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Dysbindin Modulates Prefrontal Cortical Glutamatergic Circuits and Working Memory Function in Mice

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Abstract

Behavioral genetic studies of humans have associated variation in the *DTNBP1* gene with schizophrenia and its cognitive deficit phenotypes. The protein coded for by *DTNBP1*, dysbindin, is expressed within forebrain glutamatergic neurons, where it interacts with proteins involved in vesicular trafficking and exocytosis. In order to further delineate the cellular, physiological and behavioral phenotypes associated with reduced dysbindin expression, we conducted studies in mice carrying a null mutation within the *dtmbp1* gene. Dysbindin mutants exhibited impairments of spatial working memory as compared with wild-type controls; heterozygous mice exhibited intermediate levels of cognitive dysfunction. Deep layer pyramidal neurons recorded in the prefrontal cortex of mutant mice exhibited reductions in paired-pulse facilitation, and evoked and miniature excitatory post-synaptic currents, indicating a difference in the function of pre-synaptic glutamatergic terminals, as well as elevated spike thresholds. Taken together, these data indicate that dysbindin potently regulates excitatory transmission in prefrontal cortex, potentially through a pre-synaptic mechanism, and consequently modulates cognitive functions depending upon this brain region, providing new insights into the molecular mechanisms underlying cortical dysfunction in schizophrenia.

Keywords

working memory; schizophrenia; glutamate; cognition; excitatory; pre-synaptic

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Disclosure/Conflict of Interest

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Introduction

Emerging evidence suggests that a significant number of the gene mechanisms that contribute to risk for schizophrenia modulate complex cognitive functions that depend upon the prefrontal cortex and medial temporal lobe (Arguello and Gogos, 2006; Cannon, 2005; Harrison and Weinberger, 2005; Weinberger *et al*, 2001). Indeed, some of these genes, including *DISC1*, *DTNBPI*, *COMT* and *NRG1*, are expressed normally within the cortical circuitry that subserves cognition (Lipska *et al*, 2006; Tunbridge *et al*, 2006; Weickert *et al*, 2004), and it is thought that polymorphisms within these genes consequently explain variation in cognitive phenotypes, such as declarative memory, attention and executive functions, within the human population. For example, a rare haplotype within the *DISC1* gene associates with low prefrontal cortical volume and impairments in cognitive function, specifically working memory (Cannon *et al*, 2005). These genotype-phenotype relationships further underscore the value of cognitive mechanisms as candidate endophenotypes for schizophrenia and related psychotic disorders.

More recently, attention has focused on *DTNBPI*, the gene that codes for dystrobrevin-binding protein (dysbindin) and its relationship to cognition (Burdick *et al*, 2007; Burdick *et al*, 2006; Donohoe *et al*, 2007; Luciano *et al*, 2008). In humans, variation within this gene is associated with risk for developing schizophrenia and impairments in cognitive performance. Moreover, this gene is expressed within cortical neurons, including pyramidal neurons (Talbot *et al*, 2004), suggesting that it is well-positioned to modulate functions that depend upon cortical excitatory tone (Chen *et al*, 2008; Numakawa *et al*, 2004). *DTNBPI* haplotypes that represent vulnerability markers for schizophrenia appear to associate with low dysbindin expression (Bray *et al*, 2005); given the putative role for dysbindin in vesicular trafficking and transmitter exocytosis (Chen *et al*, 2008; Numakawa *et al*, 2004; Talbot *et al*, 2006; Talbot *et al*, 2004), a reduction in dysbindin expression may lead to a decrease in glutamate output (Numakawa *et al*, 2004), a mechanism that could explain deviant patterns of prefrontal cortical activity in schizophrenia (Manoach, 2003; Weinberger and Berman, 1988; Williamson, 1987).

Neuronal microcircuits, involving glutamatergic pyramidal neurons and local, inhibitory interneurons, within the prefrontal cortex play essential roles in the encoding and maintenance of information in working memory (Fuster, 2001; Goldman-Rakic, 1995; Miller, 2000). Specifically, neurons within prefrontal cortex exhibit increased activity during maintenance of information within working memory, and this “memory-related” activity has been attributed to glutamate-dependent recurrent excitation within the local circuit (Compte *et al*, 2000; Compte *et al*, 2003; Durstewitz and Seamans, 2002). Compromised glutamate release, hypothetically secondary to low dysbindin expression, may be expected to compromise the ability of cortical circuits to maintain recurrent excitation and to “fail” during maintenance of information held in working memory. These alterations in glutamatergic transmission may relate to the altered patterns of prefrontal activity shown by patients with schizophrenia compared with controls across varying degrees of memory load and in particular to the reduced activation of cortical regions during high memory load conditions in these patients (Callicott *et al*, 2000; Karlsgodt *et al*, 2007; Karlsgodt *et al*, 2009; Manoach, 2003).

