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Der Einfluss des Gens Disrupted-in-Schizophrenia 1 (DISC1) auf den Mechanismus der tangentialen Migration cortikaler Interneurone

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der Friedrich-Schiller-Universität Jena

von Dipl. Trophologin Christin Gampe

geboren am 16.07.1985 in Jena

Jena, 2014

1. Gutachter: Prof. Jürgen Bolz
   Friedrich-Schiller-Universität Jena

2. Gutachter: Dr. Christoph Kaether
   Leibniz-Institut für Altersforschung Jena

3. Gutachter: Prof. Martin Bastmeyer
   Karlsruher Institut für Technologie

Dispuation: 24.11.2014

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Abkürzungsverzeichnis

bp	Basenpaare
BSA	Kälber-Serumalbumin
CB	Calbindin
CGE	caudale ganglionische Eminenz
DAPI	4',6-Diamidino-2-phenylindol
DISC1	Disrupted-in-Schizophrenia 1
D-MEM	Dulbecco´s Modified Eagle`s Medium
E	Embryonaltag
EB3	End-Bindungsprotein 3
EDTA	Ethyldiamintetraessigsäure
EGFP	verstärktes grünes Fluoreszenzprotein
EmGFP	Emeralds-GFP
EP	Elektroporation
F-Aktin	filamentöses Aktin
FBS	fötales Kälberserum
GABA	Gamma-Aminobuttersäure
GAD67	Glutamatdecarboxylase 67
GBSS	Gey´s balanced salt solution
GFP	grünes Fluoreszenzprotein
HBSS	Hanks´balanced salt solution
HEPES	N- (2-Hydroxyethyl)-piperazin-N'-2- ethansulfonsäure
LGE	laterale ganglionische Eminenz
Lhx6	LIM Homeobox-Gen 6
LIS1	Lissencephaly 1
MAP1A	Mikrotubuli-assoziiertes Protein 1A
MGE	mediale ganglionische Eminenz
miRNA	Mikro-RNA
MZ	Marginalzone
NMDA	N-Methyl-D-Aspartat
PBS	Phosphatgepufferte Kochsalzlösung
PFA	Paraformaldehyd

POA	präoptisches Areal
PV	Parvalbumin
RCAS	*replication-competent avian sarcoma-leukosis virus long terminal repeat with splice acceptor*
RNAi	RNA-Interferenz
ROCK	Rho-assoziierte Proteinkinase
RT	Raumtemperatur
SVZ	Subventrikularzone
TAE	Tris-Acetat-EDTA
TVA	Tumorvirus A
VZ	Ventrikularzone

1 Einleitung

Die Großhirnrinde (Cortex) stellt die Integrationszentrale jeglicher Sinneswahrnehmungen dar und ermöglicht eine der Situation entsprechende Verhaltensanpassung. Gerade der Neocortex, der entwicklungsgeschichtlich jüngste Teil des Cortex, ist beim Menschen besonders hoch entwickelt und ermöglicht z.B. eine ausgeprägte Feinmotorik der Hände, die Ausbildung eines Bewusstseins, die Fähigkeit zu logischem Denken sowie die Ausbildung eines Gedächtnisses. Mit 90% der Großhirnoberfläche bildet er beim Menschen den mit Abstand größten Bestandteil des Cortex. Er ist sechs-schichtig aufgebaut und wird auch als Isocortex bezeichnet, während ältere Strukturen mit einer geringeren Schichtung, wie der Hippocampus und das Riechhirn, als Allocortex bezeichnet werden.

Im Hinblick auf die verschiedenen Zelltypen des Gehirns kann eine Einteilung in zwei Gruppen vorgenommen werden. Bei einen Großteil der Zellen handelt es sich um Gliazellen. Man unterscheidet mehrere Typen, die eine Vielzahl von Funktionen erfüllen, wie z.B. Nährstoffaustausch, Ausbildung der Blut-Hirn-Schranke, immunologische Abwehr und Beteiligung an neuronalen Prozessen. Bei der zweiten Gruppe handelt es sich um Neurone, die für die Reizweiterleitung und -verarbeitung zuständig sind. Die Neuronen lassen sich wiederum in erregende Projektionsneurone und hemmende Interneurone unterteilen. Die Projektionsneurone rufen mit der Ausschüttung von Neurotransmittern wie Glutamat, Dopamin oder Serotonin ein erregendes postsynaptisches Potential hervor, während die Interneurone meist über den Neurotransmitter GABA ein inhibitorisches postsynaptisches Potential in den nachgeschalteten Zellen generieren. Mit 80-85% der Neurone machen die Projektionsneurone den weitaus größeren Teil aus. Im Gegensatz dazu weisen die Interneurone eine höhere Diversität auf (Kepecs & Fishell 2014). Durch die adäquate Balance zwischen Erregung und Hemmung wird die korrekte Funktion des Gehirns ermöglicht.

1.1 Die Migration cortikaler Interneurone

Während der embryonalen Entwicklung werden Neurone in bestimmten Proliferationszonen gebildet und migrieren dann in ihre Zielregionen ein, wo sie sich ausdifferen-

zieren und in das entstehende Netzwerk von Neuronen integrieren. Bei der Maus beginnt diese Zellmigration am Embryonaltag E10,5 und erreicht ihren Höhepunkt an E14,5.

Die neuronalen Vorläuferzellen glutamaterger Projektionsneurone entstehen in der Ventrikularzone des dorsalen Großhirns und wandern dann, meist entlang von Gliazellen, in den sich entwickelnden Cortex ein (Abb. 1). Abgesehen von Schicht I, in der sich die ersten Neurone einlagern, welche später Apoptose eingehen, entspricht die Entstehung des Cortex einem Inside-Out-Prinzip. Dabei kommen früh geborene Vorläufer schnell zu liegen und bilden damit die tieferen Schichten des Cortex (V und VI), während später geborene Vorläuferzellen eine größere Distanz zurücklegen und schließlich die äußeren Schichten des Cortex (II/III und IV) bilden. (Rakic 1971, 1974; McConnell 1988; Misson et al. 1991)

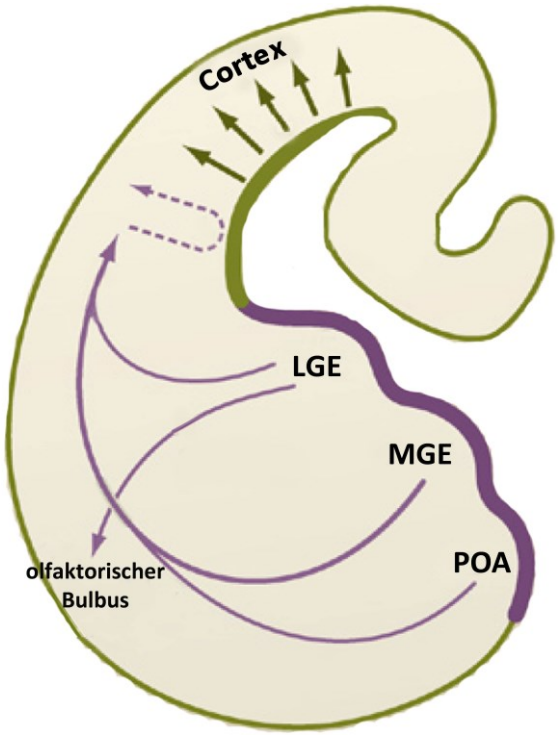

Abb. 1: Die Migration cortikaler Zellen. Projektionsneurone wandern radial in den sich entwickelnden Cortex ein (grün). Im Gegensatz dazu migrieren Interneurone aus den Proliferationszonen im basalen Telencephalon tangential in den Cortex ein (violett). (geändert nach Ayala et al. 2007)

Im Gegensatz zur sog. radialen Migration der Projektionsneurone steht die tangentiale Migration der cortikalen Interneurone. Diese werden in der Ventrikularzone des basalen Großhirns, genauer in den ganglionischen Eminenzen (laterale (LGE), mediale (MGE), caudale (CGE)) oder dem präoptischen Areal (POA), geboren und migrieren über eine große Distanz unabhängig von Gliazellen in den Cortex ein (Abb. 1). Dabei entscheidet nicht nur der Entstehungsort über den Weg in den Cortex, sondern auch der Zeitpunkt. Früher geborene Interneurone (E12,5) migrieren eher superfiziell, während zu einem späteren Zeitpunkt (E13) eine Migration auch auf tiefer gelegenen Routen zu beobachten ist (Anderson et al. 2001; Marin & Rubenstein 2001).

Da die tangentiale Migration größtenteils nicht entlang von Gliazellen stattfindet, befinden sich auf dem Migrationsweg vom basalen Telencephalon in Richtung Cortex eine

Vielzahl von Lenkungsfaktoren sowie Motogene und Stoppsignale. Eine bedeutende Proteinfamilie stellen dabei die Eph-Rezeptoren und deren Liganden, die Ephrine, dar. Durch Interaktion dieser Rezeptor-Tyrosin-Kinasen mit den Liganden werden Signalkaskaden in Gang gesetzt, die unter anderem Einfluss auf Adhäsionsprozesse sowie die Cytoskelett-Organisation haben (Kullander & Klein 2002) und darüber die Migration der Zellen beeinflussen. Weitere Lenkungsfaktoren stellen beispielsweise die Semaphorine und Neurotrophine dar. (Rudolph et al. 2014; Steinecke et al. 2014; Ruediger et al. 2013; Zimmer et al. 2011; Rudolph et al. 2010; Zimmer et al. 2010; Zimmer et al. 2008; Zimmer et al. 2007)

Neben den extrinsischen beeinflussen auch intrinsische Faktoren die Migration cortikaler Interneurone. Letztere ermöglichen den regulären Ablauf des Migrationsprozesses. Grundlage dafür ist, dass postmitotische Zellen eine Polarität ausbilden (Li & Gundersen 2008) und am vorderen Pol ein sog. Führungsfortsatz entsteht (Abb. 2). Dieser dient der Zelle zum Suchen und Erkennen von Lenkungsfaktoren in der Umgebung. Da anhand dessen die Richtungsentscheidung getroffen wird, stellt das Auswachsen dieses Fortsatzes einen dynamischen Prozess dar. (Polleux et al. 2002) Dabei wird an der Spitze des Führungsfortsatzes, welcher meist eine bifurkale Verzweigung aufweist, eine Struktur ausgebildet, die dem Wachstumskegel eines auswachsenden Axons ähnelt (Bellion et al. 2005). Im Vergleich zu glia-abhängig migrierenden Neuronen ist diese Struktur bei cortikalen Interneuronen wesentlich stärker ausgeprägt (Rakic 1971). Trifft ein Fortsatz-Ast z.B. auf ein repulsives Signal, so wird er von der Zelle eingezogen, während der andere Ast weiter auswächst (Martini et al. 2009). Wenn die Zelle aufgrund der gegebenen Faktoren eine Entscheidung bezüglich ihres Weges getroffen

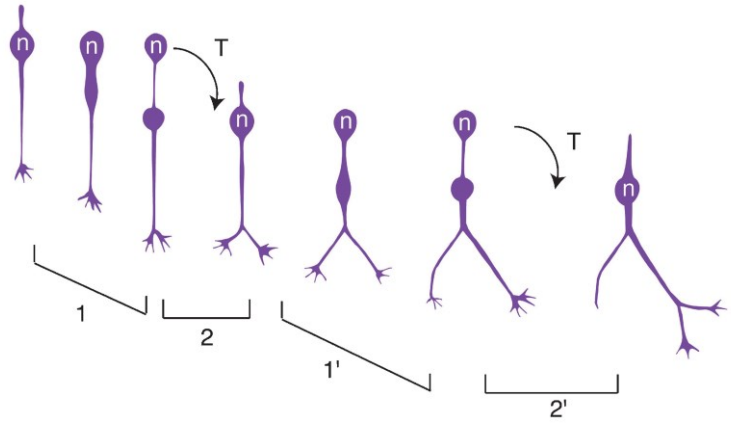

Abb. 2: Migrationsmodus tangential migrierender Interneurone. Vor der Translokation entsteht eine Schwellung im Führungsfortsatz (1), in die der Zellkern anschließend hineinbewegt wird (2). Die Verzweigung des Führungsfortsatzes spielt dabei eine wichtige Rolle, da sie es der zell erlaubt, verschiedene Lenkungsfaktoren der Umgebung wahrzunehmen. Trifft ein Fortsatzast auf ein repulsives Signal, so wird er eingezogen, während attraktive Signale das Auswachsen von Fortsätzen begünstigen (1`, 2`). n: Zellkern. T: Translokation (geändert nach Metin 2006)

hat, entsteht eine Schwellung im Führungsfortsatz, in welche der Zellkern bewegt wird. Während der Kerntranslokation, der sog. Nucleokinese, wird das Auswachsen des Fortsatzes gestoppt, wobei meist eine erneute Verzweigung der Fortsatzspitze stattfindet. Zudem entsteht am hinteren Ende der Zelle ein nachhängender Fortsatz, welcher anschließend eingezogen wird. (Abb. 2; Moya & Valdeolmillos 2004; Bellion et al. 2005; Métin et al. 2006)

1.2 Die Rolle des Cytoskeletts bei der tangentialen Migration

Die Translokation des Kerns sowie die mit der Migration verbundenen morphologischen Veränderungen cortikaler Interneurone werden vom Cytoskelett realisiert. Um die Bildung eines Fortsatzes zu initiieren, wird zunächst eine Anordnung von Mikrotubuli-Bündeln benötigt, welche sich vom Zellkörper wegbewegen und Membranausstülpungen erzeugen. Diese bilden das Fundament für den Membrantransport, der für das Auswachsen des Fortsatzes erforderlich ist. In den entstandenen Filopodien werden Aktin-Bündel gebildet, an denen die Mikrotubuli dann bevorzugt entlang wachsen. (Dehmelt & Halpain 2004) Die Interaktionspunkte von Aktin und Mikrotubuli vermitteln die Anordnung der Mikrotubuli vom Fortsatzschaft bis zur Fortsatzspitze (Schaefer et al. 2002). Dort sind Aktin-Filamente als Geflecht in Lamellipodien vernetzt und bilden seilähnliche Aktin-Bündel in die herausgestülpten Filopodien (Dehmelt & Halpain 2004).

