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Frontal cortical synaptic communication is abnormal in *Disc1* genetic mouse models of schizophrenia

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ABSTRACT

Mouse models carrying *Disc1* mutations may provide insights into how *Disc1* genetic variations contribute to schizophrenia (SZ) susceptibility. Disc1 mutant mice show behavioral and cognitive disturbances reminiscent of SZ. To dissect the synaptic mechanisms underlying these phenotypes, we examined electrophysiological properties of cortical neurons from two mouse models, the first expressing a truncated mouse Disc1 (mDisc1) protein throughout the entire brain, and the second expressing a truncated human Disc1 (hDisc1) protein in forebrain regions. We obtained whole-cell patch clamp recordings to examine how altered expression of Disc1 protein changes excitatory and inhibitory synaptic transmissions onto cortical pyramidal neurons in the medial prefrontal cortex in 4–7 month-old mDisc1 and hDisc1 mice. In both mDisc1 and hDisc1 mice, the frequency of spontaneous EPSCs was greater than in wild-type littermate controls. Male mice from both lines were more affected by the Disc1 mutation than were females, exhibiting increases in the ratio of excitatory to inhibitory events. Changes in spontaneous IPSCs were only observed in the mDisc1 model and were sex-specific, with diminished cortical GABAergic neurotransmission, a well-documented characteristic of SZ, occurring only in male mDisc1 mice. In contrast, female mDisc1 mice showed an increase in the frequency of small-amplitude sIPSCs. These findings indicate that truncations of Disc1 alter glutamatergic and GABAergic neurotransmission both commonly and differently in the models and some of the effects are sex-specific, revealing how altered Disc1 expression may contribute to behavioral disruptions and cognitive deficits of SZ.

1. Introduction

Schizophrenia (SZ) is a multifactorial psychiatric condition characterized by both positive and negative symptoms (Tamminga and Holcomb, 2005). Although the etiology of SZ remains poorly understood, the disease has a clear genetic component, with a heritability thought to exceed 75% (Wexler and Geschwind, 2011). Major efforts have identified genetic risk factors, but few have demonstrated strong biological support (Sanders et al., 2008; Moens et al., 2011). One notable exception is the gene *disrupted-in-schizophrenia-1* (*Disc1*). A positive association between SZ and a chromosomal translocation (1:11) disrupting the *Disc1* gene on chromosome 1 was reported in a Scottish family with a high rate of SZ, depression, and bipolar disorder (Millar et al., 2000). Since this discovery, studies have supported a central role for *Disc1* genetic variation in conferring susceptibility to psychiatric disease (Cannon et al., 2005; Thomson et al., 2005; Hashimoto et al., 2006; Moens et al., 2011).

Disc1 encodes the Disc1 protein, which acts as a cytosolic scaffold protein required for processes such as neurogenesis, neuronal migration, dendritic growth, and synaptic maintenance (Brandon et al., 2009; Jaaro-Peled et al., 2009; Hayashi-Takagi et al., 2010; Brandon and Sawa, 2011; Porteous et al., 2011). In mutation carriers, either haploinsufficiency (conferring reduced functional Disc1 expression) or dominant-negative effects of the mutated Disc1 may form the basis for susceptibility to psychiatric disorders (Sawa and Snyder, 2005; Porteous et al., 2006; Hikida et al., 2007).

Genetic animal models have been designed to elucidate how *Disc1* mutations produce the pathophysiology of psychiatric illnesses. These

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models include mice with abolished expression of the full-length mouse Disc1 (mDisc1) protein (Koike et al., 2006), and mice expressing a truncated human Disc1 (hDisc1) protein (Hikida et al., 2007; Li et al., 2007; Pletnikov et al., 2008). These mice exhibit disturbances in sensorimotor gating, hyperactivity, cognitive deficits, depression, and altered social interactions (Kvajo et al., 2008; Pletnikov et al., 2008).

At present, the effects of mutant Disc1 expression on the functional properties of cortical neurons remain unknown. We used electrophysiological methods to examine how expression of truncated Disc1 protein alters membrane properties and synaptic transmission in cortical pyramidal neurons from the medial prefrontal cortex, a brain region crucial to most pathophysiological hypotheses of SZ, in mDisc1 and hDisc1 mouse models.

2. Methods

2.1. Animals

Two genetic mouse models were used. In the first model, a 129S6/SvEv *mDisc1* allele with a 25-bp deletion variant in exon 6 that resulted in the introduction of a premature termination codon in exon 7 was transferred into the C57BL/6J background, resulting in a strain that expresses truncated mDisc1 (Koike et al., 2006; Kvajo et al., 2008). The second model, hDisc1, expresses truncated human Disc1 protein and was created by breeding B6;SJL-Tg(TRE-CMV-hDISC1) founders with single transgenic (control) B6;CBA-Tg(Camk2a-tTA) 1Mmay/j mice (The Jackson Laboratory, Bar Harbor, ME, USA) to generate double transgenic mutant mice of the hybrid B6;SJL;CBA background (Pletnikov et al., 2008). Expression of mutant hDisc1 was limited to forebrain regions including the cerebral cortex, hippocampus, and striatum. mDisc1 mice and wildtype (WT) littermates were obtained from breeding colonies at the University of California, Los Angeles (UCLA). Transgenic hDisc1 mice and single transgenic CaMKII tTA littermates used as controls were obtained from breeding colonies at the Johns Hopkins University School of Medicine in Baltimore, Maryland and shipped to UCLA. All mice were examined at 4–7 months of age and compared with age-matched WT littermates (mDisc1) or controls (hDisc1). All procedures were performed in accordance with the Public Health Service's *Guide for Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at UCLA. Mice were individually housed on a 12 h light–dark cycle, and food and water were available *ad libitum*. Mice were genotyped twice, once at weaning and again after experimentation. There were no significant differences in body weight between WT and mutant mice of the same sex.