To examine these relationships more directly, we examined working memory function and excitatory neurotransmission within prefrontal cortex in so-called sandy mice that possess a large deletion contained wholly within the *dtmbp1* gene (Li *et al.*, 2003). Several recent studies involving these null mutant mice have shown that loss of dysbindin function impedes excitatory neurotransmission (Chen *et al.*, 2008; Numakawa *et al.*, 2004; Talbot *et al.*, 2006), effects which likely correspond to behavioral abnormalities, including social deficits, enhanced stimulant sensitization and poor performance in certain simple memory tests (Bhardwaj *et al.*, 2008; Cox *et al.*, 2009; Feng *et al.*, 2008; Hattori *et al.*, 2008; Takao *et al.*, 2008). To complement these studies, we sought to gather direct evidence that spatial working memory function, an important endophenotype for schizophrenia (Glahn *et al.*, 2003), is affected by loss of dysbindin, as well as to determine whether physiological changes in the neural circuitry of working memory are a consequence of this mutation. We hypothesized that null mutation of the gene coding for dysbindin would impair working memory and that this cognitive endophenotype would be associated with a decrease in excitatory neurotransmission within circuits involved in this cognitive process.

Materials and Methods

Animals

Dysbindin mutant mouse breeders on the DBA2J background were obtained from Roswell Park Cancer Institute (Conyers GA). Experimental mice were generated by heterozygote crosses, and genotypes were determined by polymerase chain reaction. The wt product [472 bp] was amplified with the following primers: TGAGCCATTAGGAGATAAGAGCA and AGCTCCACCTGCTGAACATT; the *dys*⁻ product [274 bp] was amplified with the following primers: TCCTTGCTTCGTTCTCTGCT and CTTGCCAGCCTTCGTATTGT). The fragments were separated on a 3% agarose gel.

Group-housed male mice were used in the behavioral and electrophysiological studies. The mice in the behavioral experiments were 60–100 days of age during experimentation, while the mice used in the recording studies were 45–60 days of age. All experimental protocols were approved by the Chancellor's Animal Research Committee at UCLA or the Medical University of South Carolina Institutional Animal Care and Use Committee.

Working memory testing

Mice were trained and tested using a delayed non-match-to-position test (Marrs *et al.* 2005; Aarde and Jentsch 2006) in small aluminum and Plexiglas operant conditioning chambers (Med-Associates Inc., St Albans VT), fitted with a horizontal array of five nose-poke apertures on one-side of the box and a 20-mg pellet delivery magazine on the opposite side. This delayed non-match-to-position task emphasizes retrospective encoding and maintenance of spatial information in a manner analogous to spatial delayed response tests used to measure working memory in rats, monkeys and humans.

Each trial in the delayed non-match to sample task consists of both a sample phase and a choice phase. The inter-trial interval and time-out periods were 5 s and 3 s, respectively. In the sample phase, one of the 5 nose-poke apertures was chosen at random and illuminated

for up to 15 s. A response into the illuminated aperture (correct sample-phase response) caused the aperture light to extinguish and the magazine light to be illuminated. The magazine remained lit until an entry into the magazine is detected, after which the choice phase was initiated (see below). In the sample phase, the trial was aborted and a time-out ensues if the mouse makes a response in an unlit nose-poke aperture during stimulus presentation (incorrect sample-phase response) or failed to respond to the illuminated aperture during the 15 s presentation period (sample-phase omission). During the choice phase of the trial, two apertures were illuminated: the sample aperture as well as another, randomly selected aperture (the non-match location). Both of these apertures were illuminated for up to 15 s. A response in any aperture other than that of the non-match location resulted in a time-out, and an incorrect choice-phase response was recorded. Failure to make a response while the apertures were illuminated resulted in a time-out, and a choice-phase omission was recorded. Responses to the non-match location (correct response) triggered magazine illumination and pellet delivery.

During the first 30 days of training, there was no imposed delay, allowing the mice to acquire the task under relatively low memory load conditions. After this initial training, probe sessions were administered in which minimum delay periods were imposed between a correct sample-phase response and the first response into the magazine that initiated the choice phase. The imposed delays were either 0.5, 5 or 10 s and were interpolated in pseudorandom order and at equivalent frequencies across the session. The tasks ended after 75 trials are completed or 60 min passes, whichever comes first.

