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Chapter II

Candidate Dyslexia Susceptibility Genes and Disorders of Neuronal Migration: Behavioral Effects of Cortical Dysgenesis in a Rodent Model

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Abstract

Subtle alterations in the development of the neocortex are associated with cognitive deficits in humans and other mammals. A large number of genes modulate the development of the neocortex, and research into the behavioral phenotype associated with specific gene manipulations is advancing rapidly. Recent findings include evidence that anomalies in several human genes, (*DYX1C1, DCDC2, KIAA0319, MRPL19, C2ORF3, ROBO1, KIAA0319L*), may be associated with an increased incidence of dyslexia. Concurrent research has shown that the rat homologs for a subset of these genes (*DYX1C1, DCDC2, KIAA0319, ROBO1*) modulate critical parameters of early cortical development, including neuronal migration. Recent studies have utilized animal models of genetic disruption, using in utero RNA interference to knockdown expression of *Kiaa0319* and *Dyx1c1* in developing rat pups. Analyses have shown that interference with these genes leads to aberrant neuronal migration in the neocortex, resulting in the formation of ectopias and heterotopias in white matter. Interestingly, these malformations are similar to those observed in histological studies of brain tissue obtained *post mortem* from dyslexic individuals. Additionally, these genetic disruptions to neuronal migration have been associated with deficits in rapid auditory processing, spatial learning, and working memory in animal models. Parallel deficits in core processes critical to language and learning have been reported in dyslexic populations. Specifically, whereas

dyslexia is diagnostically defined overall as a reading impairment in the absence of any generalized cognitive or neurologic deficit, this disorder likely reflects complex effects of multiple genes on multiple neural systems. As such, it is not surprising that more fine-grained behavioral sub-phenotypes have been identified within the dyslexic population (also called "intermediate phenotypes" in human genetic studies; see Marino *et al.*, 2007), and these may ultimately prove easier to link to the effects of specific dyslexia-risk genes. Reported sub-phenotypes include specific deficits in short term or working memory (verbal and also non-verbal), visual attention, and rapid auditory processing. It must be noted that the mapping of these intermediate phenotypes onto "clinical sub-types" of dyslexia (e.g., phonologic versus surface or orthographic dyslexia) has proved difficult—likely due to variations in definition, classification, and approach—but it seems likely that the clinical sub-types reported for dyslexia may reflect differential intermediate phenotypes and, in turn, the influence of different genes or gene combinations. One benefit of a focus on these intermediate phenotypes is that such behavioral measures are more amenable to modeling in animals (whereas reading *per se* is not). Thus, rodent models can be used in paradigms of working memory, visual attention and rapid auditory processing. Moreover, such rodent models can be employed to link specific intermediate phenotypes with individual dyslexia-risk genes. In the present review, behavioral results and histological data are discussed, and comparisons are made between the neuroanatomical and behavioral impacts of early interference with *Dyx1c1* and *Kiaa0319* in rodents. The recent findings are summarized and discussed in the context of improving our understanding of the genetic contributions to the behavioral expression of dyslexia in humans.

Introduction

Dyslexia and Neuronal Migration

Neocortical neuronal migration is the process by which newly divided neurons travel from the ventricular zone (VZ) where they are formed to their final target layer in the cerebral cortex. This delicate and important stage of neurodevelopment requires the perfect synchrony of several complex processes such as cell adhesion, cell signaling, and a vast array of dynamic cytoskeletal modifications (for review, see Hatten, 1999). Disruption to any of these processes can have detrimental effects on neuronal migration and thus to the development of the brain, and several well-known disorders are directly related to aberrant migration of neocortical neurons (Roberts, 2007; Spencer-Smith *et al.,* 2009; Valiente and Marin, 2010; Walsh *et al.,* 2000; Wynshaw-Boris *et al*., 2010). In general, disruption to neuronal migration leads to misplaced neurons within and outside of the cortex, which often results in aberrant patterns of connectivity and synaptic transmission—ultimately creating atypical physiological and behavioral effects. In many disorders, the effects of disrupted neuronal migration are severe—often presenting as significant mental retardation, motor impairment, and in some cases even death (see Valiente and Marin, 2010; Wynshaw-Boris *et al.*, 2010 for review). Such disorders are perhaps not surprisingly accompanied by large scale changes to the structure of the brain that can easily be detected by MRI or CT scans.

Research within the last several decades has revealed that small, focal disruptions of neuronal migration are also associated with significant (though generally more specific and

subtle) behavioral deficits. For example, Galaburda and colleagues (1985) examined *post mortem* brain tissue samples from individuals diagnosed with developmental dyslexia and discovered numerous focal dysplasias indicating early disruptions in the process of neuronal migration. Indeed, each of the four brains displayed several incidences of neuronal "nests" formed by neocortical neurons that had accumulated in small clusters within Layer 1 of the cortex. Along with these ectopic collections of cells, Galaburda *et al.* observed small instances of aberrant folding of the cortical tissue. These microgyria were characterized by fused laminae (only appearing to be 4 layered instead of 6), and a lack of columnar organization across the cortical layers in the affected tissue. Interestingly, the malformations were generally concentrated in perisylvian regions in the left hemisphere. Additionally, the observed cortical changes were accompanied by cell size changes in the medial geniculate nucleus, which had fewer large cells and more small cells than the MGN in control brains (Galaburda *et al.,* 1994)**.** Although the cortical anomalies were not present in the exact same locations in each of the four brains, and some patients had more instances of disruption than others, Galaburda *et al.* concluded that these lesions had some pathogenetic role in the subjects' dyslexia. Authors suggested that the early cortical lesions may have led to widespread cortical reorganization, and in turn to the formation of anomalous connections. This was impossible to confirm in the *post mortem* tissue samples, but concurrent evidence supporting this proposed cortical rewiring comes from the use of animal models, which will be discussed later in this review. This was the first documented evidence suggesting a relationship between developmental dyslexia and aberrant neuronal migration. Unlike the neuroanatomy characteristic of many other neuronal migration disorders, the collections of aberrantly placed neurons in these brains were too small to be detected by current methods of *in vivo* imaging. This is perhaps not surprising given that developmental dyslexia—unlike disorders such as lissencephaly or double cortex syndrome—is not characterized by severe global impairments in learning. Instead, the deficits that define dyslexia are significant, but subtle and domain-specific.

