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Expression of mutant DISC1 in Purkinje cells increases their spontaneous activity and impairs cognitive and social behaviors in mice

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ABSTRACT

In addition to motor function, the cerebellum has been implicated in cognitive and social behaviors. Various structural and functional abnormalities of Purkinje cells (PCs) have been observed in schizophrenia and autism. As PCs express the gene *Disrupted-In-Schizophrenia-1* (*DISC1*), and *DISC1* variants have been associated with neurodevelopmental disorders, we evaluated the role of DISC1 in cerebellar physiology and associated behaviors using a mouse model of inducible and selective expression of a dominant-negative, C-terminus truncated human DISC1 (mutant DISC1) in PCs. Mutant DISC1 male mice demonstrated impaired social and novel placement recognition. No group differences were found in novelty-induced hyperactivity, elevated plus maze test, spontaneous alternation, spatial recognition in Y maze, sociability or accelerated rotarod. Expression of mutant DISC1 was associated with a decreased number of large somata PCs (volume: 3000–5000 μm^3) and an increased number of smaller somata PCs (volume: 750–1000 μm^3) without affecting the total number of PCs or the volume of the cerebellum. Compared to control mice, attached loose patch recordings of PCs in mutant DISC1 mice revealed increased spontaneous firing of PCs; and whole cell recordings showed increased amplitude and frequency of mEPSCs without significant changes in either R_{input} or parallel fiber EPSC paired-pulse ratio. Our findings indicate that mutant DISC1 alters the physiology of PCs, possibly leading to abnormal recognition memory in mice.

1. Introduction

It is widely believed that the cerebellum is involved in motor activity and coordination (Evarts and Thach, 1969). However, the cerebellum also has extensive connections with the brain regions (e.g., prefrontal and posterior parietal cortex) implicated in cognitive and social aspects of human behavior (Clower et al., 2005). Consistently, decreased gyrification, smaller granular and molecular layers of the vermis and loss of Purkinje cells (PCs) have been associated with social, emotional, and cognitive dysfunction in schizophrenia (Andreasen and Pierson, 2008; Martin and Albers, 1995; Schmahmann, 1991; Schmitt et al., 2010; Snider, 1982; Supprian et al., 2000; Yeganeh-Doost et al., 2011). A decreased number of PCs (Kemper and Bauman, 1998; Kern, 2003) and abnormal sizes and shapes of neurons of the deep cerebellar nuclei were observed in autism spectrum disorders (ASD) (Amaral et al., 2008; Palmen and van Engeland, 2004). High incidence of cerebellar movement disorders such as limb dysmetria were observed in children diagnosed with ASD (Papadopoulos et al., 2012).

Disrupted-In-Schizophrenia 1 (*DISC1*) is a psychiatric gene disrupted by the balanced (1:11) (q42.1; q14.3) translocation, segregating in the Scottish family with several major psychiatric disorders, including schizophrenia, depression, and bipolar disorder (Blackwood et al., 2001; Millar et al., 2000; St Clair et al., 1990). Recent studies have reported association of *DISC1* polymorphisms with ASD as well (Kanduri et al., 2016; Kilpinen et al., 2008; Zheng et al., 2011). Schurov et al. (2004) and Goudarzi et al. (2013) found that PCs were predominantly positive for DISC1. However, other small cells in the molecular and the granular layer also demonstrated some DISC1-positive immunoreactivity. Ma et al. (2002) and Austin et al. (2003) were unable to detect DISC1 expression in PCs but found it in glial cells of the molecular layer and Bergmann glia cells only (Austin et al., 2003; Bord et al., 2006; Goudarzi et al., 2013; Ma et al., 2002; Schurov et al., 2004). However, the role of DISC1 in cerebellar physiology and associated behaviors has not been evaluated. To this end, we generated a mouse model of inducible and selective expression of C-terminus truncated human DISC1

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(mutant DISC1) in PCs and assessed the morphological and electrophysiological properties of PCs as well as behavioral and cognitive phenotypes in mutant DISC1 mice.

2. Materials and methods

2.1. Animals

Our mouse model of inducible expression of human mutant DISC1 in the cerebellum is based on the Tet-off system (SFig.1) (Pletnikov et al., 2008). In order to express mutant DISC1 in PCs, heterozygous Parv2A-tTA2 single transgenic male or female mice (generated and kindly provided by Dr. Hongkui Zeng at the Allen Institute for Brain Science, Seattle, WA) were crossed with homozygous single transgenic TRE-mutant DISC1 mice (line 1001) (Pletnikov et al., 2008). This breeding protocol produces litters with ~50% single transgenic mice (TRE-mutant DISC1) that do not express mutant protein but have the transgenic insertion (control mice) and ~50% double transgenic mice (Parv2A-tTA2; TRE-mutant DISC1) that express mutant protein (mutant DISC1 mice). Thus, a balanced combination of paternal or maternal backgrounds was used; and each litter had pups of both genotypes mitigating possible effects of unequal treatment from nursing dams.

All mice were backcrossed to the C57BL/6j background for at least 12 generations. Both male and female mice were used in all experiments. Mouse pups were weaned on postnatal day (P) 21 and housed in sex-matched groups of five in standard mouse cages on a 12-h light/dark cycle at a room temperature of 23 °C with free access to food and water.

Mouse tails were used for genotyping as previously described (Pletnikov et al., 2008). The sequences of the primers are presented in Supplemental Table 1. The animal protocol was approved by the Johns Hopkins University Animal Care and Use Committee.