Incorrect choices and omissions during the sample phase and omissions during the choice phase were calculated and analyzed by one-way analyses of variance, with genotype as the factor. Accuracy of responding during the choice phase was analyzed by repeated measures ANOVA with genotype as the factor and delay length as the repeated measure.

Electrophysiological Recordings

Brain slices were prepared from male wt/wt, wt/dys⁻ and dys⁻/dys⁻ mice. Subjects were anesthetized with the inhalant isoflurane (Abbott Laboratories). The brain was then removed and coronal slices were cut at 300 μm thickness in ice-cold high-sucrose solution containing (in mM): sucrose, 200; KCl, 1.9; Na₂HPO₄, 1.2; NaHCO₃, 33; MgCl₂, 6; CaCl₂, 0.5; glucose, 10; ascorbic acid, 0.4. Slices were incubated at 33°C for at least 1 h before recordings; the incubation medium was an artificial cerebrospinal fluid solution containing (in mM): NaCl, 125; KCl, 2.5; NaH₂PO₄, 1.25; NaHCO₃, 25; MgCl, 4, CaCl, 1, d-glucose, 10; sucrose, 15; ascorbic acid, 0.4, continuously aerated with 5% CO₂/95% O₂. After incubation, slices were transferred to a submerged chamber and superfused with oxygenated artificial cerebrospinal fluid (in mM: 125 NaCl, 2.5 KCl, 25 NaHCO₃, 2.0 CaCl₂, 1.3 MgCl₂, 10 glucose and 0.4 ascorbic acid) at room temperature. Recordings were made using a Multiclamp 700B amplifier (Axon Instruments, CA), connected to a computer running Windows XP and Axograph X® software and later analyzed off-line. All recordings were obtained from neurons in layers V or VI of the prelimbic or infralimbic cortex, identified using infrared-differential interference contrast optics and video-microscopy.

Current Clamp—For current-clamp recordings, thick-walled borosilicate pipettes (3–7 M Ω tip resistance) were filled with (in mM): 125 K⁺-gluconate, 3 KCl, 2 MgCl₂, 10 HEPES, 0.1 EGTA. A series of current steps (1000 ms duration, –100 to +300 pA, at 1 Hz) were injected to evoke spike firing at various steady-state membrane potentials. In between the steps, cells were held as close to –80 mV as possible via DC current injection (referred to as holding current in Table 1). Measures of the intrinsic membrane excitability (rheobase current, holding current, number of evoked spikes, input resistance, action potential threshold, amplitude, and half-width) were compared across genotypes.

Voltage clamp—For voltage-clamp recordings, electrodes (3–7 M Ω resistance in situ) were filled with a solution containing (in mM): 135 CsCl, 10 HEPES, 2 MgCl₂, 1 EGTA, 4 NaCl; 2 Na-ATP, 0.3 tris-GTP, 1 QX-314, 10 phosphocreatine; 285 mOsmols. All the voltage-clamp experiments were performed in the presence of 100 μ M picrotoxin. Series resistances (10–20 M Ω) and input resistances were continually monitored throughout the experiment via a –1 mV (100 ms) hyperpolarizing pulse. Evoked EPSCs (eEPSCs) were elicited by applying low-intensity, square-wave pulses (50–150 μ A; 100 μ sec in duration) using a bipolar concentric electrode placed within 200 μ m of the recording electrode. The eEPSC amplitude was defined as the mean amplitude during a 1–2 ms window at the peak of the EPSC minus the amplitude during a similar window immediately before the stimulus artifact. Pulses were administered every 30 seconds and peak eEPSC amplitude was measured. Stimulus intensity was gradually increased in order to construct an input-output curve from 10 μ A up to 500 μ A (in some cases), until maximum amplitude was reached, meaning that response amplitude remained consistent regardless of increasing stimulation intensity. The responses included in the analysis were limited to those measured at 75% of maximum amplitude. Paired pulse stimuli were delivered at a frequency of 0.3Hz, with an ISI of 50 msec.

Miniature EPSCs (mEPSCs) were obtained from 50–200 sweeps per cell; TTX (1 μ M) was added to the recording buffer when mEPSCs were assessed. Amplitude and frequencies were calculated using MiniAnalysis software® with a detection threshold of 8 pA, events were then manually checked for accuracy.