Durch welche Cytoskelett-Komponenten die Nucleokinese realisiert wird, ist Gegenstand aktueller Studien. Bei cortikalen Projektionsneuronen geht man davon aus, dass das Centrosom, ein Organisationszentrum für Mikrotubuli, entscheidend an der Migration im Neocortex beteiligt ist (Abb. 3A; Higginbotham & Gleeson 2007). Dieses befindet sich vor dem Zellkern und bewegt sich in die Anschwellung des Führungsfortsatzes, gefolgt vom Zellkern. Dafür bilden Mikrotubuli ausgehend vom Centrosom eine korbähnliche Struktur, die den Zellkern umschließt. (Schaar & McConnell 2005; Tsai & Gleeson 2005) Mittels Mikrotubuli-assoziierten Transportproteinen wie dem Dynein-Motor-Komplex erfolgt die sprunghafte Vorwärtsbewegung des Zellkerns entlang der Mikrotubuli in die Anschwellung hinein (McKenney et al. 2010). Als Gegenkraft für die Zugkräfte am Zellkern wirken Tubulin-Bündel des Führungsfortsatzes, die am Zellcortex verankert sind (Asada & Sanada 2010). Der Zellcortex wiederum ist mit umgebenden

Zellen oder der extrazellulären Matrix über Adhäsionspunkte verbunden, was die Zug- bzw. Schubkräfte übermittelt.

Obwohl der Einfluss des Mikrotubuli-Cytoskeletts nicht ausgeschlossen werden kann (Bellion et al. 2005; Baudoin et al. 2012), wird ihm bei der Nucleokinese cortikaler Interneurone eher wenig Bedeutung zugeschrieben. Man geht davon aus, dass Mikrotubuli für die Bildung des Führungsfortsatzes zuständig sind und eine Art Richtungsgeber für den Kern darstellen. Zahlreiche Studien weisen dagegen auf eine entscheidende Rolle des Aktin-Cytoskeletts hin (Abb. 3B). Es konnte gezeigt werden, dass filamentöses Aktin

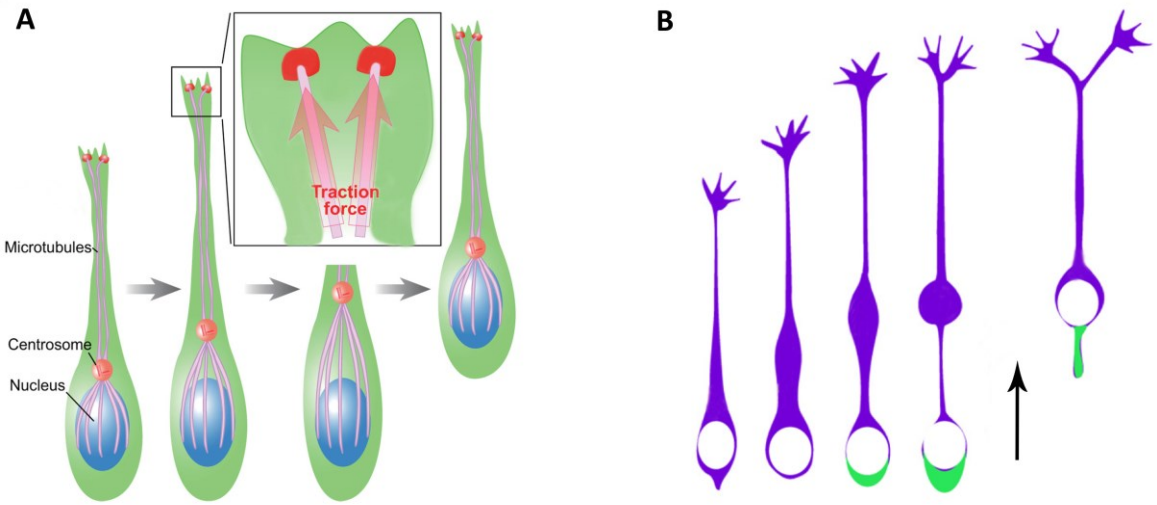

Abb. 3: Das Cytoskelett realisiert die Migration neuronaler Zellen. (A) Bei der Migration von Projektionsneuronen wird den Mikrotubuli eine besondere Bedeutung zugeschrieben. Danach bilden diese, ausgehend vom Centrosom, eine käfigartige Struktur um den Zellkern. Vor der Nukleokinese bewegt sich das Centrosom in die schwellung des Führungsfortsatzes hinein und „zieht" anschließend den Zellkern hinterher. Dabei ist die Verankerung von Mikrotubuli am distalen Zellcortex für die Ausbildung der Zugkraft essentiell. (geändert nach Asada & Sanada 2010) (B) Im Gegensatz dazu weisen viele Studien darauf hin, dass bei tangential migrierenden Interneuronen das Aktin-Cytoskelett die notwendige Kraft zur Bewegung des Zellkerns aufbringt. Dabei wird der Zellkern durch MyosinII-Kontraktionen in die Anschwellung hinein „geschoben". grün: Aktin-Kondensationen (geändert nach Bellion et al. 2005)

(F-Aktin) sowie das Aktin-assoziierte Motorprotein MyosinII hinter dem Kern akkumulieren. Dabei wird angenommen, dass die Kontraktion dieses Akto-Myosin-Netzwerkes den Zellkern von hinten „anschiebt" und am Ablösen und Einziehen des nachhängenden Fortsatzes beteiligt ist. Entsprechend führt die Behandlung von migrierenden Interneuronen mit einem MyosinII-Inhibitor zu einer eingeschränkten Nucleokinese. (Bellion et al. 2005; Schaar & McConnell 2005; Martini & Valdeolmillos 2010)

Laut einer Studie an migrierenden Neuronen des Kleinhirns könnte die benötigte Kraft zur Bewegung des Kerns jedoch auch am vorderen Zellpol aufgebracht werden. Nach He

et al. (2010) ist die Wachstumskegel-ähnliche Struktur der Fortsatzspitze nicht nur für die Richtungsentscheidung notwendig. Indem sie aktiv das Zellsoma inklusive Kern über einen F-Aktin-Fluss entlang des Führungsfortsatzes vorwärts zieht, ist sie außerdem maßgeblich an der Nucleokinese beteiligt.

Das unterschiedliche Ausmaß der Adhäsion migrierender Neurone zum Substrat könnte die Ursache für verschiedene Migrationsmechanismen der Zellen sein. Zum Beispiel entwickelt sich der nachhängende Fortsatz von cortikalen Interneuronen nicht zum Axon, wie es bei Projektionsneuronen der Fall ist. Da er nachgezogen wird, müssen die Verbindungen zu anderen Zellen sowie zur extrazellulären Matrix immer wieder gelöst werden. Im Vergleich zur glia-abhängigen Migration ist die tangentiale Migration daher durch eine geringere Adhäsion sowie eine erhöhte Akto-Myosin-Kontraktionsfähgkeit gekennzeichnet (Friedl 2004). Es wird vermutet, dass jede neuronale Zelle die gleichen Möglichkeiten besitzt, Kraft für die Nucleokinese aufzubringen, wobei die Art des Substrats ausschlaggebend ist, ob der Zellkern „gezogen" oder „geschoben" wird (Martini & Valdeolmillos 2010). Darüber hinaus kann man davon ausgehen, dass Interaktionen von Mikrotubuli und Aktin für die Zellmigration essentiell sind, auch wenn viele Studien nur den Effekt einer einzelnen Cytoskelett-Komponente untersuchen (Rodriguez et al. 2003; Fletcher & Mullins 2010).

1.3 Die Bedeutung cortikaler Interneurone

Zur Zeit wird die Einteilung der cortikalen Interneurone in rund 20 Klassen diskutiert (Ascoli et al. 2008; Defelipe et al. 2013; Kepecs & Fishell 2014). Dabei werden sowohl morphologische und elektrophysiologische Eigenschaften als auch die Expression von Markergenen berücksichtigt. So stellt Lhx6 (LIM Homeobox-Gen 6) einen Marker für Interneurone der MGE dar (Lavdas et al. 1999), aus der u.a. schnell feuernde, Parvalbumin-positive (PV^+) Korbzellen stammen (Butt et al. 2005).

Die Vielzahl verschiedenartiger Interneurone im Cortex erlaubt die Ausbildung komplexer inhibitorischer Schaltkreise (Ascoli et al. 2008; Defelipe et al. 2013). Diese sind bedeutend für die Synchronisation der neuronalen Aktivität, indem sie die Weitergabe von Informationen modulieren. Dabei kann sowohl der synaptische Eingang (dendritische Inhibition) als auch der Ausgang von Projektionsneuronen (perisomatische Inhibition) reguliert werden (Freund 2003). Die Aktivität von Neuronen und Interneuronen

führt so zur notwendigen Balance von Exzitation und Inhibition im Cortex. Ist diese Balance, z.B. durch eine fehlerhafte Migration cortikaler Interneurone, gestört, kann es zur Entstehung von neurologischen oder psychischen Krankheiten kommen. Beispiele für solche Erkrankungen sind Epilepsie, Autismus, Depression und Schizophrenie. (Sanacora et al. 2000; Levitt 2005; LeMagueresse & Monyer 2013)

1.4 Schizophrenie als psychische Erkrankung

Bei Schizophrenie handelt es sich um eine schwere psychische Erkrankung, von der ca. 1% der Weltbevölkerung betroffen ist (Jablensky et al. 1992). Studien zeigen eine klare genetische Komponente, die jedoch nicht allein für die Entstehung bzw. den Ausbruch der Erkrankung ursächlich ist. So spielen weitere Faktoren, wie beispielsweise epigenetische Prozesse oder vorgeburtliche Infektionen der Mutter, ebenfalls eine Rolle. Die Symptome von Schizophrenie-Patienten sind sehr variabel, wobei man zwischen Positiv- und Negativsymptomen unterscheidet. Unter Positivsymptomen versteht man inhaltliche Denkstörungen, Sinnestäuschungen bis hin zu Halluzinationen sowie sog. Ich-Störungen. Auffällig werden diese Patienten häufig durch die Ausbildung von Wahnvorstellungen. Im Gegensatz dazu stehen die Negativsymptome. Neben kognitiven Defiziten handelt es sich dabei vor allem um eine Abflachung des Empfindens und sozialen Rückzug. Der Ausdruck von Emotionen wird dabei durch motorische Defizite, welche die Mimik und Gestik einschränken, weiterhin erschwert. Einen Hinweis auf das Vorhandensein dieser Negativsymptome gibt die Abnahme individueller Persönlichkeitsmerkmale. Bei Schizophrenie handelt es sich folglich um eine Erkrankung, die den Alltag und die Lebensqualität der Patienten stark negativ beeinflusst. Entsprechend hoch ist auch die Suizidrate mit 10-15%.

Es gibt verschiedene Hypothesen zu den Ursachen von Schizophrenie, die sich jeweils auf einen Teilbereich der Erkrankung beziehen oder aufgrund der Wirksamkeit von Medikamenten aufgestellt wurden. Dazu zählen die Dopamin-, Glutamat-, Immun- und GABA-Hypothese. (Lang et al. 2007) Letztere wird u.a. durch eine verringerte Expression des GABA-produzierenden Enzyms GAD67 sowie des Markers Parvalbumin in Gehirnen von Schizophrenie-Patienten gestützt (Akbarian et al. 1995; Hashimoto et al. 2003). Ein Mangel an diesen Zellen könnte Einflusss auf die γ-Oszillation haben. Dabei handelt es

sich um eine Synchronisation der Aktivität von Pyramidenzellen, die durch Interneurone bewirkt wird (Cardin et al. 2009; Sohal et al. 2009). Störungen der γ-Oszillation wurden bei Schizophrenie-Patienten bereits beobachtet. Sie stellen eine mögliche Ursache für die verminderte Leistung des Kurzzeitgedächtnisses von Erkrankten dar. Weiterhin kann die Kommunikation zwischen einzelnen Cortex-Arealen gestört sein. Auch hier sind Verschaltungsprobleme ursächlich, welche möglicherweise bereits während der Entwicklung nicht angemessen ausgebildet wurden. (Lewis et al. 2005; Daskalakis et al. 2007; Uhlhaas & Singer 2010; Nakazawa et al. 2011; Lewis et al. 2012; Marin 2012; Inan et al. 2013)

Obwohl sie häufig erst im jungen Erwachsenenalter ausbricht, deuten viele Befunde auf eine frühe Grundsteinlegung der Erkrankung hin; sie bilden die Basis für die Neuro-Entwicklungshypothese von Schizophrenie. Entsprechend dieser Hypothese wird die Entwicklung des embryonalen und fötalen Gehirns gestört, was zu einer verminderten Vernetzung der Neurone und veränderten biochemischen oder physiologischen Funktion führt. (Raedler et al. 1998; Harrison 1999; Lang et al. 2007; Jaaro-Peled et al. 2009; Owen et al. 2011) So gibt eine ektopische Ansammlung cortikaler Interneurone in der weißen Substanz von Schizophrenie-Patienten einen Hinweis auf einen Migrationsdefekt dieser Zellen während der vorgeburtlichen Entwicklung (Anderson et al. 1996; Eastwood & Harrison 2003; Joshi et al. 2012).