Dyslexia Phenotypes: Rapid Auditory Processing

Developmental dyslexia is a complex disorder that is characterized by a variable constellation of underlying core behavioral deficits. The hallmark behavioral impairment in dyslexia is a deficit in reading, in spite of adequate intelligence and educational opportunity (Shaywitz, 1998). However, the behavioral deficits of dyslexia also encompass lower-order reading-related processes. For example, many dyslexic and language impaired individuals exhibit deficits in rapid auditory processing, which is the ability to detect changes in rapidly presented verbal or non-verbal acoustic cues**.** Tallal and colleagues were the first to demonstrate that verbal and non-verbal acoustic discrimination abilities were significantly correlated with performance on a nonword reading task (Tallal, 1980). These findings suggested that lower-level sensory processing abilities were associated with higher order speech and reading-related abilities. Several additional studies have since come out demonstrating a similar relationship between auditory processing and reading abilities in language impaired populations (Boets *et al.*, 2011; Boscariol *et al.*, 2010; Cohen-Mimran and

Sapir, 2007; Czamara *et al.,* 2010; Gaab *et al.*, 2007; King *et al.*, 2008; Vandermosten *et al.*, 2011)**.** Moreover, longitudinal studies by Benasich and colleagues have demonstrated that in children with a family history of language disability, deficits in the discrimination of two distinct tones (when presented at a short stimulus) can be elicited prior to emergent speech and reading capabilities, and serve as significant predictors of a future diagnosis of language impairment (Benasich *et al.*, 2002; 2006; Choudhury *et al.*, 2007). Taken together, these findings provide compelling evidence for the existence of a relationship between lower level sensory processing abilities and higher order language-related processes such as reading in dyslexia.

Dyslexia Phenotypes: Memory Impairment

Deficits in memory have also been reported in dyslexia. For example, deficits in verbal short term and working memory have been detected in some populations of dyslexics, and appear to be related to abilities such as word list recall—which is thought to tap into the phonological loop of working memory (Baddeley and Hitch, 1974). Similarly, language impaired individuals have been found to have impairments in verbal working memory: that is, the ability to process and store higher order verbal information that is required for sentence comprehension (Archibald and Gathercole 2006; Gathercole *et al.*, 2006; Smith-Spark and Fisk, 2007).

Deficits in visuospatial processing and visual memory, both of which are important for specific reading tasks, have also been occasionally reported in dyslexia. Dyslexic individuals have demonstrated impairments in visual pattern sequence recall as measured by the Corsi block span test and the visual patterns test (Smith-Spark and Fisk, 2007). Thus, the behavioral impairments that characterize dyslexia do not appear to be limited to verbal or strictly reading-related domains. It is also important to note that these deficits (and additional deficits not mentioned here, see Habib, 2000 for a complete review) are not represented homogeneously across all dyslexic individuals. In fact, a great deal of variability exists in the types and severity of impairment present from one individual to another. As such, some recent research into the etiology of developmental dyslexia has focused on the underlying behavioral deficits, or "intermediate phenotypes," that, in variable combinations with each other, make up a holistic diagnosis of dyslexia (Grigorenko, 2009).

Animal Models of Dyslexia: Behavioral Tasks

The interpretation of dyslexia as a clinical diagnosis overlying a pattern of causal intermediate phenotypes has opened the door for the use of animal models to study some of these lower-order behavioral processes, as well as their underlying neural substrates. For example, rapid auditory processing (RAP) is one behavioral facet of dyslexia that is amenable to animal modeling. In one set of studies, an operant conditioning paradigm was used to assess auditory processing capabilities of rats. Briefly, the rats were trained to discriminate

and respond to a specific temporal order of a pair of high and low frequency tones in a go/no go paradigm. The interstimulus interval (ISI)—or the amount of time between the two tones in the pair—was gradually reduced over the course of testing to allow examination of the animals' acoustic processing abilities as a function of temporal parameters of the task (Fitch *et al.*, 1994).

More recent work from our lab has utilized a modified prepulse inhibition paradigm to assess temporal acoustic discrimination thresholds in rats as a function of attenuation of their acoustic startle reflex (see Fitch *et al.*, 2008a). The task relies on the basic tenets of prepulse inhibition, in which a startling stimulus is preceded by a non-startling prepulse or cue that the animal is able to detect and process, causing the animal's resulting acoustic startle reflex (a gross motor reflex) to be reduced in amplitude. During testing, animals are presented with a series of acoustic programs that are progressively more complex and difficult to discriminate, beginning with a basic silent gap detection task and progressing to a two-tone Oddball discrimination task and a frequency modulated (FM) sweep discrimination task. In all tasks, during cued trials the prepulse or cue is presented at variable rates, from long (easy to discriminate) to short (difficult to discriminate) interstimulus intervals. In all RAP tasks, an animal's relative ability to discriminate a cue is calculated as an "attenuation score" (ATT), which is a ratio of the amplitude of the animal's acoustic startle reflex during cued trials to the amplitude during uncued trials. A ratio of less than 1 (or 100%) indicates discrimination of the cue.

Fundamental short term and working memory abilities similar to those reported in dyslexia can also be tested using animal models. For example, recent work from our lab utilized a delayed match-to-sample radial arm water maze to assess rodent working memory abilities (see Chrobak *et al.*, 2008). Briefly, the task requires the animals to remember the position of a goal platform located at the end of one arm in an eight arm radial water maze. Each subject is initially presented with the location of the goal during a forced choice sample, in which all arms are blocked off besides the start arm and the goal arm (both of which varied systematically across days of testing). Following a retention interval of 10 minutes, the animal is returned to the maze in a new start position, and all of the arms are open. The animal's ability to retrieve the stored goal location from memory is measured as a function of the number of incorrect arms entered before finding the goal arm.