Statistical Analysis

Parametric analyses of variance (ANOVA) were used to examine main effects (e.g., genotype) and interactions with repeated measures, when appropriate. Significant main effects or interactions were followed up with post hoc tests. In some cases, paired t-tests were used to evaluate within subject, *a priori* hypothesized effects. All figures present data as mean \pm SEM.

Results

Spatial Working Memory

We first sought to determine whether null mutation of the *dtbpl* gene associates with working memory dysfunction in mice. Working memory performance of wt/wt, wt/*dys*[–] and *dys*[–]/*dys*[–] mice was evaluated using an operant delayed-non-match-to-position task (Figure

1). During each trial in this procedure, mice first respond to a single illuminated aperture (the sample), experience a delay during which no cues are presented and then must make a response into one of two apertures: the sample stimulus and a non-match stimulus. Across an initial 30 day training period in which the conditional rules of the task were trained, all groups acquired and performed the task equivalently, exhibiting above chance levels of choice accuracy. Probe sessions were then delivered in which delays of 0.5–10 s were introduced; as expected, a main effect of delay was found for choice accuracy ($F_{(2,56)}=4.9$, $p=0.01$), indicating that performance was sensitive to the length of the retention interval. Considering all completed trials, choice accuracy was significantly affected by genotype (Figure 1; $F_{(2,28)}=4.2$, $p=0.02$) but the genotype x delay interaction did not reach significance; *post hoc* Tukey tests confirmed that mutants exhibited poorer choice accuracy than did controls ($p<0.01$), but that the difference between wild-type and heterozygous mice only reached the trend level ($p=0.07$).

To assess whether sensorimotor phenotypes could contribute to the observed impairments in *dys⁻/dys⁻* mice, we examined other aspects of task performance. We first calculated correct responding in the sample phase that only requires animals to respond to a single illuminated light; this measure was not affected by genotype ($F_{(2,28)}=0.04$, $p=0.95$), indicating that the two groups were equally able to process the sensory properties of the stimuli and to emit a conditionally appropriate response. Furthermore, omission rates did not differ between the two groups for either the sample ($F_{(2,28)}=1.4$, $p=0.26$) or choice phases (Figure 1; $F_{(2,28)}=0.3$, $p=0.7$), indicating that both groups were equally motivated to perform the task and were equally capable of responding within the time constraints of the two components of each trial. Therefore, the poor response accuracy in mutants observed during the non-matching phase of the trial appears to depend upon the need to encode and retain information about the sample cue across the delay period.

Physiological Properties of Prefrontal Cortical Pyramidal Neurons

Given the important role for delay-related, glutamate-dependent activity of prefrontal cortical pyramidal neuron networks in the representation of visuospatial information maintained in working memory (Goldman-Rakic, 1995), we next examined whether genetic dysbindin deficiency altered glutamatergic transmission in prefrontal cortex, using *in vitro* recordings from deep layer pyramidal neurons within the medial frontal cortex in wt/wt, wt/*dys⁻* and *dys⁻/dys⁻* mice.

We first measured basic membrane properties in pyramidal neurons from all three genotypes and found that while spike amplitude, spike half-width, input resistance and cell capacitance showed no significant effect of genotype, rheobase and spike threshold were significantly affected (Table 1). Rheobase current was significantly affected by genotype ($F_{(2,24)}=5.1$; $p=0.009$, Table 1) with *dys⁻/dys⁻* mice exhibiting an approximately 50% reduction compared to wt/wt. This change in rheobase current can be attributed to a significant reduction in spike threshold in neurons recorded from *dys⁻/dys⁻* mice (-56.7 ± 1.5 mV) compared to wt/wt (-47.2 ± 2.0 mV; $F_{(2,24)}=5.6$, $p=0.006$) but not to differences in the amount of holding current injected to maintain a membrane potential of -80 mV (Table 1), suggesting that resting membrane potentials were not different between genotypes. For

rheobase and membrane threshold, the corresponding values for heterozygous mice were intermediate between *wt/wt* and *dys⁻/dys⁻* mice. These data suggest that dysbindin deletion or reduction may increase the excitability of pyramidal neurons.

We next sought to examine whether null mutation of the *dtbpl* gene affected excitatory transmission within the local circuitry of the prefrontal cortex; our *a priori* hypothesis, stemming from recently published work (Chen *et al*, 2008; Numakawa *et al*, 2004; Talbot *et al*, 2004), was that dysbindin deletion would impair excitatory synaptic transmission. Assessment of eEPSC amplitude showed a significant difference amongst the three genotypes (Figure 2A; $F_{(2,44)}=3.2$, $p<0.05$), with a significant reduction in evoked responses in both *wt/dys⁻* and *dys⁻/dys⁻* mice, relative to *wt/wt* controls ($p<0.05$). These initial data suggested that evoked synaptic responses were lower in mutant, as compared with wild-type, animals.