2.2. Slice preparation

Mice were anesthetized using isoflurane, decapitated, and the brains were rapidly removed to ice-cold dissection artificial cerebrospinal fluid (ACSF), containing 130 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 1.25 mM $NaHPO_4$, 10 mM glucose, 5 mM $MgCl_2$ and 1 mM $CaCl_2$; aerated with 95% O_2/5% CO_2 (pH = 7.2; 290–300 mOsm/L). The brain was trimmed and glued to the stage of a vibrating microtome (model VT1000S; Leica), and 300 μm coronal slices of the cerebral cortex were cut. Slices were stored and allowed to recover at room temperature in a chamber submerged in oxygenated standard ACSF (same composition as dissection ACSF, except that $MgCl_2$ and $CaCl_2$ were 2 mM) for at least 1 h before experimentation.

2.3. Electrophysiology

We performed whole-cell patch clamp recordings from layer II/III pyramidal cells of the medial prefrontal cortex. Neurons in layers II/III of the prefrontal cortex are critically involved in working memory and in SZ models a reduction of gamma oscillations along with a loss of parvalbumin interneurons occurs selectively in superficial but not deep cortical layers (Cunningham et al., 2006). Furthermore, the inputs to and the morphology of cortical layer II/III pyramidal cells have been shown to undergo pathophysiological alterations in SZ (Lewis and Gonzalez-Burgos, 2000; Pierri et al., 2001; Hill et al., 2006). Pyramidal neurons were visualized with infrared videomicroscopy and differential interference contrast optics and identified by somatic size and typical basic membrane properties (Cummings et al., 2009). Recordings were made with borosilicate glass micropipettes filled with a cesium methanesulfonate (CsMeth)-based internal solution containing 125 mM cesium methanesulfonate, 4 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 9 mM EGTA, 8 mM HEPES, 5 mM MgATP, 1 mM Tris/GTP, 10 mM disodium phosphocreatine and 0.1 mM leupeptin (pH = 7.2; 270–280 mOsm/L). In some experiments QX-314 (4 mM) was included in the internal solution to prevent Na^+ channel activation when holding at depolarized membrane potentials. Voltage clamp recordings were performed using a Multiclamp 700B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA). During electrophysiological recordings, slices were continuously perfused in 95% O_2/5% CO_2 ACSF at a flow rate of ~2 mL/min at room temperature.

Spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs, respectively) were recorded, filtered at 1 kHz and digitized at 100–200 μs using Clampex 10.2 in gap-free mode (Molecular Devices). To assess basic membrane properties and sEPSCs, cells were voltage-clamped at −70 mV. Cell membrane capacitance was determined from a depolarizing step voltage command (10 mV) using the membrane test function integrated in the pClamp10 software.

To more completely isolate glutamate receptor-mediated sEPSCs, bicuculline methobromide (BIC, 5 μM) was applied in order to block γ-aminobutyric acid type A ($GABA_A$) receptor-mediated currents. This concentration of BIC completely abolishes sIPSCs in cortical pyramidal neurons (Cummings et al., 2009). To isolate sIPSCs, membranes were stepped to a holding potential of +10 mV and currents were recorded in regular ACSF. No glutamate receptor antagonists were used for this experiment as this would have precluded estimation of glutamate-GABA ratios and also because quinoxaline derivatives such as CNQX and NBQX alter sIPSC frequency (McBain et al., 1992; Brickley et al., 2001). Nevertheless, after addition of BIC no spontaneous synaptic activity was observed at +10 mV indicating that glutamate synaptic events did not contribute to sIPSCs at this holding potential.

Spontaneous EPSCs and IPSCs were analyzed offline using the automatic detection protocol within the MiniAnalysis program (Justin Lee, Synaptosoft, version 6.0) and subsequently checked manually for accuracy. The threshold amplitude for the detection of an event (5 pA for EPSCs; 10 pA for IPSCs) was set above the root mean square noise (<2 pA at V_{hold} = −70 mV and <4 pA at V_{hold} = +10 mV). Event kinetic analysis used the MiniAnalysis program, and EPSCs and IPSCs with peak amplitudes between 10–50 pA and 10–100 pA, respectively, were grouped, aligned by half-rise time, and normalized by peak amplitude. In each cell, grouped events were averaged to obtain rise times, decay times, and half-amplitude durations. We calculated the ratio of excitatory to inhibitory events by dividing the frequency of sEPSCs by the frequency of sIPSCs for each cell.

2.4. Statistical analyses

Values in figures and text are means ± SEMs. Differences between group means were assessed with appropriate Student's t-tests (unpaired), or Mann–Whitney rank sum test when distributions were not normal, and two-way analyses of variance (ANOVA) with one repeated measure (RM), followed by Bonferroni *post hoc* tests. Differences were considered statistically significant if $p<0.05$. Microsoft Excel and Sigma Stat 3.5 were used to perform all statistical analyses.