Animal Models of Dyslexia: Focal Freeze Lesions

Galaburda *et al.'s* findings of disrupted neuronal migration in the brains of dyslexics, combined with the ability to model some of the core behavioral processes involved in dyslexia, led to the development of subsequent animal models of migrational disruption. For example, one of the first animal models of "dyslexia" used in our lab was created via mechanical disruption to neuronal migration in rat pups. This was achieved through application of a freezing probe to the animal's skull plate on postnatal day 1 (P1), which produced a necrotic injury to the subjacent cortical tissue, characterized by the formation of a microgyric fold over time. The induced microgyric lesion was strikingly similar to those observed in the brains of dyslexic individuals, and was characterized by fused laminae and

disrupted columnar organization (Rosen *et al.*, 1992). Anatomical studies of the brains of microgyric animals revealed widespread changes related to the disruption of neuronal migration, including significant changes in synaptic connections between the microgyric cortex and other areas of the cortex and the thalamus (Rosen *et al.*, 2000). Additional evidence for widespread changes following induction of microgyria came from another study that demonstrated that microgyria induced in the whisker barrel fields via focal freezing in P1 rat pups lead to an increase in size in the contralateral barrel field (Rosen *et al.,* 2001). Moreover, analysis of thalamic cells in microgyric animals revealed a shift in cell size distribution in the MGN of male animals following induction of the lesion in somatosensory cortex (Peiffer *et al.,* 2002a; Rosen *et al.*, 2006). The MGN of male microgyric animals was observed to have more small cells and fewer large cells than their sham counterparts—a finding highly similar to observations from dyslexic human *post mortem* brains (Galaburda *et al.,* 1994).

Given the neuroanatomical similarities between the brain tissue from dyslexic patients and microgyric rats, questions arose as to the behavioral phenotype of these animals. Subsequently, behavioral assessments of microgyric rats revealed interesting parallels to the clinical deficits observed in dyslexic humans. For example, when tested on the previously discussed operant conditioning go/no-go acoustic discrimination task, male microgyric rats showed impaired performance on short interstimulus interval trials only, indicating a specific impairment in rapid auditory processing (Clark *et al.,* 2000; Fitch *et al.*, 1994). Similarly, when tested on the modified prepulse inhibition task, male microgyric rats consistently showed less attenuation of their acoustic startle response (and thus less cue discrimination) when tested with rapidly presented cues (Peiffer *et al.*, 2002b; 2004a-c; Threlkeld *et al.,* 2006). Further behavioral studies of microgyric animals assessed the working memory abilities of lesioned animals, since working memory impairments are detected in some populations of dyslexics and language impaired individuals (Jeffries and Everatt, 2004; Smith-Spark and Fisk, 2007). A study using the previously described eight arm radial water maze found that male microgyric rats consistently made more incorrect arm entries before locating the goal platform throughout testing, indicating impaired spatial working memory as compared to shams (Fitch *et al.,* 2008b).

Animal Models of Dyslexia: NZB and BXSB Mice

More naturalistic animal models of disrupted neuronal migration similar to that observed in developmental dyslexia were derived from populations of inbred mice—specifically, NZB and BXSB mice—that displayed spontaneously occurring neocortical ectopias. The use of these mice allowed for detailed investigation of the electrophysiological properties of cells within Layer 1 ectopias, similar to those observed in the brains from human dyslexics (Galaburda *et al.,* 1994). These focal disruptions to neuronal migration were found to contain both glutamatergic and GABAergic neurons (Gabel and LoTurco, 2001), and cells within ectopias were found to receive both excitatory and inhibitory inputs from surrounding normatopic cortex. Tracing studies also revealed that ectopic cells formed aberrant connections with other regions of the cortex and the thalamus (Sherman *et al.,* 1990; Jenner *et*

al., 2000). Given the observed patterns of connectivity associated with these malformations, it is perhaps not surprising that they were shown to be vulnerable to epileptiform firing patterns (Gabel and LoTurco, 2001). These studies formed a critical foundation for understanding the electrophysiological properties (and potential functional implications) of cortical malformations similar to those observed in human dyslexic patients.

The behavioral profile of these ectopic mice is similar to that of microgyric rats. Studies have consistently demonstrated working memory impairments in ectopic mice when compared to non-ectopic littermates (Boehm *et al.*, 1996; Hyde *et al.,* 2001; Waters *et al.*, 1997). Additionally, male ectopic mice were significantly impaired compared to shams when discriminating rapidly presented acoustic cues, indicating temporally-specific acoustic processing impairments (Clark *et al.,* 2000; Peiffer *et al.,* 2002b). Studies of mice with spontaneously occurring ectopias thus provided a further link between focal disruptions of neuronal migration similar to those observed in the brains of dyslexics, and parallel behavioral deficits, using an animal model.

The Genetics of Dyslexia

Currently, animal models of dyslexia are being created through the use of genetic manipulations. This work has been made possible by recent advances in epidemiological studies of developmental dyslexia, which have detected several regions on the human genome that are significantly associated with the disorder. The initial discovery that dyslexia is a heritable trait was derived from studies of twins, with concordance rates from 50-100% for dyslexia in monozygotic twins, in contrast to dizygotic twins' concordance rates of only 29-54% (Willcutt *et al.*, 2010). With the knowledge of dyslexia's heritability, more recent epidemiological studies of dyslexia have utilized genetic linkage studies to detect chromosomal loci that are inherited along with the dyslexia phenotype in affected families (Cardon *et al.,* 1994; Fisher *et al.,* 1999; Grigorenko *et al.,* 1997; 2000; Nopola-Hemmi *et al.,* 2000; Smith *et al.,* 1983). Genetic linkage studies have been followed up by genetic association studies, which seek out changes or mutations within specific genes located in the chromosomal loci identified in the linkage studies. Genetic association studies have been carried out in populations of dyslexics in several countries, and from these studies a handful of genes have been identified as candidate dyslexia susceptibility genes (CDSGs) (Francks *et al.*, 2004; Hannula-Jouppi *et al.*, 2005; Konig *et al.,* 2011; Meng *et al.*, 2005; Poelmans *et al.*, 2009; Scerri *et al.*, 2010; Taipale *et al.*, 2003). The first identified CDSG is located on Chromosome 15. Specifically, a translocation on the long arm of Chromosome 15 interrupting the genetic sequence of a gene—*DYX1C1*—was found to associate with specific reading disability in a single family in Finland (Taipale *et al.,* 2003). Further support for associations between *DYX1C1* and dyslexia has been reported in other populations as well (Brkanac *et al.,* 2007; Dahdouh *et al.,* 2009; Marino *et* al., 2007; Wigg *et al.,* 2004). However, there have also been reports of failed replication attempts (Bellini *et al.,* 2005; Marino *et al.,* 2005; Scerri *et al.,* 2004). Two additional CDSGs were identified on the short arm of Chromosome 6. Specifically, association analyses using markers for loci identified on Chromosome 6 revealed consistent correlations between dyslexia and single nucleotide

polymorphisms (SNPs) within the sequences of the genes *KIAA0319* and *DCDC2* (Cope *et al.,* 2005; Elbert *et al.,* 2011; Francks *et al.,* 2004; Harold *et al.,* 2006; Ludwig *et al.,* 2007; Meng *et al.,* 2005; Newbury *et al.,* 2010; Schumacher *et al.,* 2006; Wilcke *et al.,* 2009). Additional CDSGs have recently been identified, but replications of these findings and further characterizations of the identified genes are limited (Hannula-Jouppi *et al.,* 2005; Konig *et al.,* 2011; Matsson *et al.,* 2011; Poelmans *et al.,* 2008; Scerri *et al.,* 2010). Thus, for the purposes of this review we will focus on findings concerning *DYX1C1* and *KIAA0319*, two well-studied candidate dyslexia risk genes.