2.2. Western blotting

In order to evaluate protein levels of mutant DISC1 across postnatal development, control and mutant male and female mice were euthanized at postnatal days (P) 0, 10, 21, 60 or 150. Brains were quickly removed and frontal cortex, hippocampus and cerebellum were isolated in ice-cold phosphate buffered saline (PBS), frozen on dry ice and were kept at −80 °C until used. Expression of mutant DISC1 was measured using our published protocol (Pletnikov et al., 2008). Briefly, membranes were incubated overnight at 4 °C with mouse anti-*c-myc* antibody (Roche Applied Science, Madison, WI, Cat#11667149001, 1:1000) to assess expression of *myc*-tagged mutant DISC1 followed by peroxidase-conjugated goat anti-mouse (Sigma-Aldrich, St. Louis, MO; Cat # NA931-1ML, 1:1000) secondary antibody. Optical density (O.D.) of protein bands on each digitized image was normalized to that of loading control (β-tubulin or β-actin, Cell Signaling, Danvers, MA; 1:3000). Densitometry was done using the ImageJ software (https://imagej.nih.gov/ij/). Normalized values of 3–4 mice per group of both sexes were used for analysis.

2.3. Behavioral tests

Behavioral tests were performed on 2–5 month old mice. The interval between each behavioral test was at least one week. The tests were performed in the following order: elevated plus maze, open field test, spontaneous alteration, spatial recognition memory test, novel place recognition, sociability and social novelty test, fear conditioning, and accelerating rotarod test. The behavioral protocols are described in detail in the Supplemental Materials.

2.4. Histopathological analyses

For the histopathological evaluation, we used a separate cohort of control and mutant DISC1 male and female mice (P21) and mice employed in the behavioral tests (P150). Mice were deeply anesthetized with Forane (isoflurane USP, NDC 10019-360-60, Baxter Healthcare Corporation, Deerfield, IL, USA) and transcardially perfused with 0.1 M phosphate buffer (PBS; pH 7.4) with heparin (10,000 U/L), and then perfused with 4.0% paraformaldehyde in 0.1 M PBS. The brains were dissected out and post-fixed in 4.0% paraformaldehyde in 0.1 M PBS for 24 h at 4 °C. After cryoprotection with 30% sucrose in 0.1 M PBS for 48 h, the brains were cut into 40 μm thick parasagittal sections. Sections were stained with cresyl violet or H&E for stereology and histopathological assessments.

2.5. Stereology assessments

We evaluated the effect of mutant DISC1 on volume of the cerebellum, total number of Purkinje cells and Purkinje cell size. Measurements and analyses were performed by an investigator blind to the groups' identities using the Cavalieri and the Optical Disector/Fractionator and Nucleator methods (Stereo-Investigator; MBF Bioscience, Williston, VT) as previously described (Manaye et al., 2007; Subbiah et al., 1996; West, 1993). The detailed protocols are described in the Supplemental Materials.

In order to assess possible neuroinflammation in the brain of mutant mice, adjacent sections were stained with avidin–biotin immunohistochemistry (Vector, Burlingame, CA, USA) with rabbit anti-Iba1 (Wako Chemicals USA, Inc., Richmond, VA, Cat # 019-19741, 1:1000) or mouse anti-GFAP (Abcam PLC, Cambridge, MA, Cat # ab10062, 1:1000) antibodies followed by biotinylated anti-rabbit or anti-mouse Immunoglobulin G (IgG, Vector, Burlingame, CA, USA) antibodies.

2.6. Fluorescent immunostaining

Separate cohorts of male and female mice were used for immunostaining at P21. In order to visualize PCs that express mutant DISC1 for single cell immunostaining analyses and electrophysiological recording (described below), control or mutant DISC1 mice were crossed with TIGRE-Ins-TRE-tdT-D-554 (Ai63; generated and kindly provided by Dr. Hongkui Zeng at the Allen Institute for Brain Science, Seattle, WA) mice that express tdTomato under the same promoter. This breeding protocol produced double transgenic mice (Parv-2AtTA2; TIGRE-Ins-TRE-tdT-D-554) that express tdTomato only in PCs (control-tdTom mice) or triple transgenic mice (Parv2A-tTA2; TIGRE-Ins-TRE-tdT-D-554; TRE-mutant DISC1) that express both mutant DISC1 and tdTomato in the same PCs (mutant DISC1-tdTom mice). Briefly, after incubating brain sections in the blocking solution for 1 h at room temperature (RT), the sections were incubated overnight at 4 °C with the primary antibodies: mouse monoclonal anti-SC35 splicing factor, (Thermo Fisher Scientific, Waltham, MA; 1:200); goat anti-mCherry, (SicGEN, Cantanhede, Portugal, Cat # AB0040-200; 1:200) or rabbit anti-Calbindin D28K (EMD Millipore, Billerica, MA, Cat # AB1778; 1:400). Afterwards, the sections were incubated for 1 h at RT with the corresponding Alexa 488-, 568-, 633-labeled species-specific secondary antibodies (Thermo Fisher Scientific, Waltham, MA former Life Technologies, Carlsbad, CA, 1:500). Images were taken with a Zeiss LSM 510 confocal laser scanning microscope with 40×/1.3 oil DIC objective at the Johns Hopkins University Neuroscience Multiphoton/Electrophysiology Core Facility.

2.7. Image analysis

To measure levels of SC35-immunioreactivity (SC35-ir) of individual PCs in confocal images of the cerebellum in mutant DISC1-tdTom and control-tdTom mice, the Imaris software (Bitplane AG, Zurich, Switzerland) was used. Using mCherry (tdTomato) channel, we selected the entire surface of the soma of a PC to generate a region of interest (ROI) defined by the program as "3D soma surface" for the selected PC. We then generated a new "SC35in" channel that included SC35-ir