To further investigate changes in synaptic transmission, mEPSCs were recorded from pyramidal neurons in the presence of picrotoxin (100 μ M) and TTX (1 μ M), and the frequency of mEPSCs across a broad range of amplitudes (8–100 pA) was analyzed. We observed an overall significantly lower frequency of mEPSCs (main effect of genotype: $F_{(2,33)}=4.1$, $p=0.01$) in *dys⁻/dys⁻* (0.3 ± 0.03 Hz; $n=10$) and *wt/dys⁻* mice (0.27 ± 0.1 Hz; $n=7$) animals, compared with *wt/wt* mice (0.6 ± 0.1 Hz; $n=16$, Figure 2B); these genotypic differences were most obvious for events in the range of 20–30 pA. Additionally, mEPSC amplitude ($F_{(2,33)}=2.2$, $p=0.04$) was also significantly reduced in *dys⁻/dys⁻* (12.7 ± 0.8 ; $n=16$) compared to *wt/wt* neurons (15.8 ± 1.4 ; $n=10$; Figure 2C). These data suggested that reduced dysbindin expression leads to significant decreases in excitatory transmission within the prefrontal cortex.

Because of the extant data indicating that dysbindin is expressed within glutamatergic terminals and interacts with vesicle-trafficking proteins, we hypothesized that the reduction in mEPSCs may result from decreased pre-synaptic release of glutamate in dysbindin mutants. Therefore, we next examined paired-pulse facilitation. The overall ANOVA examining the effects of genotype on the paired-pulse ratio revealed a significant main effect of genotype ($F_{(2,26)}=7.4$, $p=0.0009$; Figure 2D) that was largely attributable to a reduction in the paired-pulse ratio in *dys⁻/dys⁻* mice (ratio= 1.1 ± 0.06 ; $n=9$) compared with *wt/wt* controls (ratio= 1.6 ± 0.1 ; $n=8$). These results further support the idea that dysbindin deletion or reduction impairs cortical glutamate release.

Discussion

Null mutation of the gene encoding dysbindin led to specific changes in performance of a delayed non-match to position test of spatial working memory, as well as the associated, underlying neural mechanisms within prefrontal cortex believed to mediate this cognitive function. Although dysbindin null subjects were as capable as wild-type controls in processing and responding in a conditionally appropriate way to visual cues, they were unable to respond correctly in a delayed non-match-to-position task, indicating that they had difficulty encoding or maintaining the item in spatial working memory. These data suggest

that poor working memory maintenance is a phenotypic consequence to loss of dysbindin expression.

Encoding and maintenance of visuospatial information in working memory is thought to involve the selective recruitment of prefrontal cortical neuronal circuits, which exhibit persistent activity during the active maintenance of the information (Compte *et al*, 2000; Compte *et al*, 2003; Durstewitz *et al*, 2000; Seamans *et al*, 2003). Because dysbindin has been shown to modulate glutamate outflow (Chen *et al*, 2008; Numakawa *et al*, 2004), we hypothesized that the poor working memory exhibited in dysbindin mice was due to a compromised glutamate transmission. Electrophysiological recordings from deep layer pyramidal neurons in the medial prefrontal cortex confirmed this hypothesis by revealing that the amplitude of eEPSCs and the frequency of miniature EPSCs were decreased and moreover, paired-pulse facilitation was not present in dysbindin mutant mice. Collectively, these data support the conclusion that the pre-synaptic release of glutamate is impaired in dysbindin deficient mice.

Of importance, hippocampal circuits also contribute to performance of delayed match/non-match to sample tasks such as the one used here (Deadwyler *et al*, 1996; Hampson and Deadwyler, 1996, 2000; Hampson *et al*, 1993), so neural dysfunction within the hippocampus could also contribute to the working memory phenotype in dysbindin mutant mice. Other behavioral studies in dysbindin null mutant mice have found deficits in tasks that predominantly measure hippocampal function (Bhardwaj *et al*, 2009; Cox *et al*, 2009; Takao *et al*, 2008), so the current behavioral effects may not rely upon prefrontal cortical dysfunction alone. Future studies directly assessing hippocampal circuitry, using electrophysiology and gene expression, are underway.