Various behavioral assessments have been utilized in the genetic association studies of dyslexic populations, and some discernible patterns between impairments in specific behavioral domains and involvement of specific genes have emerged. For example, there is some evidence suggesting that variation in *DYX1C1* contributes to impairments in reading-related processes such as phonological awareness and phonological decoding—both of which characterize an individual's ability to break words down into smaller acoustic units (phonemes) (Wigg *et al.,* 2004). Additionally, a strong body of evidence has recently emerged indicating that *DYX1C1* likely plays a significant role in short term memory in dyslexics, as measured by tests of verbal short term memory such as nonword repetition and single letter backward span, (both of which require subjects to maintain acoustic information in short term memory during task performance; Dahdouh *et al.,* 2009; Marino *et al.,* 2007; Newbury *et al.,* 2010; Wigg *et al.,* 2004). A recent study in an Australian population also demonstrated that a mutation in *DYX1C1* was significantly associated with memory abilities in a non-affected group of individuals, thus indicating that the gene may be involved in core memory processes in populations of normal readers as well (Bates *et al*., 2010). Taken together, these findings suggest a role for *DYX1C1* in the memory domain, which may account for its main contribution to the development of dyslexia.

Genotype-phenotype relationships between behavior and *KIAA0319* have also been recently reported. For example, various genetic association studies found significant correlations between variants of *KIAA0319* and impairments in reading-related tasks such as single word reading, phonological decoding, phonemic awareness, and orthographic coding (Cope *et al.,* 2005; Francks *et al.*, 2004; Harold *et al.,* 2006). Each of these tasks is phonologically based, and is dependent upon the subject's perception and understanding of letter sounds. Interestingly, there is also evidence that *KIAA0319* may play a role in normal variation in reading abilities in the general population as well (Luciano *et al.,* 2007; Paracchini *et al.,* 2008). In contrast to *DYX1C1,* there is no evidence for or against a role of *KIAA0319* in short term memory to date. Taken together, these data provide compelling evidence for a specific role of *KIAA0319* in phonological reading-related processes.

Animal Models of Dyslexia: RNA Interference

Following the advent of these advances in our genetic understanding of dyslexia, genetically altered animal models have been created to better characterize the rodent homologs of these genes and their protein products. Specifically, rats have been used to create models of a genetic knockdown of the rodent homologs of both *DYX1C1* and *KIAA0319*. In

contrast to a genetic knockout, a genetic knockdown results in reduced amounts of a given gene's protein product, rather than a total loss. Moreover, some genetic knockdown methods allow researchers to assess the effects of genetic alterations in specific bodily systems—such as brain regions—as opposed to the system-wide effects attained with a complete genetic knockout. Recently, several researchers have used RNA interference (RNAi) to create targeted genetic knockdowns of the rodent homologs of *DYX1C1* and *KIAA0319* in populations of neocortical pyramidal progenitor neurons in the ventricular zone during fetal development. Briefly, the RNA interference method involves the creation of a short hairpin RNA (shRNA) plasmid that corresponds to a specific genetic sequence of interest in the rat genome (see LoTurco *et al.,* 2009 for review). When used to study genes in the brain, the shRNA vector is delivered to the population of migrating neurons in the brains of rats during embryonic development via injection into the lateral ventricles. Electroporation creates a temporary, favorable electrical environment that allows the shRNA vector to be taken into the nucleus of cells, where it is transcribed along with the cells' own genetic material. *In vivo*, the shRNA interferes with the translation of the protein that corresponds to the gene of interest. The RNAi method has provided researchers the unprecedented ability to characterize the brain-specific cellular and physiological functions of the Dyx1c1 and Kiaa0319 proteins (the rodent homologs of the human DYX1C1 and KIAA0319 proteins) in live models.

Brain expression profiles have shown that both *DYX1C1* and *KIAA0319* are expressed widely throughout the brain from early in development through adulthood in both rodents and humans. In rodents, *Dyx1c1* is expressed ubiquitously throughout the brain at modest levels, with the highest concentrations found in the neocortex, hippocampus, and choroid plexus (Rosen *et al.,* 2007). *KIAA0319* expression seems to be more specific to the brain than that of *DYX1C1* (which is expressed in several other tissues; see Rebhan *et al.*, 2011). Specifically, *KIAA0319* is expressed in the neocortex, ganglionic eminence, hippocampus, midbrain, and cerebellum of rats and humans (Paracchini *et al.,*2006). Animal models and cell culture lines have allowed researchers to investigate the cellular roles of the Dyx1c1 and Kiaa0319 proteins, both of which remain largely unknown. Recent investigations into the role of the Kiaa0319 protein have suggested that it may be involved in cell adhesion and cell signaling, both processes that play important roles during neuronal migration (Velayos-Baeza *et al*., 2007; 2008; 2010). The potential role of the Kiaa0319 protein in cell adhesion is also supported by the observation that the physical neural-glia relationship—which is known to be mediated by cell adhesion proteins—is completely disrupted in neurons transfected with *Kiaa0319* shRNA. Following transfection, affected neurons exhibited orthogonal orientations to nearby radial glial fibers (Paracchini *et al.*, 2006). Work by Wang and colleagues suggested that the rodent homolog of DYX1C1 may be involved in regulation of cytoskeletal dynamics (Wang *et al.,* 2006). Early interference with *Dyx1c1* caused neurons to remain in the multipolar stage. Typically, migrating neurons transform from the multipolar stage to the bipolar stage. Thus it is possible that the Dyx1c1 protein is required for transformation out of the multipolar stage during migration. Other recent investigations into the cellular function of Dyx1c1 have also indicated a potential role for this protein in estrogen receptor metabolism (Massinen *et al.,* 2010). The potential functional implications of this role in terms of neurodevelopment are a topic of ongoing investigation.