1.5 *Disrupted-in-Schizophrenia 1 (DISC1)* ist ein Risikogen für Schizophrenie

Wie bereits erwähnt, sind an der Entstehung von Schizophrenie genetische sowie umweltbedingte Faktoren beteiligt. Ein Risikogen stellt *Disrupted-in-Schizophrenia 1 (DISC1)* dar. Dieses wurde in einer schottischen Familie mit gehäuftem Auftreten von Schizophrenie und anderen psychischen Erkrankungen entdeckt. Bei einigen Familienmitgliedern kam es durch eine somale Translokation zum Abbruch des Gens, woher dieses seinen Namen erhielt (zu deutsch: *Unterbrochen-bei-Schizophrenie 1*). Als Folge dieser Mutation liegt das DISC1-Protein bei diesen Personen in vekürzter Form vor. (Millar et al. 2000; Blackwood et al. 2001; Chubb et al. 2008)

Daraufhin wurde in zahlreichen Studien die Funktion von DISC1 untersucht. Es handelt sich um ein cytosolisches Protein, welches eine Vielzahl von möglichen Bindungs-

partnern aufweist (Bradshaw & Porteous 2012**;** Yerabham et al. 2013) und in vielfältiger Weise die Entwicklung von Neuronen beeinflusst. So ist es für die Proliferation, die Differenzierung, das Auswachsen von Neuriten sowie die Bildung von Synapsen notwendig (Miyoshi et al. 2003; Mao et al. 2009; Brandon et al. 2009; Hayashi-Takagi et al. 2010; Hattori et al. 2010; Ishizuka et al. 2011; Narayan et al. 2013). Auch für die Migration und die Positionierung cortikaler Neurone sowie neugeborener Zellen im adulten Hippocampus wurde bereits eine Funktion von DISC1 beschrieben (Kamiya et al. 2005; Tsai et al. 2007; Duan et al. 2007; Enomoto et al. 2009; Meyer & Morris 2009; Young-Pearse et al. 2010). Dass DISC1 über diese Funktionen auch eine Rolle bei der Entstehung von Schizophrenie spielen könnte, zeigen Studien an Mäusen mit *DISC1*-Mutationen. Neben Veränderungen der Neuro-Architektur weisen diese Tiere vor allem Verhaltensweisen auf, die mit den Symptomen von Schizophrenie-Patienten verglichen werden (Hikida et al. 2007; Clapcote et al. 2007; Pletnikov et al. 2008; Kvajo et al. 2008).

1.6 Zielstellung

In dieser Arbeit soll der Einfluss von DISC1 auf die tangentiale Migration cortikaler Interneurone während der Entwicklung des Gehirns dargelegt werden. Die übergeordnete Fragestellung bezieht sich auf den Mechanismus, wie DISC1 die tangentiale Migration beeinflusst.

Dafür sollte zunächst geklärt werden, ob DISC1 endogen in der zu untersuchenden Subpopulation cortikaler Interneurone, genauer parvalbuminerger Interneurone aus der MGE an E14,5, exprimiert ist. Der Nachweis erfolgte dabei sowohl auf mRNA- (*In-Situ*-Hybridisierung) als auch auf Proteinebene (Immunfärbung, Veröffentlichung 1).

Mit Hilfe von RNAi- sowie verschiedenen DISC1-Expressionskonstrukten wurde daraufhin das Proteinlevel von DISC1 in diesen Zellen verändert, um die daraus resultierenden Effekte auf die Migration *in vitro* und *in vivo* zu untersuchen. Als *In-Vitro*-Versuche wurden zunächst Outgrowth-Assays durchgeführt sowie Schnittkulturen angefertigt (Veröffentlichung 1). Um Effekte einer veränderten DISC1-Expression *in vivo* zu zeigen, erfolgten *In-Utero*-Elektroporationen (Veröffentlichung 2). Jeder Versuch wurde hinsichtlich der Migration sowie der Zellmorphologie ausgewertet.

Um Hinweise auf den zugrundeliegenden Mechanismus zu erhalten, durch den ein Mangel an DISC1 zu Defekten bei der Migration führt, sollte das Migrationsverhalten

vereinzelter Interneurone untersucht werden. Dazu wurden Feeder-Layer-Kulturen angefertigt und die Migration cortikaler Interneurone videomikroskopisch aufgezeichnet (Veröffentlichung 2).

Da die dynamischen Prozesse des Cytoskeletts für die Realisierung der Migration notwendig sind, sollte abschließend untersucht werden, ob ein DISC1-Mangel Veränderungen des Cytoskeletts nach sich zieht. Hierfür wurden Immunfärbungen an Interneuronen sowie videomikroskopische Analysen an NIH3T3-Fibroblasten durchgeführt (Veröffentlichung 2).

Die hier vorgestellten Manuskripte sollen zum Verständnis über die Rolle von DISC1 bei der tangentialen Migration cortikaler Interneurone beitragen. Dabei soll nachgewiesen werden, dass DISC1 endogen in tangential migrierenden Interneuronen exprimiert ist und eine Reduktion des Proteinlevels zu einer Verzögerung der Migration führt, die möglicherweise über Defekte des Cytoskeletts zustande kommt. Diese Ergebnisse sollen zum einen Erklärungsansätze dafür liefern, wie Veränderungen des DISC1-Levels eine Prädisposition für Schizophrenie hervorrufen können, und zum anderen die neuroentwicklungsbiologische These zur Entstehung von Schizophrenie untermauern.

2 Übersicht Manuskripte

Manuskript 1: Steinecke A*, Gampe C*, Valkova C, Kaether C, Bolz J (2012) **Disrupted-in-Schizophrenia 1 (DISC1) is necessary for the correct migration of cortical interneurons**. J Neurosci 32:738-745.
*geteilte Erstautorenschaft

- inklusive Cover-Illustration
- gelistet als Key-Research-Artikel bei *Psychology Progress*

In diesem Manuskript erfolgt der Nachweis der DISC1-Expression in tangential migrierenden Interneuronen der MGE. Ein Mangel an DISC1 führt zu einem Migrationsdefekt *in vitro* sowie morphologischen Veränderungen der Führungsfortsätze.

		Eigenanteil:
Zellkultur 1	Pflege von NIH3T3, Transfektion, Immuncytochemie	*100%*
Zellkultur 2	Primärkulturen, In-Vitro-Assays, Immuncytochemie	*0%*
In-Vivo-Daten	In-Utero-Elektroporation	*80%*
Histologie 1	Immunhistologie	*0%*
Histologie 2	In-Situ-Hybridisierung	*100%*
Molekularbiologie 1	RT-PCR	*0%*
Molekularbiologie 2	Vektorklonierung	*100%*
Proteinbiochemie	Western Blot	*100%*
Auswertung 1	Migration	*0%*
Auswertung 2	Morphologie	*100%*

Manuskript 2: Steinecke A*, Gampe C*, Nitzsche F, Bolz J **DISC1 knock-down impairs the tangential migration of cortical interneurons by affecting the actin cytoskeleton**. (angenommen zur Veröffentlichung in Front Cell Neurosci am 20.06.2014)
* geteilte Erstautorenschaft

In dieser Arbeit wird gezeigt, dass ein Mangel an DISC1 zu einem Defekt bei der tangentialen Migration im lebenden Tier führt, der auf einer verringerten Anzahl somaler Translokationen beruht. Untersuchungen am Cytoskelett lassen vermuten, dass Veränderungen der Aktin-Reorganisation die Ursache dafür sind.

Eigenanteil:

Zellkultur 1	Pflege von NIH3T3, Transfektion	*100%*
Zellkultur 2	Primärkulturen, In-Vitro-Assays, Immuncytochemie	*10%*
In-Vivo-Daten	In-Utero-Elektroporation	*20%*
Molekularbiologie	Vektorklonierung	*100%*
Proteinbiochemie	Western Blot	*100%*
Auswertung 1	Migration	*0%*
Auswertung 2	Morphologie	*100%*
Auswertung 3	Zellkultur 1	*100%*
Auswertung 4	Immuncytochemie	*25%*

3 Manuskripte

3.1 Steinecke A, Gampe C, Valkova C, Kaether C, Bolz J (2012) **Disrupted-in-Schizophrenia 1 (DISC1) is necessary for the correct migration of cortical interneurons.** J Neurosci 32:738-745.

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Development/Plasticity/Repair

Disrupted-in-Schizophrenia 1 (DISC1) Is Necessary for the Correct Migration of Cortical Interneurons

André Steinecke,[1]* Christin Gampe,[1]* Christina Valkova,[2] Christoph Kaether,[2] and Jürgen Bolz[1]
[1]Institut für Allgemeine Zoologie und Tierphysiologie, Universität Jena, 07743 Jena, Germany, and [2]Leibniz Institut für Altersforschung-Fritz Lipmann Institut, 07745 Jena, Germany

***Disrupted-in-Schizophrenia 1* (*DISC1*) is a prominent susceptibility gene for major psychiatric disorders. Previous work indicated that DISC1 plays an important role during neuronal proliferation and differentiation in the cerebral cortex and that it affects the positioning of radial migrating pyramidal neurons. Here we show that in mice, DISC1 is necessary for the migration of the cortical interneurons generated in the medial ganglionic eminence (MGE). RT-PCR, *in situ* hybridizations, and immunocytochemical data revealed expression of DISC1 transcripts and protein in MGE-derived cells. To study the possible functional role of DISC1 during tangential migration, we performed *in utero* and *ex utero* electroporation to suppress DISC1 in the MGE *in vivo* and *in vitro*. Results indicate that after DISC1 knockdown, the proportion of tangentially migrating MGE neurons that reached their cortical target was strongly reduced. In addition, there were profound alterations in the morphology of DISC1-deficient neurons, which exhibited longer and less branched leading processes than control cells. These findings provide a possible link between clinical studies reporting alterations of cortical interneurons in schizophrenic patients and the current notion of schizophrenia as a neurodevelopmental disorder.**

Introduction

Disrupted-in-Schizophrenia 1 (*DISC1*) is considered as one of the most compelling risk genes for schizophrenia, but also for other major psychiatric diseases. The biological functions attributed to the DISC1 protein are complex and highly diverse. For example, previous work suggested that DISC1 plays an important role during neuronal proliferation, differentiation, neurite outgrowth, synapse formation, as well as in the genesis and integration of new neurons in the adult hippocampus (for review, see Brandon et al., 2009). There are also several studies that report that DISC1 is a necessary component for the correct positioning of radially migrating cortical pyramidal neurons (Kamiya et al., 2005; Young-Pearse et al., 2010).

We were interested whether DISC1 plays a role in the migratory behavior of cortical interneurons. There are distinct differences in the mode of migration of pyramidal neurons and interneurons. In contrast to cortical projection neurons, interneurons are generated in the medial ganglionic eminence (MGE), the caudal eminence, and the preoptic area (POA) of the basal telencephalon and perform a glia-independent long-range migration in a saltatory fashion (Marín and Rubenstein, 2001). They first extend a highly dynamic branched leading process and thereby scan for extracellular guidance cues that determine their migratory pathway (Valiente and Marín, 2010). Neurite elongation then stops and the nucleus moves forward, a process called nucleokinesis, and the migratory cycle starts again (Métin et al., 2006).

In this study, we first demonstrate that DISC1 transcripts and proteins are present in the MGE of embryonic day (E) 14.5 mouse embryos. We then manipulated DISC1 expression by RNA interference in individual MGE-derived interneurons to characterize cell-autonomous effects of DISC1. Results indicate that interneuron migration is severely delayed after DISC1 suppression. In addition to these migratory deficits, DISC1 knockdown also leads to distinct morphological changes of interneurons, suggesting a causal relationship between alterations in the cytoskeleton and the impaired migration behavior.

Materials and Methods

Mice. Animals used were timed pregnant C57BL/6 mice. The day of insemination was considered as E1. Mice were killed using peritoneal injection of 10% chloral hydrate. All animal procedures were performed in agreement with the institutional regulations of the University of Jena.

Plasmids. For DISC1 knockdown, the BLOCK-iT Pol II miR RNAi expression vector kit (Invitrogen) was used to design miRNA-expressing vectors targeting the sequence AGGCAAACACTGTGAAGTGCA, as described previously (Kamiya et al., 2005). As a control vector, we used a scrambled miRNA sequence that is predicted not to target any known vertebrate sequence. For rescue experiments, the DISC1 miRNA vector (0.75 $\mu g/\mu l$) was coelectroporated with either a pCMV6-XL5 encoding the human DISC1 transcript Lv (SC301729; OriGene Technologies) or a pCAX including mouse DISC1 (a gift from Dr. Sawa, Johns Hopkins University, Baltimore, MD), each at 2.25 $\mu g/\mu l$. For overexpression of mouse DISC1 in NIH3T3, the DISC1 coding region from pCAX was cloned into pmRFP-C1.

Antibodies. The following primary antibodies were used: αDISC1 (1:50 for staining and 1:200 for Western Blotting; SC-47990, Lot #2011;

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*A.S. and C.G. contributed equally to this work.
The authors declare no competing financial interests.
Correspondence should be addressed to Jürgen Bolz, Institut für Allgemeine Zoologie und Tierphysiologie, Universität Jena, Erbertstrasse 1, 07743 Jena, Germany. E-mail: jurgen.bolz@uni-jena.de.
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Santa Cruz Biotechnology), αGAPDH (1:1000; a gift from Dr. Morrison, Fritz Lipmann Institute, Jena, Germany), α-Lim-homeobox-gene 6 (αLhx6; 1:200; SC-98607, Lot #B-1609; Santa Cruz Biotechnology), αCalbindin (1:2000; Swant), and αGFP (1:1000; Invitrogen). Nuclei were stained with 4′,6-diamidin-2-phenylindol (DAPI).

RT-PCR. Tissue from the MGE of E14.5 mice and tissue from the hippocampus of the mother were homogenized in trizol and RNA was isolated using chloroform and isopropyl alcohol. cDNA synthesis was performed using oligo dT primer. For RT-PCR, cDNA was amplified by the following primer pair: ACCCAGGATAGCCTGCCTGCA and ATCAGGTCACAGCCCGGCCA, using a HotStarTaq DNA Polymerase (Qiagen).

In situ *hybridization.* For *in situ* hybridization, a fragment of the mouse DISC1 (bases 1355–2031) was cloned into a pBluescript vector and DIG-labeled sense and antisense probes were generated. *In situ* hybridizations were performed as described previously (Zimmer et al., 2008).