Relationship to Cognitive Endophenotypes for Schizophrenia

The ability to maintain and manipulate information held in working memory appears to be a quantitative trait, and the heritability of working memory performance amongst unaffected relatives of individuals diagnosed with schizophrenia supports the conclusion that this aspect of cognition is an endophenotype and/or marker of genetic liability for the disorder (Glahn *et al*, 2002; Glahn *et al*, 2003). Genetic variation in other schizophrenia risk genes, most notably *DISC1*, associates with spatial working memory function in humans (Burdick *et al*, 2005; Cannon *et al*, 2005; Koike *et al*, 2006; Kvajo *et al*, 2008; Li *et al*, 2007), and studies in mouse genetic models has further supported the linkages between these particular genes and cognitive performance (Cox *et al*, 2009; Takao *et al*, 2008). Further to that, the current results support a role for dysbindin in the modulation of spatial working memory and possibly other prefrontal cortical-dependent cognitive functions. Notably, however, dysbindin is expressed in brain regions other than the prefrontal cortex; its considerable expression in hippocampus suggests that it could (through cellular actions similar to those reported here to occur in the prefrontal cortex) strongly affect synaptic plasticity and associated memory functions (Cox *et al*, 2009; Takao *et al*, 2008).

Dysbindin and Its Relation to Glutamatergic Mechanisms

Earlier findings regarding the function of dysbindin suggested either no, or an inverse, correlation between levels of dysbindin and VGluT-1 protein levels in the dentate gyrus of hippocampus in humans (Talbot *et al.*, 2004) and in younger animals (<30 days old) (Chen *et al.*, 2008). However, recent studies using more selective antibodies suggest that there is a positive correlation between dysbindin-1 and VGluT-1 levels in older animals, such as the ones used in this study (45–60 days of age), as well as in humans (Talbot, personal communication). Therefore, it is likely that glutamate release is reduced in the *dys⁻/dys⁻* and *wt/dys⁻* animals due to an impairment of the cells' ability to package glutamate into vesicles; this hypothesis is supported by our observation of reduced amplitude of eEPSCs and frequency of mEPSCs *in vitro*. Additionally, we observed a significant decrease in the amplitude of mEPSCs in the *dys⁻/dys⁻* animals, suggesting that dysbindin deletion may further impair glutamatergic transmission via postsynaptic mechanisms, as well. This issue requires further investigation in order to specify the relevant mechanisms involved.

Interestingly, although dysbindin deletion appears to decrease glutamate release at the axon terminal, it appears that the absence of dysbindin may also result in an increase in excitability, as shown by a decrease in the amount of intra-somatic current injection required to evoke an action potential (rheobase). When the basic physiological properties of prefrontal cortical pyramidal neurons were examined, significant difference between the genotypes were found in spike threshold and rheobase current, as well as a decrease of more than 50% in cell capacitance in the *wt/dys⁻* and *dys⁻/dys⁻* groups. Since $C=1/R$, a decrease in capacitance will result in an increase in resistance, therefore rendering the cell more excitable. Changes in capacitance may result from a reduction in cell size, neuronal dendritic branching or spine density. Another possibility is that loss of dysbindin may impact GABAergic interneurons, eliciting concomitant reductions in GABA release and resulting in a disinhibition of pyramidal cells, a possibility that deserves future exploration. Regardless of the mechanisms underlying this increase in excitability, it appears that any effects that might result from an increase in the generation of action potentials in the *dys⁻/dys⁻* mice may be counteracted by the relevant impairments in glutamate release. Future experiments will investigate changes in dendritic branching as well as alterations in GABA release and interneuron function in dysbindin deficient mice.

Recordings during performance of delayed alternation tasks have shown an increase in pyramidal cell firing during the delay period of the task (Fuster and Alexander, 1971; Goldman-Rakic, 1995; Miller, et al, 1996). This recurrent excitatory activity has been proposed to be the cellular correlate for working memory, suggesting that decreases in glutamatergic transmission, such as the decreases reported here in the *dys⁻/dys⁻* and *wt/dys⁻* mice, may underlie the cognitive deficits observed in some neuropsychiatric disorders such as schizophrenia and bipolar disorder. A promising new avenue for pharmacological treatments is aimed at targeting modulators of NMDA receptors, such as the glycine transporter, or more recently, positive allosteric modulators of the mGluR5 glutamate receptor. Several studies have shown that mGluR5 antagonists not only decrease burst firing of PFC pyramidal neurons but also impair spatial working memory (Ballard *et al.*, 2005; Homayoun and Moghaddam, 2006; Homayoun *et al.*, 2004; Locchi *et al.*, 2007), suggesting

that mGluR5 agonists could restore normal levels of activity in dysfunctional cortical circuits affected in schizophrenia.