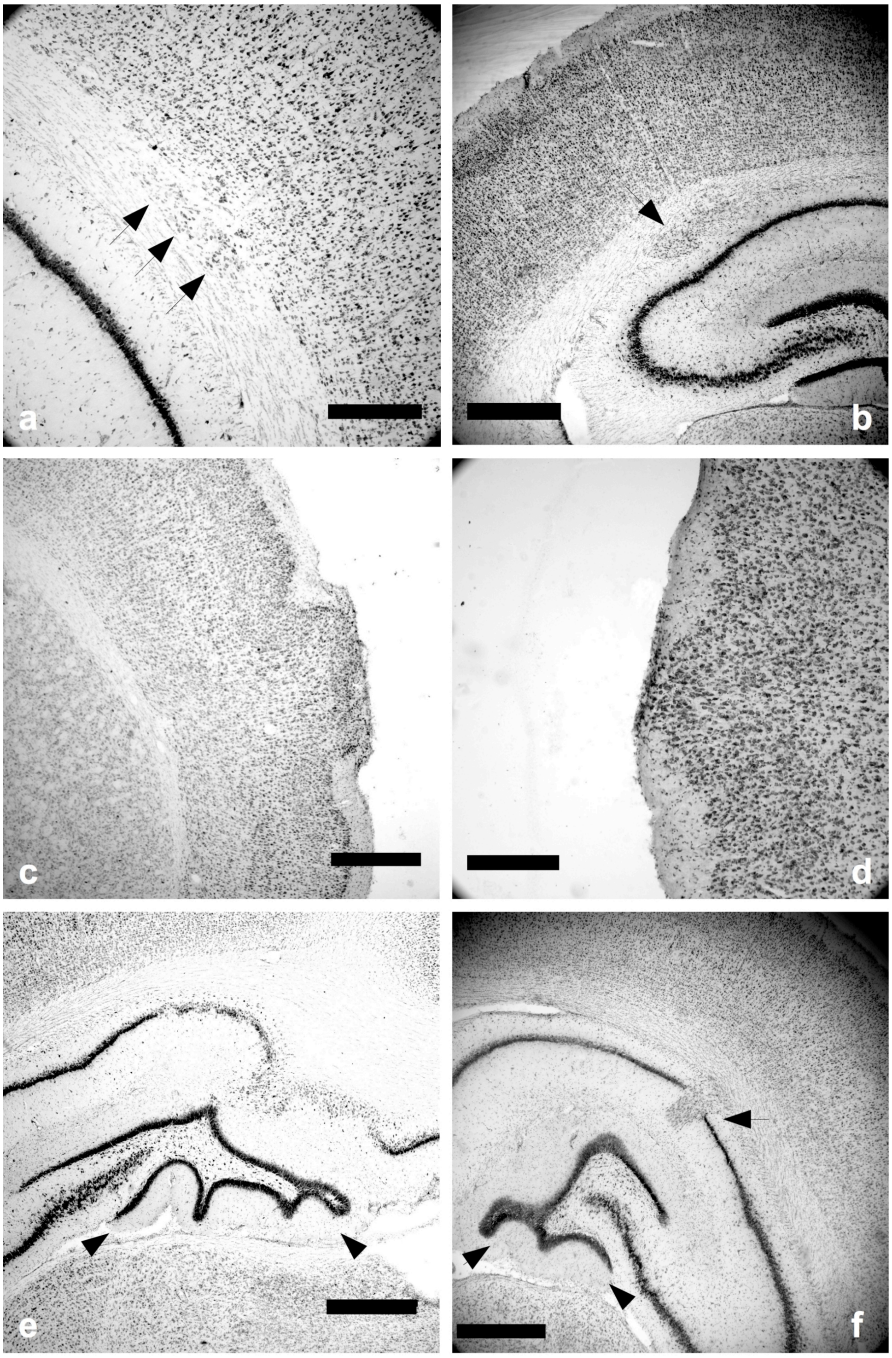

Figure 1. Neuroanatomical disruptions in rats following *in utero* transfection of *Dyx1c1* shRNA and *Kiaa0319* shRNA. Panels A, C, and E are representative images from coronal sections of adult rat brains following *in utero* transfection of *Dyx1c1* shRNA, while panels B, D, and F are representative images from rat brains following *in utero* transfection of *Kiaa0319* shRNA. All sections were stained for Nissl substance with cresyl violet. A., B. (Scale bars = 500 μm and 1 mm, respectively) Heterotopic collections of neurons in white matter (black arrows). C., D. (Scale bars = 1 mm) Layer I ectopia resulting from the injection wound

during the transfection. E., F. (Scale bars = 1 mm) Hippocampal dysplasia, characterized by both heterotopic disruption of the CA1 region (black arrow) and abnormal folding of the dentate gyrus (black arrowheads).

Subsequent studies of these genes utilized the RNAi method to knockdown levels of each protein in the neocortex of developing rat pups, which led to the discovery that each protein plays a role in neuronal migration. For example, animals transfected embryonically with *Dyx1c1* shRNA showed significant delays in migration in a population of transfected Layer 2/3 pyramidal neurons, which were arrested in the white matter four days after the electroporation procedure (Wang *et al.,* 2006). In adult animals, a small subpopulation of transfected neurons were found to form heterotopic collections in the white matter and in Layer VI, while the majority of transfected neurons had migrated to supragranular layers—most of them traveling beyond their appropriate target layer (i.e., over migration; Rosen *et al.,* 2007). One quarter of the transfected animals also exhibited ectopic nests of neurons in Layer 1, similar to those previously observed in dyslexic brains (Galaburda *et al.,* 1985). Additionally, early interference with this gene created migrational anomalies within the hippocampus. Similarly, embryonic knockdown of *Kiaa0319* lead to an initial arrest of transfected neurons in the white matter (Paracchini *et al.,* 2007). Interestingly, the *Kiaa0319* shRNA transfection also caused a marked change in neuronal morphology, and the neuronal-radial glial cell interaction. That is, instead of being aligned parallel to one another with the migrating neuron's leading process attached to the radial glial fiber, the neuron attained a largely orthogonal orientation with respect to the radial glial cell (Paracchini *et al.,* 2007). The adult neuroanatomical phenotype following transfection of *Kiaa0319* shRNA is characterized by the presence of white matter heterotopia, abnormal laminar placement of individual transfected neurons, and migrational disruptions to the hippocampus (Peschansky *et al.,* 2009).