Primary cell culture. For dissociated neurons from the MGE of E14.5 embryos, MGEs were dissected and collected in ice-cold HBSS supplemented with 0.65% glucose. After incubation with 0.025% trypsin in HBSS for 17 min at 37°C, tissue was dissociated into single cells by trituration and filtered through a nylon gauze to remove cell aggregates. Neurons were cultured in DMEM [supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.4 mM L-glutamine] at 37°C and 5% CO_2 for 2 d.

To approve the antigen specificity, *ex utero* electroporation was performed and neurons were extracted from the MGE as described above.

Fibroblast cell culture and Western blotting. NIH3T3 cells were grown in DMEM-F12 with 10% FBS and 5% penicillin/streptomycin under standard cell culture conditions and transfected with plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Cells were lysed after 32 h in STEN buffer (150 mM sodium chloride, 50 mM Tris, 2 mM EDTA, and 0.2% NP-40). Lysates were separated on 12% SDS-polyacrylamide gels and transferred to PVDF membranes. Membranes were blocked in TBS-T buffer (300 mM NaCl, 10 mM Tris, pH 7.6, and 0.1% Tween 20) for 30 min and then incubated with the primary antibody for 1 h at room temperature (RT) or overnight at 4°C. After washing in TBS-T (four times for 8 min), membranes were incubated for 1 h at RT with horseradish peroxidase-conjugated secondary antibodies. Membranes were washed again in TBS-T and the signal was detected using enhanced chemiluminescence.

Immunocytochemistry. Neurons and NIH3T3 were fixed in 4% paraformaldehyde (PFA), washed with PBS containing 0.2% Triton X-100, and blocked for 1 h with 5% bovine serum albumin (BSA), 0.3% Tween 20 in PBS containing 0.2% Triton X-100. For immunostainings against Lhx6, cells were washed in heated citrate buffer and a blocking solution containing sodium azide was used (1% milk, 10% FBS, 1 mg/ml BSA, and 52 μg/ml sodium azide in PBS). Primary antibodies were applied overnight. After washing with PBS, secondary antibodies were incubated for 1 h followed by washes in PBS and DAPI staining.

Immunohistochemistry. For immunohistochemistry, freshly prepared cryosections (18 μm) were fixed in 4% PFA in PBS for 30 min (for DISC1: 2% PFA, 10 min) and washed in 0.2% Triton X-100 in PBS, blocked in 10% goat serum, 5% BSA, and 0.2% Triton X-100 in PBS for 1 h, followed by the incubation of the primary antibodies overnight. For immunostainings against Lhx6, slices were cooked in citrate buffer and a special blocking solution containing sodium azide was used (1% milk, 10% FBS, 1 mg/ml BSA, and 52 μg/ml sodium azide in PBS). After washing, sections were incubated for 2 h with the secondary antibody followed by washing, incubating in DAPI, and embedding.

Ex utero *electroporation.* Brain hemispheres from E14.5 embryos were dissected in ice-cold sterile Krebs buffer (126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM MgCl, 2.1 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose). *Ex utero* electroporation was performed as described previously (Yozu et al., 2005). In brief, <1 μl of a 1 μg/μl miRNA solution was pressure injected into the ventricular zone of the MGE, followed by electroporation of two pulses of 100 ms duration and 100 V using a BTX ECM 830 (Harvard Apparatus).

Outgrowth assay. After *ex utero* electroporation, MGE were dissected in methyl cellulose medium (MC) (0.4 g of methyl cellulose, 5% FBS, 1% penicillin/streptomycin, 1% L-glutamine, 0.1% glucose) and cut in 200 × 200 μm pieces using a tissue chopper. The MGE explants were precultured at 37°C and 5% CO_2 for 1 h, embedded in chicken plasma that was cross-linked with thrombine, and cultured in MC medium for 2 DIV before fixation with 4% PFA in PBS. Before analyzing, we made certain that there were transfected cells in the explant and that the outgrowth of nontransfected cells was normal.

Slice cultures. After *ex utero* electroporation, hemispheres were embedded in 30% low-melt agarose in PBS, cut into 250 μm slices using a vibratome, and kept in Krebs buffer for postholding sections (supplemented with 10 mM HEPES, 1% penicillin/streptomycin, and 0.1 mg/ml gentamicin) on ice. Slices were transferred on membranes (Whatman) lying on neurobasal medium (supplemented with 2% B-27, 1% penicillin/streptomycine, 1% L-glutamine, and 0.5% glucose), and cultured for 3 DIV at 37°C and 5% CO_2 before fixation with 4% PFA in PBS.

In utero *electroporation.* Timed pregnant female mice were treated with 4 mg/kg Carprofen for 30 min before deeply anesthetizing with a mixture of fentanyl (0.05 mg/kg), midazolam (5 mg/kg), and metedomidine (0.5 mg/kg). Afterward the uterine horns were exposed. Various constructs (1 μl of total volume, 1 μg/μl DNA plus 2% fast green to monitor the injection) were injected into the lateral ventricles of the embryos and electroporation (5 pulses at 40 V, 100 ms duration) was performed with a forceps electrode connected to a BTX ECM 830 (Harvard Apparatus). After 2 d *in utero*, brains were fixed in 4% PFA in PBS and cryosections were made, followed by immunostaining against GFP.

Cell analysis. The transfected cells were scanned with a Zeiss LSM 510 and analyzed using ZEN 2009 software. Cells from at least three independent experiments were examined.

Results

DISC1 is expressed in MGE-derived interneurons

We first used RT-PCR on MGE tissue isolated from E14.5 embryos to examine the expression of DISC1 mRNA in this subdivision of the subpallium. As a positive control, we used adult hippocampus, where DISC1 remains highly expressed (Austin et al., 2004). RT-PCR showed similar signals in these two brain regions (data not shown). To analyze the spatial distribution of DISC1 transcripts in the telencephalon, we performed *in situ* hybridizations. As illustrated in Figure 1*A*, DISC1 mRNA was detected in the cerebral cortex with highest expression levels in the ventricular zone (VZ) and the subventricular zone (SVZ), consistent with previous findings (Ma et al., 2002; Austin et al., 2004). In the basal telencephalon, DISC1 transcripts were found in the VZ and SVZ of the lateral ganglionic eminence (LGE), MGE, and the POA (Fig. 1*A*).

Next, we wanted to examine the distribution of DISC1 at the protein level. We first tested the antigen specificity of the DISC1 antibody. For this, NIH3T3 fibroblasts were transfected with pmRFP-DISC1 or pmRFP-C1 (control) and immunostainings were performed. The exogenous DISC1 completely overlapped with the immunocytochemical signal (Fig. 1*E*). Analysis of the mean gray values showed a significant increase of the DISC1 signal in cells overexpressing DISC1 compared with cells expressing RFP only (Fig. 1*E′*). In addition, we performed immunostainings with dissociated MGE cells that were transfected with either control miRNA or DISC1 miRNA (Fig. 1*F*) to downregulate endogenous DISC1. Compared with control transfected interneurons, there was a significant decrease in the DISC1 signal in cells transfected with DISC1 miRNA (Fig. 1*F′*). Together, these findings indicate that the antibody reacts with both endogenous and exogenous DISC1 protein. We then used this antibody to perform Western blot analysis to quantify the knockdown efficiency of the DISC1 miRNA. Compared with control miRNA, the knockdown was not complete, but it suppressed ~65% of both DISC1 isoforms known in mice (Fig. 1*G*).

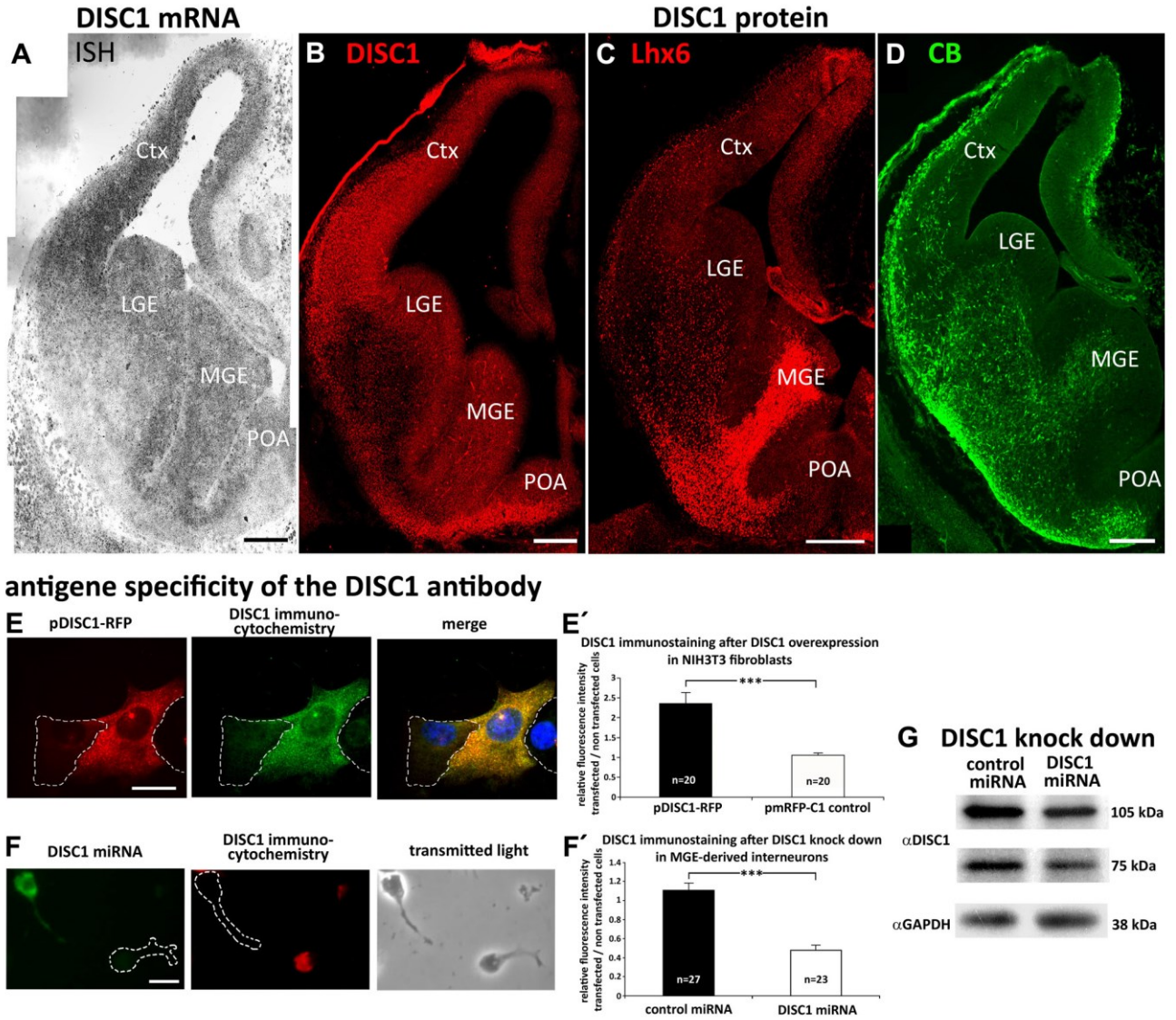

Figure 1. DISC1 mRNA and protein is expressed in E14.5 brains. ***A***, *In situ* hybridization (ISH) demonstrates the expression of DISC1 mRNA in the cortex (Ctx), MGE, LGE, and POA of E14.5 embryos. ***B–D***, Immunohistochemistry with E14.5 cryosections of DISC1 (***B***), Lhx6 (***C***), and CB (***D***). The DISC1 signal overlaps with both markers for interneurons. Scale bars, 200 μm. ***E–F***, Antigen specificity of the DISC1 antibody and knockdown of DISC1 by miRNA. Scale bars, 10 μm. ***E***, Immunocytochemistry with NIH3T3 fibroblasts transfected with pmRFP-DISC1. ***E′***, Analysis of the mean gray value. There was a significant increase of the DISC1 signal in cells overexpressing DISC1 compared with cells expressing RFP only (RFP: 1.07 ± 0.05; DISC1-RFP: 2.36 ± 0.28; each 20 cells). ***F***, Immunocytochemistry on MGE-derived cells transfected with DISC1 miRNA. ***F′***, Comparison of the mean gray value. In interneurons transfected with DISC1 miRNA, the DISC1 signal was significantly decreased compared with control transfected cells (control miRNA: 1.11 ± 0.07, 27 cells; DISC1: miRNA 0.74 ± 0.05, 23 cells). ***G***, Western blot to quantify the DISC1 knockdown efficiency in NIH3T3 fibroblasts. ***$p < 0.001$; *t* test. Error bars are SEM.

We then performed immunostainings of E14.5 cryosections. Results indicated DISC1 protein expression in the basal telencephalon concentrated in the VZ and the SVZ of the MGE, LGE, and POA (Fig. 1*B*), which is consistent with the findings of the *in situ* hybridization (Fig. 1*A*). In the SVZ of the basal telencephalon, the distribution of DISC1-immunoreactive cells overlapped with Lhx6-positive neurons (Fig. 1*C*), a transcription factor that is expressed in the vast majority of MGE neurons that migrate to the pallidum (Alifragis et al., 2004), and calbindin (CB)-positive neurons (Fig. 1*D*), another early marker for immature interneurons (Anderson et al., 1997). To confirm a coexpression of DISC1 with interneuron markers on a cellular level, we used dissociated MGE-derived cells to perform double immunostainings. As shown in Figure 2, first and second lane, DISC1 is expressed in Lhx6-positive as well as CB-positive cells.