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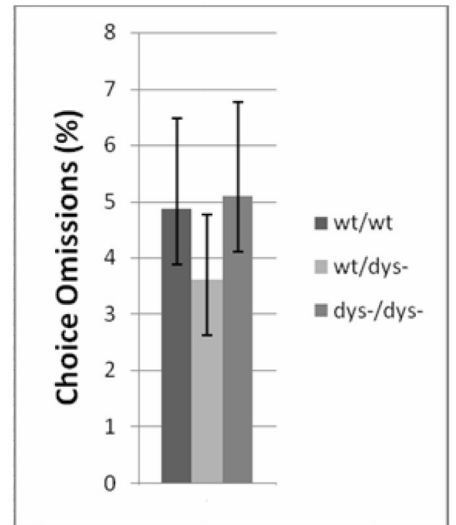
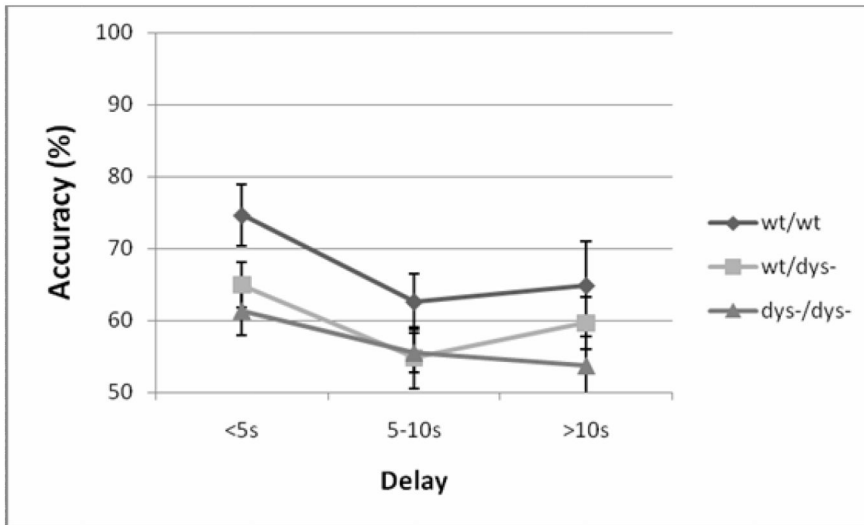
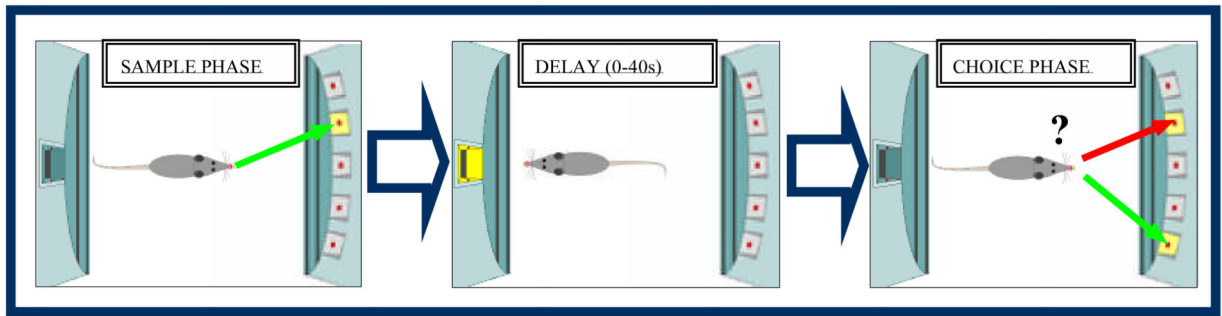


Figure 1.