It is worth noting that some recent epidemiological studies have provided evidence that a subset of the variants of *KIAA0319* previously linked to dyslexia have base pair mutations that result in reduced (rather than eliminated) expression of the gene, which is similar to what is being modeled in the RNAi animals (Dennis *et al.,* 2009; Paracchini *et al.,* 2006). Although there is not currently an overwhelming body of evidence specifically linking reduced expression of CDSGs to dyslexia, the genetic knockdown rodent model has provided invaluable information on the developmental processes that these proteins are normally involved in, and the implications of disrupting these functions for subsequent morphology and behavior.

Dyslexia and RNAi: Rapid Auditory Processing

The epidemiological evidence of a role for *DYX1C1* and *KIAA0319* in dyslexia, combined with the evidence from animal models that these genes are involved in neurodevelopment and neuronal migration, led our lab to perform assessments on behaviors that parallel some of the intermediate phenotypes involved in dyslexia—namely, rapid auditory processing and working memory. [It is important to note that *post mortem* tissue analysis of all subjects following each behavioral study revealed consistent patterns of aberrant neocortical neuronal migration in shRNA treated animals, similar to those previously

reported (Figure 1; see Peschansky *et al.*, 2009; Rosen *et al.*, 2007)]. Initially, we utilized the previously discussed modified pre-pulse inhibition paradigm to assess rapid and complex acoustic processing abilities of rats following embryonic interference with either *Dyx1c1* or *Kiaa0319* (see Figure 2).

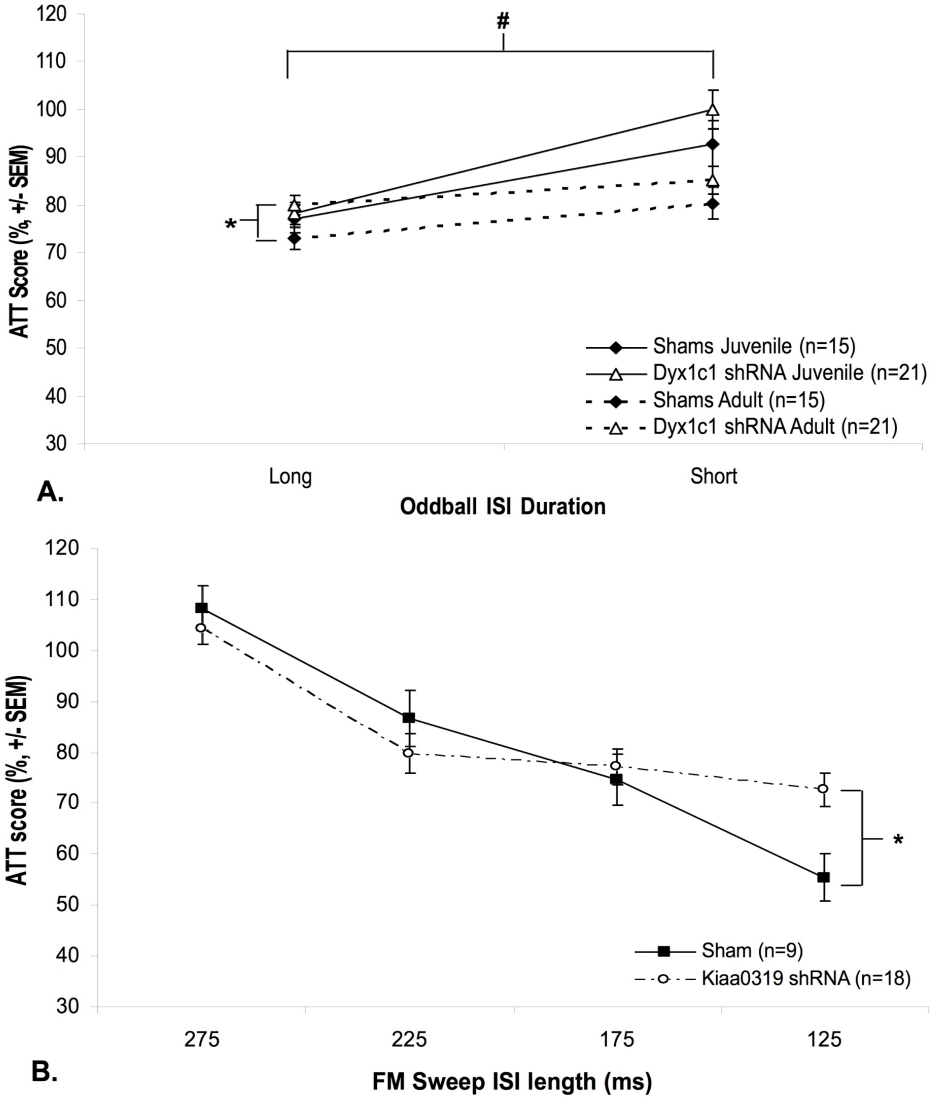

Figure 2. Auditory processing impairments in *Dyx1c1* and *Kiaa0319* shRNA treated animals. A. *Dyx1c1* shRNA animals: (adapted from Threlkeld *et al.*, 2007) On the Oddball test (a complex, two-tone frequency discrimination task), there was an overall near-significant Treatment effect (#, $p = .051$, one-tailed), suggesting that the *Dyx1c1* shRNA treated animals performed worse than shams overall on the task—at both long and short stimulus durations, as indicated by their higher attenuation scores. Additionally, there was a significant main effect of Treatment in adulthood on the long Oddball task [* indicates $p < .05$], with *Dyx1c1* shRNA animals performing worse than shams (Threlkeld *et al.,* 2007). B. *Kiaa0319* shRNA animals: (adapted from Szalkowski *et al.,* submitted) On FM Sweep (a complex frequency sweep discrimination task), there was a significant Treatment x ISI interaction ($p < .05$) and simple effects analysis revealed a significant

Treatment effect at the shortest duration ISI, 125 ms, with *Kiaa0319* shRNA treated animals performing worse than shams ($p < .01$).