Immunocytochemistry also allowed us to identify the subcellular distribution of DISC1. Previous studies demonstrated that the subcellular expression pattern of DISC1 is complex and cell type-specific. Endogenous DISC1 has been found within the nuclei of certain cell types (Sawamura et al., 2008), the cytoplasm, where it colocalizes with centrosomal proteins or F-actin (Morris et al., 2003), and also in the growth cones, the tips of growing axons (Shinoda et al., 2007). In MGE-derived interneurons, we found that DISC1 is consistently expressed in the cell body and in most cases also in the tips of cell processes, particularly in the filopodia of the leading process (Fig. 2*C*, arrow). In migrating interneurons, the cell body consists of a very thin layer of cytoplasm surrounding the nucleus. Confocal imaging of DISC1-immunoreactive cells counterstained with the nuclear marker DAPI revealed that in the cell body, DISC1 was typically confined

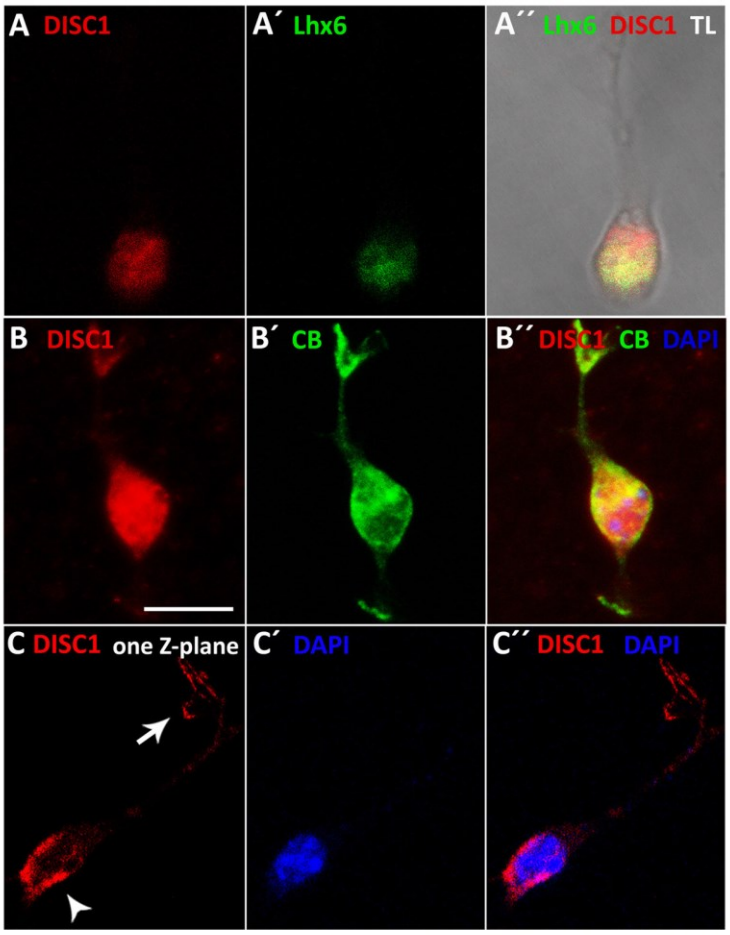

Figure 2. DISC1 is expressed in MGE-derived cortical interneurons. ***A***, ***B***, Dissociated cells from the MGE of E14.5 embryos show that DISC1 is coexpressed with Lhx6 (***A***) and CB (***B***). ***C***, Analysis of single Z-planes reveals that DISC1 is concentrated behind the nucleus (arrowhead). In addition, DISC1 is also concentrated in the tips of the leading process (arrow). Scale bar, 10 μm, TL, Transmitted light.

to the cytoplasm of the cell body. In several cases, however, DISC1 was not evenly distributed in the cytoplasm around the cell nucleus, but was concentrated at the rear of the nucleus, opposite to the leading process (Fig. 2*C*, arrowhead). This subcellular distribution of DISC1 suggests that it might play a functional role in the migration of these neurons, for example by influencing nuclear translocation or the correct formation of leading processes.

DISC1 knockdown leads to migration deficits in MGE-derived neurons

To test the hypothesis that DISC1 is involved in the migration of MGE-derived cells, we first performed a cell migration assay combined with DISC1 RNA interference. For this, brain hemispheres of E14.5 embryos were *ex utero* electroporated with either a DISC1 miRNA vector or a control vector expressing control miRNA. Both vectors coexpressed EmGFP to visualize the transfected cells. After electroporation, small tissue blocks from the MGE were prepared and cultured for 2 d in a 3D matrix of cross-linked chicken plasma that allowed cell migration out of these explants. In this assay, downregulation of DISC1 with miRNA decreased the number of cells exiting the explants by 62% compared with cells transfected with control miRNA (from 4.7 ± 0.6 cells to 1.8 ± 0.4 cells per explant; Fig. 3*A*,*B*,*C*).

We next attempted to rescue the migration defects after DISC1 knockdown by coelectroporation DISC1 miRNA with expression constructs of human DISC1 (hDISC1), which are insensitive to the miRNA (Young-Pearse et al., 2010). As an additional control, we also coelectroporated DISC1 miRNA with expression constructs of murine DISC1 (mDISC1), which are susceptible to the miRNA. As illustrated in Figure 3*C*, after coexpression of DISC1 miRNA with mDISC1, the number of cells migrating out of MGE explants was still reduced compared with control conditions. In fact, there was no difference in the impaired migration between MGE cells transfected with DISC1 miRNA alone and cells cotransfected with mDISC1 plasmids, suggesting that DISC1 miRNA efficiently interferes with endogenous and exogenous murine DISC1. In contrast, coelectroporation of DISC1 miRNA with hDISC1 increased the number of interneurons leaving the explants by 67%, from 1.80 ± 0.18 (DISC1 miRNA + mDISC1) to 3.02 ± 0.27 cells per explant (DISC1 miRNA + hDISC1). This indicates that hDISC1 significantly rescued the effects of DISC1 knockdown, although the rescue was not complete.

Next, we wished to examine directly how DISC1 knockdown affects interneuron migration in the basal telencephalon. It has been reported that ganglionic eminences can be specifically electroporated *in utero* by adjusting the angle of electrode paddles relative to the horizontal plane of the brain (Borrell et al., 2005). However, as others previously noticed (Gelman et al., 2009), we found that it is not possible to restrict *in utero* electroporation to MGE cells and most attempts result in transfections of cells either scattered throughout the entire subpallium or more or less focused clusters of transfected cells at random places in the basal telencephalon. Thus, when these brains are analyzed a few days after *in utero* electroporation, it is virtually impossible to know the exact origin of the transfected cells. We therefore used an *ex utero* electroporation approach, where brains were first removed from E14.5 embryos and miRNA plasmid solution was then pressure injected directly into the MGE before electroporation (Yozu et al., 2005). Adding Alexa 546 as a fluorescent marker to the miRNA solutions, we confirmed that the injections were spatially restricted to the MGE. We then prepared slice cultures from the *ex utero* electroporated brains, kept them for 3 DIV, and analyzed the distribution of the migration pattern of the transfected MGE cells (scheme Fig. 4*A*).

In slices prepared from *ex utero* electroporated brains, after 3 DIV, there was still a dense focus of transfected cells in the MGE, which made it difficult to quantify the exact number of these neurons. However, transfected MGE cells that had migrated to the LGE and transfected neurons that had continued their migra-

tion to the cortex were clearly discernable. We therefore compared the number of cells transfected with DISC1 miRNA or control miRNA that had migrated from the MGE to the LGE with those that continued their migration to the cortex in each slice culture. The total number of labeled cells in the LGE and cortex was set as 100%. After transfection with control miRNA, 58% of the labeled cells were found in the LGE while 42% of the labeled cells continued their migration to the cortex. In contrast, after transfection with DISC1 miRNA, 75% were found in the LGE and only 25% reached the cortex (Fig. 4*B–D*). Thus, reduction of DISC1 in MGE neurons strongly impairs their tangential migration toward the cerebral cortex.

DISC1 depletion changes the morphology of migrating interneurons

The migration of cortical interneurons is a complex process that goes along with distinct morphological changes. It is known that the leading process stops growing while the nucleus moves forward. When nucleokinesis was blocked or reduced, interneurons exhibited prolonged leading processes (Bellion et al., 2005). We therefore measured the length of the leading process of transfected cells in the outgrowth assay as well as in the slice cultures. In both assays, the length of the leading processes from DISC1-deficient cells were significantly longer than that of control transfected cells (Fig. 5*A–D*,*G*). Abnormally prolonged leading processes were not observed when DISC1 miRNA was cotransfected with hDISC1 (40.49 ± 1.67 μm for control transfected cells; 35.65 ± 1.76 μm for DISC1 miRNA + hDISC1 transfected cells). As expected, there was no rescue effect for leading process length when cells were cotransfected with mDISC1 (Fig. 5*H*).

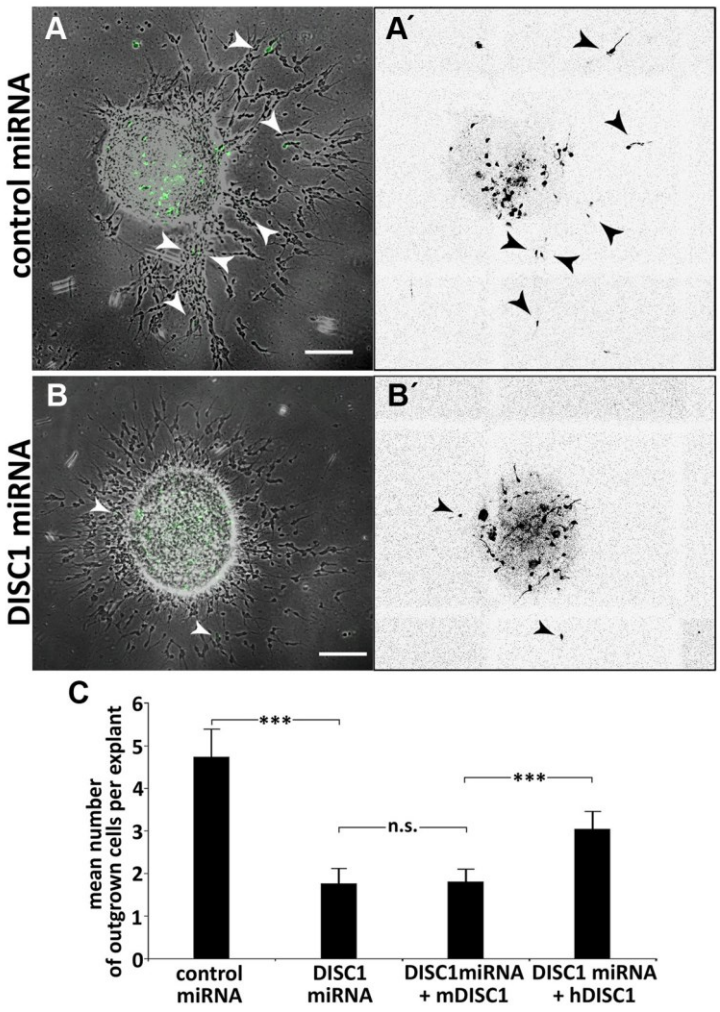

Figure 3. DISC1 knockdown leads to migration defects in the outgrowth assay. ***A***, ***B***, Photomicrographs of MGE explants transfected with control miRNA (***A***) or DISC1 miRNA (***B***), arrows point to transfected neurons (fluorescence) that migrated out of the explants (transmitted light). ***A′***, ***B′***, Inverted fluorescence pictures of ***A*** and ***B*** for better visualization of transfected cells. ***C***, Quantitative analysis shows that the number of cells exiting the explants is reduced after DISC1 knockdown (control miRNA: 4.7 ± 0.6 cells per explant, $n = 84$ explants; DISC1 miRNA: 1.8 ± 0.4 cells per explant, $n = 82$ explants) that can be rescued by human but not mouse DISC1 (DISC1 miRNA + mDISC1: 1.80 ± 0.18 cells per explant, $n = 116$ explants; DISC1 miRNA + hDISC1: 3.02 ± 0.27 cells per explant, $n = 104$ explants; *t* test). ***$p < 0.001$, *t* test; n.s. $p \geq 0.05$. Scale bars, 200 μm. Error bars are SEM.

The leading processes of migrating interneurons are typically branched and it is generally assumed that they sample the environment for guidance cues (Martini et al., 2009). We therefore counted the number of side branches of cells transfected with DISC1 and control miRNA in both *in vitro* assays. We observed that control transfected cells branched significantly more often than DISC1-deficient cells. The highest number of side branches in cells transfected with DISC1 miRNA was three, while control transfected cells had up to six branches (Fig. 5*A–D*). On average, control transfected cells had 3.4 ± 0.14 branches; after DISC1 knockdown, the mean number of branches was reduced to 2.05 ± 0.12 ($p < 0.001$; Fig. 5*I*). After cotransfection of DISC1 miRNA with hDISC1, the number of sides branches significantly increased compared with transfections with DISC1 miRNA alone ($p < 0.001$; Fig. 5*J*). However, cotransfections of DISC1 miRNA with mDISC1 did not increase the number of side branches observed after DISC1 knockdown (Fig. 5*J*). Thus, precise expression levels of DISC1 seem necessary for the formation of appropriate leading processes in migrating cortical interneurons.