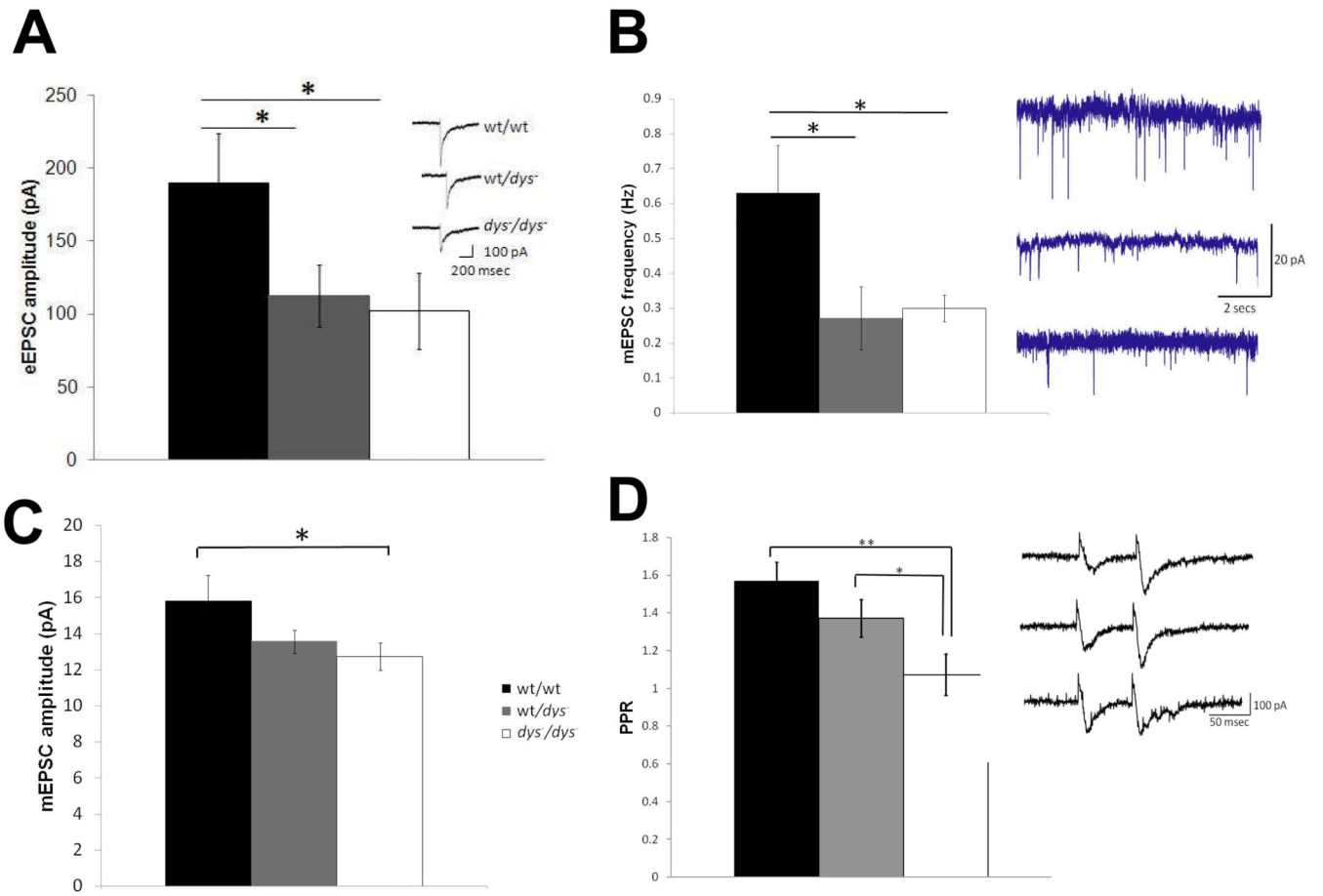


Figure 2.

Membrane properties of prefrontal cortex neurons recorded in wild-type and heterozygous or homozygous dysbindin null mutant mice.

Table 1

genotype	rheobase (pA)	spike threshold (mV)	spike amplitude (mV)	spike half-width (msec)	input resistance (mOhms)	cell capacitance (pF/cm2)	Holding current (pA)
wt/wt	135 +/- 7.2	-47.2 +/- 2.0	79.05 +/- 1.6	2.9 +/- 0.1	126.7 +/- 10.5	165.1 +/- 16.1	-178.2 +/- 21.5
wt/dys ⁻	121.4 +/- 22.5*	-49.6 +/- 2.6*	78.2 +/- 3.9	2.2 +/- 0.1	118.6 +/- 12.9	79.5 +/- 19.8	-169.7 +/- 36.2
dys ⁻ /dys ⁻	67.1 +/- 11.7**	-56.7 +/- 1.5**	76.3 +/- 5.9	2.9 +/- 0.5	125 +/- 6.5	54.1 +/- 7.4	-192.4 +/- 40.9

Astrices indicate statistically significant differences between wild-type controls and mutant groups.

* p<0.05

** p<0.01.