only observed for the predictor /bad/ and not for all distractors, and is observed across genotypes, this effect is likely due to anticipation of the stimulus rather than due to a stimulus generalization effect. At a presentation rate of 4 syllables per second (sps), the *Dcdc2*-variant rats responded less to the

predictor than the wild type. This prediction deficit could indicate cerebellar deficit, as the cerebellum generates predictions throughout language processing (Argyropoulos, 2016).

The largest limitation of this study was sample size. Only five rats were tested, and of these only one was a homozygous *Dcdc2*-knockout. Regardless of the small sample size, we observed deficits in speech sound discrimination of rapidly-presented stimuli and observed an anticipation effect in responding to the predictor rather than the target. However, it is possible that a larger sample would further illuminate deficits of *Dcdc2*-knockout rats in a prediction task.

A further limitation to this study is the inability to generalize findings in rodents to humans. Since the speech sounds used in the study do not have ecological relevance in rodents, it is possible that we would observe a different effect in humans. Further study is required to determine the role of *DCDC2* in contributing to dyslexia phenotypes in humans. Larger sample size would provide a better understanding of the effect of *Dcdc2*-knockout on prediction in a rat model, and further study could include a prediction study in humans with *DCDC2*-variant genotypes.

The results of the study are somewhat contradictory. The lack of significant difference between genotypes in response to the target in the presence or absence of the predictor could indicate that *Dcdc2* variation does not impact the cerebellum or prediction ability. In contrast, the finding that *Dcdc2*-variant rats responded less to the predictor /bad/ at a speed of 4 sps could indicate an inability of these rats to anticipate the predictor, which could be due to cerebellar deficit. Further study is required to confirm the results of the study and understand the effect of Dcdc2 variation on cerebellar function in prediction.

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