To directly compare these *in vitro* results with the *in vivo* situation, we performed *in utero* electroporation of E14.5 embryos using control and DISC1 miRNA and examined the brains after 2 d. As mentioned above, it is not possible to selectively transfect the MGE with this technique. We therefore restricted our morphometric analysis to cells that were still located in the MGE. Under these *in vivo* conditions, we could observe very similar morphological alterations as in the *in vitro* assays. MGE cells transfected with DISC1 miRNA had a leading process that was almost twice as long as in control transfected neurons (55 ± 7 μm compared with 28 ± 2 μm; Fig. 5*E–G*). We also observed a modified branching pattern in DISC1-deficient cells after *in utero* electroporation. Over 90% of these cells had unbranched processes and the remaining cells had only one side branch. In contrast, approximately half of the control transfected cells had two or more side branches. The average number of branches was

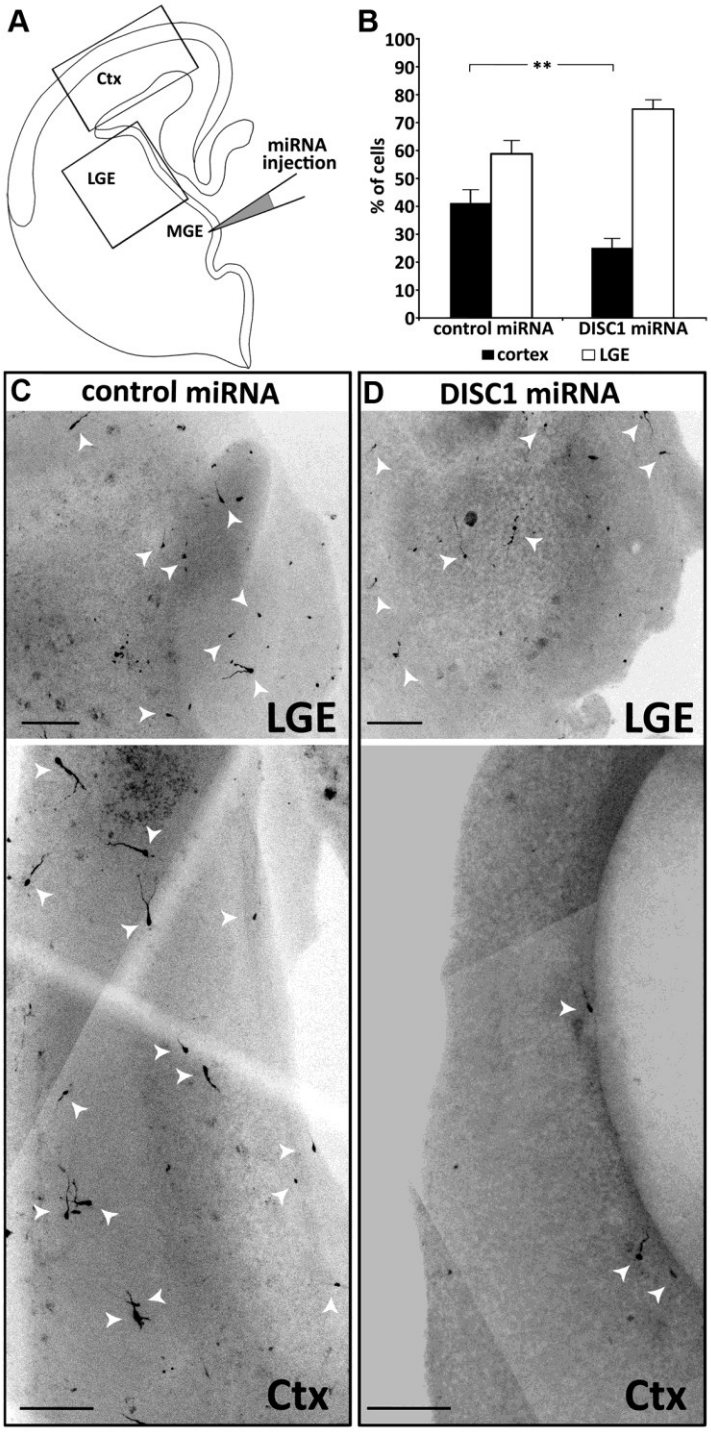

Figure 4. Focal DISC1 knockdown in the MGE leads to migration defects in slice cultures. ***A***, Schematic drawing showing the injection of miRNA solution into the MGE of whole hemispheres followed by electroporation. The hemispheres were sliced and kept for 3 DIV. Rectangles indicate the regions of the photomicrographs shown below. ***B***, Quantification of the number of cells which migrated to the LGE and cells that migrated further to the cortex (Ctx). Under control conditions, significantly more cells reached the cortex compared with DISC1 knockdown slices (control miRNA: 41.2 ± 4.7% cells in the cortex, $n = 17$; DISC1 miRNA: 25.2 ± 3.2 cells in the cortex, $n = 24$; *t* test: $^{**}p < 0.01$). ***C–D***, Photomicrographs from cortex and LGE of control miRNA (***C***) and DISC1 miRNA (***D***) transfected slices. Scale bars, 200 μm. Error bars are SEM.

2.7 ± 0.2 for control transfected cells and 1.1 ± 0.1 for DISC1 miRNA transfected cells ($p < 0.001$; Fig. 5*E–F,I*).

Together, the *in vitro* assays demonstrate that DISC1 knockdown leads to migratory deficits that correlate with specific changes in cell morphology. The fact that the same morphological alterations after DISC1 suppression also occurred *in vivo* strongly suggests that DISC1 is a necessary component for the correct migration of interneurons in the intact brain.

Discussion

In this study, we found that transcripts of the psychiatric illness risk gene *DISC1* are expressed in the MGE, a major source of cortical interneurons. DISC1 protein was detected in MGE neurons that coexpress the early interneuron markers Lhx6 and CB. In these neurons, DISC1 is concentrated in the tips of the leading process and its branches as well as in the somatic cytoplasm. Downregulation of DISC1 via miRNA increased the length of the leading process and at the same time decreased the number of side branches in MGE-derived interneurons. Finally, cell migration assays revealed that DISC1 is a necessary component for the correct tangential migration of interneurons to the cerebral cortex.

Several previous studies have implicated DISC1 in regulating neuronal migration. For example, after *in utero* electroporation of DISC1 shRNA in cortical precursor cells in the ventricular zone, which gives rise to pyramidal neurons, transfected cells were retained in the intermediate zone and failed to migrate into the cortical plate (Kamiya et al., 2005). In contrast, knockdown of DISC1 in newly generated granule cells in the adult hippocampus resulted in an overextended migration (Duan et al., 2007; Enomoto et al., 2009; Kim et al., 2009). It has therefore been suggested that DISC1 does not directly mediate neuronal migration but rather relays positional cues to the migratory machinery (Duan et al., 2007). Consistent with this view, a recent study demonstrated that NMDA receptor antagonists in adult mice lead to an overextension in migration of newborn granule cells and a concomitant reduction in DISC1 expression (Namba et al., 2011). This overextended migration induced by NMDA receptors was rescued by exogenous expression of DISC1, demonstrating that extracellular signals can regulate DISC1 levels and thereby influence neuronal migration. In the present study, we found that after DISC1 suppression in the MGE, interneurons were able to migrate to the LGE and further to the cortex. However, the portion of DISC1-deficient MGE cells that reached their final destination in the cortex was much lower than in control transfected MGE neurons. Thus, DISC1 does not seem to control the positioning of

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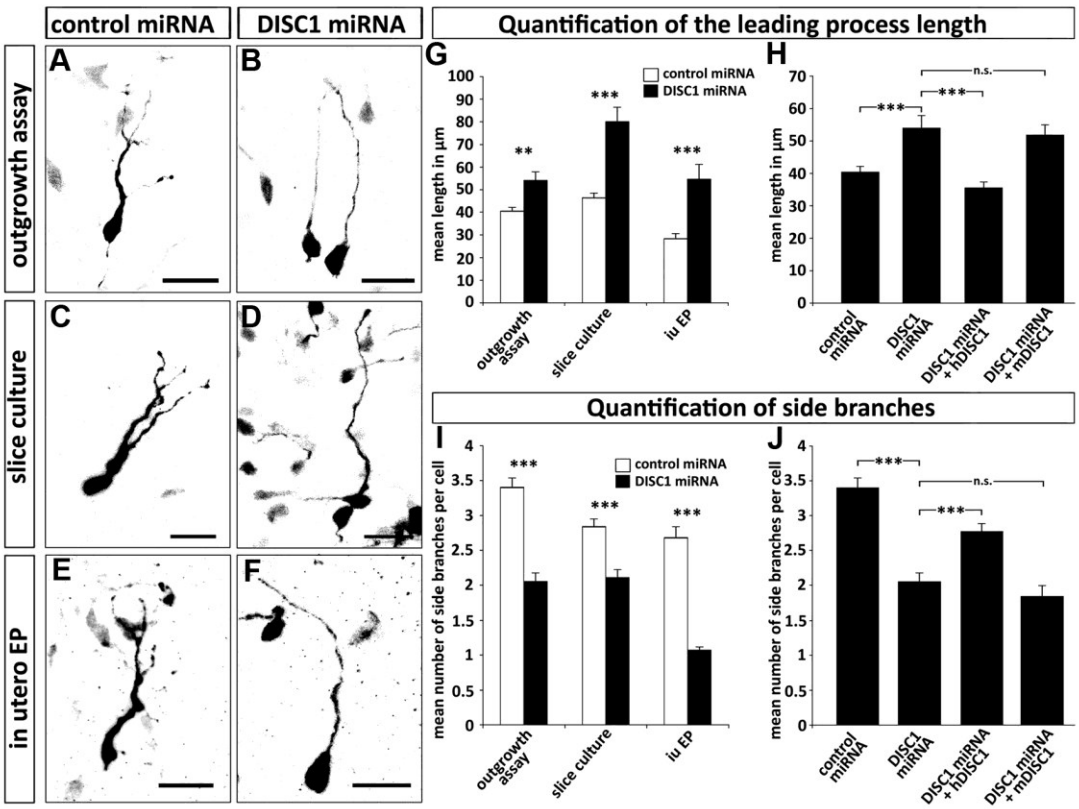

Figure 5. DISC1 knockdown leads to alterations in the morphology of MGE-derived interneurons. ***A–F***, Photomicrographs of cells transfected with control miRNA or DISC1 miRNA in outgrowth assays (***A***, ***B***), slice cultures (***C***, ***D***), and after *in utero* electroporation (iu EP) (***E***, ***F***). ***G–J***, Quantification of cell morphology. Under all experimental conditions, DISC1-deficient cells show longer, but less branched leading processes (***G***, ***I***). Cotransfection of miRNA with mDISC1 or hDISC1 in the outgrowth assay shows that the effect of DISC1 knockdown can be rescued by a miRNA insensitive DISC1 (***H***, ***J***). For statistical analysis, the means were compared with a Student's *t* test. Outgrowth assay: leading process length: control miRNA, 41 ± 2 μm; DISC1 miRNA, 54 ± 4 μm; DISC1 miRNA + mDISC1, 52 ± 3 μm; DISC1 miRNA + hDISC1, 36 ± 2 μm; number of side branches: control miRNA, 3.4 ± 0.1; DISC1 miRNA, 2.1 ± 0.1; DISC1 miRNA + mDISC1, 1.85 ± 0.15; DISC1 miRNA + hDISC1, 2.77 ± 0.11; control miRNA, $n = 127$ neurites from 30 cells; DISC1 miRNA, $n = 60$ neurites from 30 cells; DISC1 miRNA + mDISC1, $n = 33$ neurites from 21 cells; DISC1 miRNA + hDISC1, $n = 71$ neurites from 24 cells. Slice culture: leading process length: control miRNA, 46 ± 2 μm; DISC1 miRNA, 80 ± 6 μm; number of side branches: control miRNA, 2.8 ± 0.1; DISC1 miRNA, 2.1 ± 0.1; control miRNA, $n = 92$ neurites from 30 cells; DISC1 miRNA, $n = 66$ neurites from 30 cells. *In utero* electroporation: leading process length: control miRNA, 28 ± 2 μm; DISC1 miRNA, 55 ± 7 μm; number of side branches: control miRNA, 2.7 ± 0.2; DISC1 miRNA, 1.1 ± 0.1; control miRNA, $n = 53$ neurites from 18 cells; DISC1 miRNA, $n = 28$ neurites from 26 cells). ***$p < 0.001$; **$p < 0.01$; n.s. $p > 0.05$, *t* test. Scale bars, 20 μm. Error bars are SEM.

cortical interneurons, but rather directly interferes with the ability of these neurons to migrate properly.

The dynamic behavior of different classes of migrating neurons has been described in detail (for review, see Ayala et al., 2007). The molecular mechanisms underlying these distinct cellular events, however, are still under debate. Alterations in the cytoskeletal proteins tubulin and actin, motor proteins of the dynein and kinesin family, and numerous regulatory proteins have been implicated to control the migration of diverse populations of neurons.

The subcelluar distribution of DISC1 in MGE neurons and the morphological alterations after DISC1 knockdown in these cells provide some clues how DISC1 might contribute to the migration of cortical interneurons. In migrating MGE neurons, before the initiation of nucleokinesis, actin, and the nonmuscle myosin II accumulate at the rear of the cell, forming a cup-like structure (Bellion et al., 2005). Blocking myosin II with belbbistatin reduced nuclear movement, suggesting that contraction of actin-myosin at the rear pushes the nucleus forward and presumably breaks adhesion and the trailing process at the cell rear. Since DISC1 is often localized in a cup-like shape at the trailing edge of MGE neurons, DISC1 might interact with F-actin and/or myosin motors. A colocalization of DISC1 with actin filaments and actin binding proteins has been reported previously (Morris et al., 2003).

We found that DISC1 is consistently expressed in the tips of the leading processes of MGE-derived interneurons. It has been suggested that dynein anchored in the membrane of the leading process is pulling microtubules attached to the centrosome and leading process. Recent work with migrating pyramidal neurons has shown that microtubule plus-end binding protein adenomatous polyposis coli (APC) is required to anchor microtubules to the distal end of the leading process (Asada and Sanada, 2010). If the localization of APC to the distal tips of the leading process is prevented, radial migration is impaired and the neurons exhibited longer leading processes. For migrating interneurons, where DISC1 is localized in the tips of the leading process, DISC1 might be involved in the attachment of microtubules to the distal ends of these neurites. This could explain why MGE neurons after DISC1 knockdown, in addition to their migratory deficits, also exhibit prolonged leading processes.