In the first study (assessing rapid auditory processing abilities of rats following transfection of *Dyx1c1* shRNA) we found that embryonic interference with *Dyx1c1* led to an auditory processing impairment. However, this impariment was not specific to rapid presentation of auditory stimuli. Rather, on an "Oddball task," (a complex, two-tone frequency discrimination task), an overall repeated measures ANOVA was found to show a near-significant Treatment effect ($p = .051$, one-tailed), suggesting that the *Dyx1c1* shRNA treated animals performed worse than shams overall on the task—at both long and short stimulus presentations. Additionally, there was a significant main effect of Treatment in adulthood on the long Oddball task [* indicates $p < .05$], with *Dyx1c1* shRNA animals performing worse than shams (Threlkeld *et al.,* 2007).

In a subsequent study testing the rapid auditory processing abilities in *Kiaa0319* shRNA treated rats, we found that on FM Sweep, a complex auditory processing task involving discrimination of a high-low frequency sweep, a repeated measures ANOVA revealed a significant Treatment x ISI interaction ($p < .05$). In this case, simple effects analysis revealed a significant Treatment effect only at the shortest duration ISI, 125 ms, with *Kiaa0319* shRNA treated animals performing worse than shams ($p < .01$). This indicated that adult *Kiaa0319* shRNA treated rats exhibited specific RAP deficits with rapid cue presentation on a complex frequency discrimination task (Szalkowski *et al.,* submitted).

Interestingly, in each case the transfected animals failed to show deficits on easier RAP tasks, such as silent gap detection. It was not until the animals were presented with more complex or rapid tasks (e.g., with temporal order discrimination, and/or stimuli at high rates (short durations) of presentation) that impairments in performance were evident. Moreover, it is interesting to speculate that there may be subtle differences in the RAP abilities of *Dyx1c1* and *Kiaa0319* transfected animals. Specifically, when presented with the complex FM sweep frequency discrimination task, *Kiaa0319* transfected animals showed significantly impaired detection only at the shortest interstimulus interval (i.e., the most rapid presentation of the acoustic cue (see Figure 2b)). This indicates that the auditory processing impairment induced by knockdown of *Kiaa0319* in these animals is temporally-specific. In contrast, when presented with a similar complex frequency discrimination RAP task (Oddball two-tone discrimination), the *Dyx1c1* transfected animals show impairment across long *and* short interstimulus intervals, suggesting that the deficit is not limited to processing of rapid stimuli, and may instead be a by-product of a disruption to core executive function or short term working memory (see Figure 2a).

Dyslexia and RNAi: Learning and Simple Spatial Memory

Following each RAP study, animals were tested for basic spatial learning and memory abilities using a Morris water maze (Szalkowski *et al.,* submitted; Threlkeld *et al.,* 2007). The results of the Morris water maze testing are strikingly similar for the *Dyx1c1* and

Kiaa0319 studies, with significant impairment in task performance observed exclusively in those animals with specific disruptions to the hippocampus (see Figure 4). Interestingly, the presence of cortical malformations alone did not lead to significant spatial memory impairments in either the *Dyx1c1* or *Kiaa0319* shRNA animals, with cortically disrupted animals performing at a similar level to shams. In addition to providing further characterization of the functional effects of the genetically-induced hippocampal anomalies observed in these animals, the data derived from the Morris water maze testing serve as evidence that the shRNA treated animals are not "globally dysfunctional" as a result of their disrupted neurodevelopment, and thus their acoustic processing deficits are not simply the result of an all-encompassing impairment.

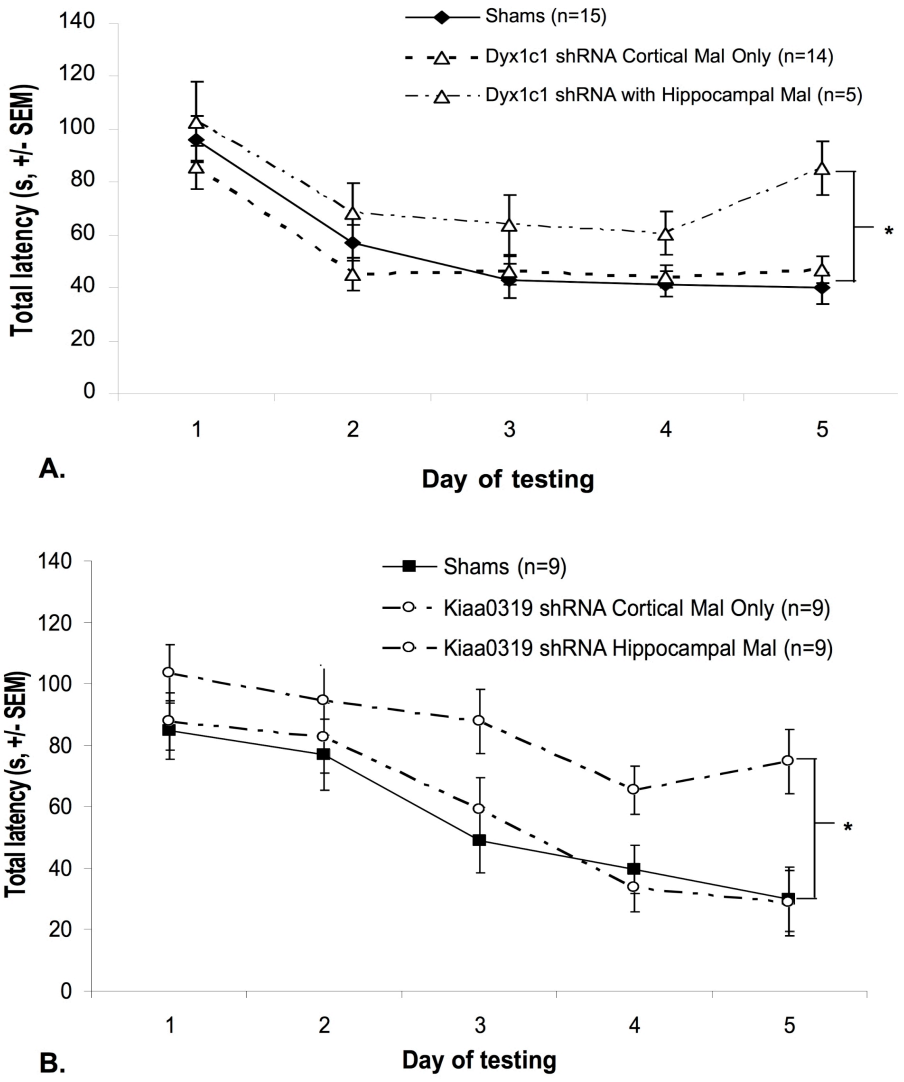

Figure 3. Simple spatial memory abilities in *Dyx1c1* and *Kiaa0319* shRNA treated rats. Simple spatial memory was assessed via Morris water maze testing. In each study, rats transfected with *Dyx1c1* shRNA or

Kiaa0319 shRNA with specific disruptions to the hippocampus took significantly longer to locate the platform during Morris water maze testing than both shams and shRNA treated animals without hippocampal malformations. (Adapted from Threlkeld *et al.,* 2007 and Szalkowski *et al.*, submitted, respectively; * indicates $p < .05$).