Obviously, the precise mechanisms how DISC1 is involved in interneuron migration remain to be determined. However, our results indicate that DISC1 has an impact on the migratory behavior of interneurons during early development that might lead to deficits in the number or composition of GABAergic neurons

in the cortex. Dysfunctions of local GABAergic circuits have often been associated with the pathophysiology of schizophrenia (Benes and Berretta, 2001; Lewis et al., 2005). Thus, our findings support the notion that schizophrenia is a neurodevelopmental disease that may result at least in part from defects in neuronal integration (Lewis and Levitt, 2002).

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3.2 Steinecke A, Gampe C, Nitzsche F, Bolz J **DISC1 knockdown impairs the tangential migration of cortical interneurons by affecting the actin cytoskeleton**.

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DISC1 knockdown impairs the tangential migration of cortical interneurons by affecting the actin cytoskeleton

André Steinecke[1], Christin Gampe[1], Falk Nitzsche, and Jürgen Bolz*

Institut für Allgemeine Zoologie und Tierphysiologie, Universität Jena, 07743 Jena, Germany.

[1]These authors contribute equally to this work.

* Correspondence:

Dr. Jürgen Bolz
Universität Jena
Institut für Allgemeine Zoologie und Tierphysiologie
Erbertstrasse 1
07743 Jena, Germany
jurgen.bolz@uni-jena.de

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Abstract
Disrupted-in-Schizophrenia 1 (DISC1) is a risk gene for a spectrum of major mental disorders. It has been shown to regulate radial migration as well as dendritic arborization during neurodevelopment and corticogenesis. In a previous study we demonstrated through in vitro experiments that DISC1 also controls the tangential migration of cortical interneurons originating from the medial ganglionic eminence (MGE). Here we first show that DISC1 is necessary for the proper tangential migration of cortical interneurons in the intact brain. Expression of EGFP under the Lhx6 promotor allowed us to analyze exclusively interneurons transfected in the MGE after in utero electroporation. After 3 days in utero, DISC1 deficient interneurons displayed prolonged leading processes and, compared to control, fewer neurons reached the cortex. Time-lapse video microscopy of cortical feeder-layers revealed a decreased migration velocity due to a reduction of soma translocations. Immunostainings indicated that DISC1 is co-localized with F-actin in the growth cone-like structure of the leading process. DISC1 knockdown reduced F-actin levels whereas the overall actin level was not altered. Moreover, DISC1 knockdown also decreased levels of phosphorylated Girdin, which cross-links F-actin, as well as the Girdin-activator pAkt. In contrast, using time-lapse video microscopy of fluorescence-tagged tubulin and EB3 in fibroblasts, we found no effects on microtubule polymerization when DISC1 was reduced. However, DISC1 affected the acteylation of microtubules in the leading processes of MGE-derived cortical interneurons. Together, our results provide a mechanism how DISC1 might contribute to interneuron migration thereby explaining the reduced number of specific classes of cortical interneurons in some DISC1 mouse models.

key words: DISC1, Schizophrenia, interneuron, cortical interneuron migration, cortical development

Introduction

Disrupted-in-Schizophrenia 1 (DISC1) was originally discovered in a Scottish family in which a chromosomal translocation breaks this gene. More than 70% of those family members with DISC1 disruption were diagnosed as being schizophrenic, unipolar depressive or bipolar depressive (Millar et al., 2000). Many studies afterwards firmly established that DISC1 plays a major role in early brain development by regulating a number of essential neurodevelopmental events, including cell proliferation, neurite outgrowth, synapse formation and neuronal migration (for reviews see Ishizuka et al., 2006; Brandon et al., 2009; Soares et al., ; Narayan et al., 2013; Thomson et al., 2013). These pleiotropic actions of DISC1 are attributed to its many interaction partners, the DISC1 interactom (Camargo et al., 2007), making DISC1 a scaffold protein that impacts on many diverse brain functions.

We recently provided in vitro evidence that during embryonic development DISC1 is a necessary component for the correct tangential migration of cortical interneurons from the medial ganglionic eminence (MGE) to their target regions in the cortex (Steinecke et al., 2012). This is in accordance with previous studies which found a reduced number of parvalbumin-positive interneurons in a mouse line that expresses the truncated human DISC1, as in the Scottish pedigree. (Pletnikov et al., 2008; Ayhan et al., 2011). In the present study we first demonstrate the importance of DISC1 during tangential migration in the intact brain. For this we performed in utero electroporations and used constructs that either reduce endogenous DISC1 levels or that express a truncated murine form of DISC1 in combination with a vector that was only expressed in MGE-derived neurons. We then examined how DISC1 interferes with the cellular and the molecular machinery that drives glia-independent neuronal migration. We found that DISC1, by interacting with Girdin (girders of actin filaments) and Akt (also known as protein kinase B), regulates actin polymerization. In addition, DISC1 also influences the stability of microtubules. Thus both cytoskeletal elements that have been implicated in interneuron migration, actin filaments and micotubuli, are modified by DISC1.

Our results indicate that DISC1 has an impact on the migratory behaviour of interneurons during early development that might lead to changes in the number or composition of interneurons in the cortex. Together, this work is supporting the hypothesis that subtle perturbations in the developing brain may increase the risk for neuropsychiatric diseases later in life (Murray and Lewis, 1987; Weinberger, 1987; Cannon et al., 2002; Owen et al., 2011).

Material and Methods

Mice

Animals used were timed pregnant C57BL/6 mice. The day of insemination was considered as embryonic day (E) 1. Mice were killed using peritoneal injection of 10% chloral hydrate. All animal procedures were performed in agreement with the institutional regulations of the University of Jena.

Plasmids

Vectors expressing miRNA for DISC1 knockdown and control transfection were described previously (Steinecke et al., 2012). EmGFP has been removed for cotransfection. Additional vectors used: pLhx6-IRES-GFP (gift from Dr. Anderson), pCAX 1-597 (short DISC1, gift from Dr. Sawa), pEB3-GFP (gift from Dr. Galjart), pTubulin-GFP (coding sequence for α-tubulin cloned into pEGFP-C1 between XhoI and BamHI), pActin-RFP (coding sequence for ß-actin cloned into pmRFP-C1 between XhoI and BamHI).

In utero electroporation

Timed pregnant mice (E13.5) were treated with 4 mg/kg Carprofen for 20 min before deeply anesthetizing with a mixture of fentanyl (0.05 mg/kg), midazolam (5 mg/kg) and metedomidine (0.5 mg/kg). After the uterine horns were exposed various constructs together with pLhx6-IRES-GFP in a 4:1 ratio (2 µg/µl DNA at all) were injected into the lateral ventricles of the embryos and electroporation (5 pulses 40 V, 100 ms duration) was carried out with a forceps electrode connected to a BTX ECM 830 (Harvard Apparatus). After 3 days in utero embryonic brains were fixed in 4% paraformaldehyde and vibratome sections (150 µm) were immunostained against GFP (A6455, Invitrogen, 1:1000).

Ex utero electroporation

Brain hemispheres from E14.5 embryos were dissected in ice-cold sterile Krebs buffer [126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM MgCl, 2.1 mM $CaCl_2$, 25 mM $NaHCO_3$ and 11 mM glucose]. Ex utero electroporation was performed as described previously (Yozu et al., 2005). In brief, miRNA solution was pressure injected into the ventricular zone of the MGE, followed by electroporation of 2 pulses a 100 ms duration and 100 V using a BTX ECM 830 (Harvard Apparatus).

Primary cell culture and immunostaining

After ex utero electroporation MGE were dissected and collected in ice-cold Hank`s balanced salt solution (HBSS) supplemented with 0.65% glucose. After incubation with 0.025% trypsin in HBSS for 17 min at 37° C, tissue was dissociated into single cells by trituration and filtered through a nylon gauze to remove cell aggregates. Neurons were cultured in Dulbecco`s Modified Eagle Medium (DMEM) [supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin and 0.4 mM l-glutamine] at 37° C and 5% CO_2.
For immunocytochemistry cells were incubated on coated cover slips (19.5 µg/ml Laminin 5 µg/ml poly-l-lysin) and fixed after 2 div in 4% PFA for 30 min and washed in 0.2% TritonX-100 in PBS, blocked in 10% serum, 5% BSA and 0.2% TritonX-100 in PBS for 1 h followed by the incubation of the primary antibodies for 1 h or overnight. After washing cells were incubated for 1 h with the secondary antibody. After washing, nuclei were stained with 4′,6-Diamidin-2-phenylindol (DAPI). Following antibodies have been used: anti-DISC1 (SantaCruz, DISC-1(N-16): sc-47990, 1:50); anti-Actin (Hybridoma Bank, 1:50); anti-acTubulin (gift from Dr. Kessels, 1:400); anti-Akt/PKB[pS^{473}] (Invitrogen, 1:100); anti-Girdin (S1416 phos) (IBL, 1:50). For staining of F-actin cells were incubated in phalloidin (Biotium, 1:100).
For cells stained against F-actin and actin an Axiovert-S100, 40x objective NA: 0.45 Plan Neofluar in combination with a Spot camera was used. Cells stained against DISC1, pGirdin

and pAkt were scanned using a LSM510 (60x objektive NA: 1.4 Plan Apochromat; argon laser with 488 nm excitation, bandpass filter 500 – 550 nm emission; HeNe laser with 543 nm axcitation, longpass filter 560 nm). Cells stained against acTubulin were scanned using a LSM510 (10x objective NA:1.2 C-Apochromat; argon laser with 488 nm excitation, bandpass filter 500-550 nm; HeNe laser 543 nm excitation, longpass filter 560 nm).

Cortical feeder-layer
Feeder-layers were prepared as described previously (Bortone and Polleux, 2009). In brief, cortex cells of E14.5 embryos were cultured on coated cover slips and ex utero electroporated cells of the MGE were placed on top. After incubation at 37° C and 5% CO_2 for 36 h time lapse analysis was started using a ZEISS LSM510 (20x objective NA: 0.75 Zeiss Plan Apochromat; argon laser with 488 nm excitation, bandpass filter 500 – 550 nm emission) in combination with an incubation chamber (37° C and 5% CO_2).

Outgrowth assay
For interneuron migration in a 3D substrate of plasma MGE of E14.5 embryos were dissected in MZ medium [0.4 g methyl cellulose, 5% FBS, 1% penicillin/streptomycin, 1% L-glutamine, 0.1% glucose] and cut in 200x200 µm pieces using a tissue shopper. The MGE explants were precultured at 37° C and 5% CO2 for 1 h, embedded in chicken plasma which was cross-linked with thrombine and cultured in MZ medium for 2 div before fixation with 4% paraformaldehyde. For staining of F-actin cells were incubated in phalloidin (Biotium, 1:100). For immunostaining following antibodies have been used: anti-DISC1 (SantaCruz, 1:50); anti-Tubulin (Hybridoma Bank, 1:200).

Fibroblast cell culture
NIH3T3 fibroblasts were grown in Dulbecco`s Modified Eagle Medium (DMEM)-F12 with 10% fetal bovine serum (FBS), 5% Penicillin/Streptomycin (P/S) under standard cell culture conditions, transfected with various constructs using Lipofectamine2000 (Invitrogen) according to the manufacturer`s protocol and incubated for 48 h. For time lapse analysis cells were scanned using a ZEISS LSM510 (Tubulin-GFP: 40x objective NA: 1.3 Plan Neofluar, EB3-GFP: 63x objective NA: 1.4 Plan Apochromat; argon laser with 488 nm excitation, longpass filter 505 nm emission) in combination with an incubation chamber (37° C and 5% CO_2).

Cell analysis and statistics
Transfected cells were scanned using a Zeiss LSM510. Anaylsis of feeder-layer experiments was performed using the ZEN2011-LE software (ZEISS). Analysis of interneuron morphology as well as EB3 in fibroblasts was performed using ZEN2009 software (ZEISS). Fluorescence intensities were analysed using ImageJ. At least 3 independent experiments were performed and analysed. Significance was calculated using Student's t test (Excel). For analysis of migration defects after in utero electroporation one way ANOVA ($F_{2.99}$=33.826, $p<0.001$) and post-hock test (p-value indicated in the text) were performed using SPSS.

Results

Decreased tangential migration of DISC1 deficient cortical interneurons in vivo

Our initial hypothesis of compromised interneuron migration after DISC1 knockdown was based on functional in vitro assays (Steinecke et al., 2012). Therefore, in the present study, our first goal was to examine the migration pattern of DISC1 deficient interneurons in the intact brain. For this, one experimental strategy would be to electroporate different constructs that suppress DISC1 specifically in the MGE of embryonic brains in utero. It has been reported that the ganglionic eminences can be selectively electroporated with ventrolateral placement of the positive electrode (Borrell et al., 2005). However, we and others could not exclusively target the MGE with this technique, instead transfected cells were found scattered or in clusters throughout the basal telencephalon and at the corticostriatal junction (Bai et al., 2008; Steinecke et al., 2012). To overcome this problem, Brown et al. (2011) used transgenic mice that express the TVA receptor selectively in the VZ of the MGE and POA and then performed in utero intraventricular injections of RCAS retroviruses to target MGE and POA neurons.

We developed a different technique to target cortical interneurons in the embryonic brain. For this we used the vector pLhx6-EGFP as reporter construct which contains the lim-homeobox gene 6 (Lhx6) promoter. With this vector, EGFP labeling should occur only in those transfected cells in which Lhx6 expression is stimulated by the transcription factor Nkx2.1 (Fig. 1A). To test this we performed ex utero electroporation on brain hemispheres of E14.5 mouse embryos using a mixture of pLhx6-EGFP and alexa555-conjugated control siRNA that was injected into the MGE as well as the cortex before electroporation. Alexa555 of the siRNA was used to mark the tissue where plasmid solution was injected. After preparation of slices and culturing for 1 day in vitro EGFP was only expressed in cells of the MGE, but not in the cortex (Fig. 1B). Thus co-transfection of pLhx6-EGFP with control or DISC1 miRNA encoding constructs (Steinecke et al., 2012) in a 1:4 ratio was performed at E13.5 to study exclusively the migration of cells transfected within the MGE. The overrun of miRNA constructs prevented false-positive cells which were only transfected with the marker construct. After 3 days in utero the brains were fixed and a dense focus of EGFP+ cells was visible in the MGE, making it difficult to determine the precise number of these neurons. We therefore counted the neurons that have migrated from the MGE into the LGE and compared this with the number of neurons that have migrated further and reached the cortex. Under control conditions, the number of cells in the LGE and in the cortex was roughly the same (cortex: 54.46 ± 2.10%, LGE: 45.54 ± 2.10%, 60 slices from 12 brains; Fig. 1C, C', D). In contrast, expression of DISC1 miRNA caused a significant reduction of labeled cells that arrived in the cortex. Merely 24% of the cells reached the cortex at this time point, most of the cells were stuck in the LGE (cortex: 23.99 ± 3.44%, LGE: 76.01 ± 3.44%, 17 slices from 5 brains, $p<0.001$; Fig. 1C, C', D).