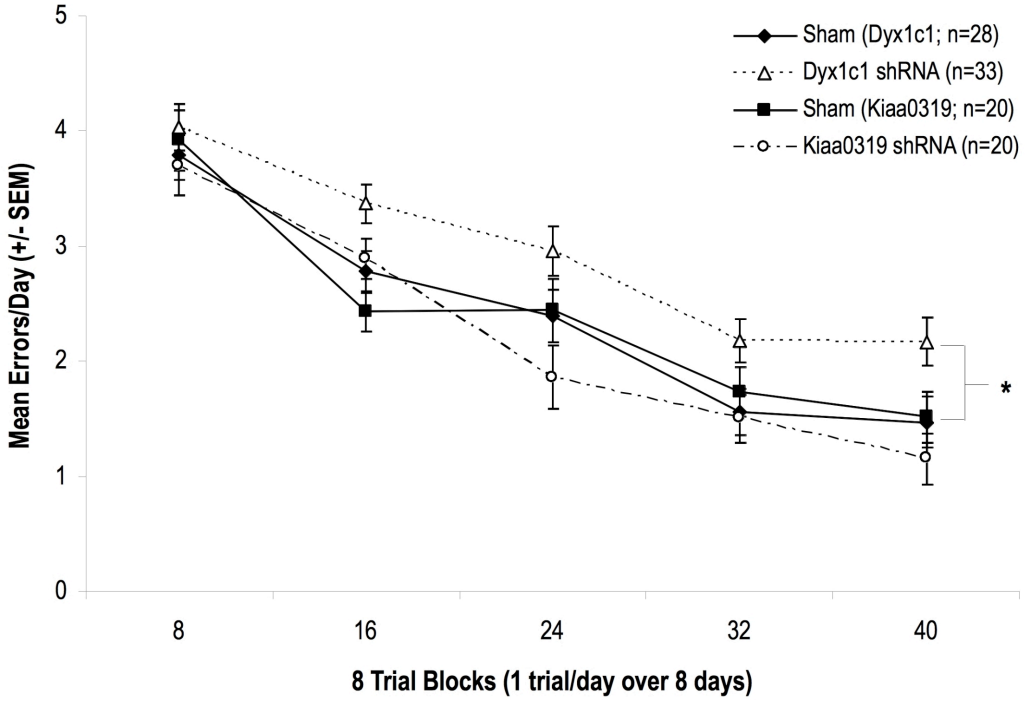

Figure 4. Working memory performance in *Dyx1c1* and *Kiaa0319* shRNA treated rats. Working memory abilities were assessed via delayed match-to-sample testing on an 8 arm radial water maze. *Dyx1c1* shRNA animals performed significantly worse than shams as indicated by an increased number of average incorrect arm entries (* indicates $p < .05$). Conversely, *Kiaa0319* shRNA treated animals did not exhibit any deficits on this task as compared to shams.

Dyslexia and RNAi: Working Memory

Working memory was also assessed in groups of genetically disrupted animals using the previously discussed delayed match-to-sample 8 arm radial water maze task. Briefly, working memory performance is measured in this task as a function of the number of incorrect arm entries animals make as they attempt to navigate to a previously presented goal arm. In Study 1 assessing the effects of *Dyx1c1* knockdown on working memory, we found that across 12 weeks of testing *Dyx1c1* shRNA treated animals consistently made more incorrect arm entries during the test trial than shams, indicating a significant yet subtle impairment in working memory (Szalkowski *et al.*, 2011) (see Figure 4a). Interestingly, the results of Study 2, which assessed working memory in *Kiaa0319* shRNA treated animals, were strikingly different than those of Study 1. That is, throughout testing, the *Kiaa0319* shRNA treated animals performed the task equivalently to shams (Szalkowski *et al.*, submitted). Taken together, these data suggest that *Dyx1c1* may have a more salient role in

working memory in rats as compared to *Kiaa0319*. This is especially interesting given two recent clinical findings that variants of the *DYX1C1* gene are specifically related to short term memory abilities in dyslexic humans (Dahdouh *et al*., 2009; Marino *et al.,* 2007). This finding fits with the proposal that early interference with *Dyx1c1* may result in disruption to core executive function, which could result in impaired working memory and could cause the observed temporally-independent acoustic processing deficit, while *Kiaa0319* may have a more specific effect on temporal processing abilities. It is important to note that replications of these findings are necessary before firm conclusions can be drawn about these potential differences in the behavioral phenotypes associated with *Dyx1c1* and *Kiaa0319*.

Conclusion

The recent use of animal models to neuroanatomically model dyslexia has allowed researchers to discover links between disruption of neuronal migration and behavior. The even more recent advances in genetic studies of dyslexia (and the technology that allows for the creation of genetically altered animals) has brought us even closer to understanding the relationship between genes, neuroanatomy, and behavior as they relate to a complex cognitive disorder such as dyslexia in humans. The interpretation of dyslexia as a summation of variable intermediate phenotypes has been a key contributor to these recent advances. For example, strict examination of the intermediate phenotype of short term memory impairment, which is reported in some populations of dyslexics, has lead to a replicated association between *DYX1C1* and a specific, well-defined dyslexia-related behavior in different populations (Dahdouh *et al.,* 2009, Marino *et al.,* 2007). Moreover, relatively straightforward genotype-phenotype relationships such as the one between *DYX1C1* and short term memory are highly amenable to animal modeling. In closing, animal models provide an essential tool allowing researchers to draw connections between microscopic aspects of neuroanatomy and behavior that are not currently possible to attain through clinical studies alone. In the case of dyslexia, animal models also provide an avenue for defining relationships between risk genes and neuroanatomical and behavioral phenotypes, which could eventually lead to better definition of subtypes of dyslexia in humans. Further characterization of the candidate dyslexia genes may result in improved screening for the disorder, more accurate diagnoses, and ultimately the development of specifically tailored behavioral therapies and more effective interventions.

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