The truncated form of DISC1 that was found in a Scottish pedigree has been described as dominant negative (Kamiya et al., 2005). Transfecting MGE cells with expression vectors for shortened murine DISC1 led to a significant decrease in the number of cells in the cortex after 3 days in utero (cortex: 34.55 ± 2.58%, LGE: 65.45 ± 2.58%, 25 slices from 7 brains, $p<0.001$ compared to control; Fig. 1D). However, with 35% of cells reaching the cortex, truncated DISC1 caused a weaker migration defect compared to the DISC1 knockdown by miRNA.

As our previous results showed alterations in the cell morphology after DISC1 miRNA expression (Steinecke et al., 2012), we analyzed the processes of transfected cells that had left the MGE and reached the LGE after 3 days in vivo. In accordance with this earlier study, cells that expressed DISC1 miRNA or a short form of DISC1 exhibited prolonged leading process-

es compared to control transfected cells (control: 38 ± 4 µm, 27 cells; DISC1 miRNA: 51 ± 5 µm, 24 cells, $p<0.05$; short DISC1: 59 ± 5 µm, 27 cells, $p<0.01$; Fig. 2A, 2B).

Thus using a reporter construct for Lhx6 positive cells we could demonstrate that DISC1 is an essential component for the correct migration of cortical interneurons in the intact brain. Knockdown of DISC1 as well as the expression of a dominant negative form led to a delayed migration after 3 days in vivo. Considering that for technical reasons we could only analyze cells that were able to leave the MGE after transfection, the migration abnormalities caused by DISC1 deficiency might be even more pronounced than described here.

Dynamics of soma translocations after DISC1 knockdown in cortical interneurons
To understand the underlying cellular mechanism of how DISC1 regulates interneuron migration we went back from the in vivo approach to in vitro systems. Therefor we injected plasmid solution into the MGE of preparated hemisphäres and electroporated them ex utero. Afterwards single cells were generated and cultured on cortical feeder-layers. Using time-lapse video microscopy to visualize the complex migration behavior of interneurons it was possible to analyze the dynamic of individual migrating cells on a two-dimensional substrate. Fig. 3A depicts a typical cell transfected with control miRNA. As illustrated in Fig. 3B, two soma translocations occurred within the 40 min recording period. In addition, a highly dynamic growth cone-like structure is visible at the leading process tip exhibiting bifurcations (arrow) and swellings (arrow head). In contrast, the DISC1 miRNA transfected cell in Fig. 3A' exhibited a thin, prolonged and less branched leading process and the growth cone-like structure was greatly reduced compared to control cells. Although swellings (arrow heads) appeared several times, a soma translocation did not occur. A graphic representation of the trajectories of these two cells is illustrated in Fig. 3B. Whereas the control transfected cell exhibits the typical saltatory pattern of interneuron migration, with leading process extension followed by soma translocation and retraction of the trailing process, there was almost no translocation of the DISC1 deficient interneuron, while the leading process was constantly moving. Consistently, in contrast to the control, no trailing process was identifiable in these cells (Fig. 3A').

Analyzing all cells monitored within 40 min we found a significant reduction of soma translocations after DISC1 knockdown. About half of the control cells performed at least one soma translocation within the 40 min recording period (30 out of 65), whereas only 14 out of 69 DISC1 deficient neurons exhibited a soma translocation. The mean number of soma translocations was 0.6 ± 0.1 after transfection with control miRNA and 0.2 ± 0.1 after transfection with DISC1 miRNA ($p<0.001$; Fig. 3C). In accordance with this observation, the mean velocity of DISC1 deficient interneurons (19 ± 1 µm/h) was significantly lower than for control neurons (33 ± 2 µm/h; $p<0.001$, Fig. 3D). However, when translocations occurred in DISC1 deficient interneurons, this arose at the same speed as in control cells (control miRNA: 30 ± 2 µm/h , 30 cells; DISC1 miRNA: 32 ± 2 µm/h , 14 cells; $p>0.05$, n.s.; Fig. 3E).

In conclusion, after DISC1 knockdown MGE-derived interneurons display less soma translocations on a cortical feeder-layer than control cells. The velocity of soma translocation is not altered in DISC1 deficient interneurons, suggesting that their migration defects are caused by alterations of the intracellular coordination between several components of the migration machinery.

Dynamics of the actin cytoskeleton after DISC1 knockdown in cortical interneurons
Previous studies provided evidence that the actin cytoskeleton is essential for glia-independent migration of neurons, exerting pushing and/or pulling forces (He et al., 2010; Martini and Valdeolmillos, 2010). Therefore we examined the distribution of actin and DISC1

at the subcellular level in cortical interneurons. For this phalloidin staining of interneurons migrating in a 3D substrate of plasma was used to label filamentous (F)-actin in combination with DISC1 immunostainings using an antibody raised against a peptide mapping near the N-terminus of the DISC1 protein. Confirming our previous results (Steinecke et al., 2012), both DISC1 and F-actin were located behind the nucleus and in the tips of the leading processes (Fig. 4A). Within the growth cone-like structure, DISC1 is in front of F-actin in the filiopodia, as illustrated at high magnification in Fig. 4B.

To monitor actin dynamics in migrating interneurons we performed time-lapse video microscopy of actin-RFP expressing MGE cells on a cortical feeder layer. In this experiment, the fluorescence RFP signal reports both globular (G-) actin as well as F-actin. However, counterstaining of transfected cells with phalloidin indicated that highly condensed RFP indicates predominantly F-actin (Lee et al., 2013). Our time-lapse movies reveal that during migration on cortical feeder-layers dynamic condensations of actin filaments occurred within the cell soma as well as in the leading process tip of interneurons. F-actin signals appeared in front of the nucleus and moved sidewise to the back during soma translocation (Fig. 4C and supplementary movie). Within the highly dynamic growth cone-like structure accumulation of actin-RFP occurred frequently.

The growth cone-like structure of interneurons is more elaborated than in radial migrating cortical projection neurons (Rakic, 1971; Bellion et al., 2005). It has been demonstrated that severing the tip of the leading process or restricting its dynamics stopped soma translocation (He et al., 2010). To study if DISC1 knockdown induced alterations of the actin cytoskeleton we analyzed the growth cone-like structure of fixed control miRNA and DISC1 miRNA expressing interneurons and compared their relative fluorescence intensities. In DISC1 deficient cells phalloidin staining was considerably less intense than in control miRNA transfected cells (control miRNA/untransfected: 1.21 ± 0.07, 78 cells; DISC1 miRNA/untransfected: 0.81 ± 0.04, 73 cells; $p<0.001$; Fig. 5A, 5E) indicating a decrease of F-actin in the leading process tips. To examine whether this reduction of filaments resulted from a decline in protein levels we performed immunocytochemistry using a pan antibody, binding both to G- and F-actin. There was no difference in the fluorescence intensities between cells expressing control or DISC1 miRNA (control miRNA/untransfected: 1.03 ± 0.05, 43 cells; DISC1 miRNA/untransfected: 1.05 ± 0.04, 57 cells; n.s. $p>0.05$, n.s.; Fig. 5B, 5E).

Previous studies indicated that Girdin (girders of actin filaments) is one of the many interaction partners of DISC1, which cross-links actin filaments (Enomoto et al., 2006; Enomoto et al., 2009). Moreover, it has also been demonstrated that Girdin is a regulator of neuroblast chain migration in the rostral migratory stream of the postnatal brain and the number of cortical interneurons is significantly decreased in Girdin$^{-/-}$ mice compared with wild-type animals (Wang et al., 2011). We therefore analyzed the amount of activated, phosphorylated Girdin (pGirdin) in leading process tips of cortical interneurons. After DISC1 knockdown the pGirdin signal was reduced by 30% compared to control levels (control miRNA/untransfected: 1.32 ± 0.97, 44 cells; DISC1 miRNA/untransfected: 0.91 ± 0.70, 42 cells; * $p<0.05$; Fig. 4C, 4E).

Although DISC1 was discovered as a binding partner of Girdin, so far there is no evidence that DISC1 directly activates Girdin. However, one known activator of Girdin is Akt, also known as protein kinase B (PKB), a serine/threonine kinase. Activated Akt (pAkt) phosphorylates serine at position 1416 in Girdin, and activated pGirdin accumulates at the leading edge of migrating cells (Enomoto et al., 2005; Enomoto et al., 2009). Therefore we examined the effect of DISC1 knockdown on pAkt levels in the growth cone-like structure of migrating

cortical interneurons and found a significant reduction compared to control cells (control miRNA/untransfected: 1.19 ± 0.08, 56 cells; DISC1 miRNA/untransfected: 0.97 ± 0.07, 56 cells; $p<0.05$; Fig. 4D, 4E).

Taken together, these results indicate that leading process tips of DISC1 deficient interneurons display lower F-actin levels compared to control. The decrease of activated forms of Girdin and Akt, which are responsible for the cross-linking of actin filaments, supports the idea that DISC1 knockdown influences the actin cytoskeleton in the growth cone-like structures of migrating interneurons and thereby interferes with their migration.

Alterations of microtubules of DISC1 deficient cortical interneurons

So far we analyzed the effects of a DISC1 knockdown on the actin cytoskeleton in the leading process tip. But the growth cone-like structure is unlikely characterized by actin only. In axonal growth cones different forms of actin are functionally linked to microtubules which also play an essential role during growth cone dynamics. Immunostaining of MGE cells migrating in a 3D substrate revealed that the leading process shaft is completely filled with microtubules which partially enter the growth cone-like structure where DISC1 is expressed (Fig. 6, Steinecke et al., 2012). This as well as the oberserved alterations of the leading process morphology (Fig. 2, Steinecke et al., 2012) imply effects on the microtubule cytoskeleton after DISC1 knockdown. We therefore analyzed the microtubule cytoskeleton in DISC1 deficient cells.

First, we examined the overall tubulin level of NIH3T3 that were transfected with control or DISC1 miRNA encoding vectors using western blot analysis. Reduction of DISC1 led neither to an increase nor a decrease of tubulin on the protein level in those cells (data not shown).

As the tubulin level seemed not to be changed, we investigated the effect of the DISC1 knockdown on microtubule polymerization and network integrity. For this we visualized the microtubule cytoskeleton in NIH3T3 fibroblasts and performed time lapse video microscopy. Using fluorescence-tagged tubulin we were able to monitor the whole microtubule cytoskeleton of living miRNA expressing fibroblasts. Like under control conditions DISC1 deficient cells displayed a compact and shape filling tubulin network involving elongated and curled regions with stable as well as dynamic microtubules. Conspicuities like microtubules that did not reach the cell cortex or instable microtubules that were consistently retracted could not be observed. (Fig. 7A)

Next we analyzed the velocity of tubulin polymerization using GFP-tagged EB3 (end-binding protein 3). This microtubule binding protein is specifically associated with the ends of growing microtubules and therefor enables the visualization of microtubule growth in living cells (Stepanova et al., 2003). Analyzing these EB3-GFP signals that appear over time (“shooting stars”) we found no difference between control and DISC1 miRNA expressing NIH3T3 fibroblasts which means neither an increased nor a reduced velocity of microtubule polymerization after DISC1 knockdown (Fig. 7B, 7C; control miRNA: 62.5 ± 2.8 nm/s, 100 shooting stars, 14 cells; DISC1 miRNA: 56.8 ± 4.1 nm/s, 90 shooting stars, 15 cells; n.s. $p>0.05$).

In growing axons the stabilization of microtubules plays an essential role in the correct movement of the growth cone. Stable bundles of microtubules fill the axon shaft and extend into the growth cone. There they are belt by actin arcs and stabilized by microtubule-associated proteins. Single dynamic filaments reach out of the stable bundles and explore the periphery. Since dynamic microtubules act as guidance sensors, stable bundles are necessary for axonal forward movement. Locally induced stabilization and dynamic are required for growth cone turning and branching. (reviewed in Lowery and Van Vactor, 2009; Kalil and

Dent, 2014). To examine whether a DISC1 knockdown effects the selective stabilization of microtubules in the growth cone-like structure of migrating interneurons, we analyzed the acetylation of microtubules in fixed MGE cells. Although this post-translational modification seems not the have an influence on polymerisation or depolymerisation of microtubules by itself, it is presumed to be a marker for stabilization (Schulze et al., 1987; Westermann and Weber, 2003). We performed immunocytochemistry on MGE cells and found a significant decrease of acetylation at the distal end of the leading process after DISC1 knockdown. In control cells from the cell body into the growth cone-like structure. In DISC1 deficient cells only 87% of the leading process length from the cell body towards the leading process tip was acetylated (Fig. 8A, 8B; control miRNA: length of acetylated tubulin signal relative to the leading process length: 0.96 ± 0.01, 48 cells; DISC1 miRNA: length of acetylated tubulin signal relative to the leading process length: 0.87 ± 0.02, 52 cells; *** $p<0.001$). Thus stable microtubule bundles are no longer reaching into the growth cone-like structure in DISC1 deficient cortical interneurons.

Taken together, in interneurons with decreased DISC1 levels microtubule stabilization is reduced at the leading process tip where stable microtubule bundles reach into the growth cone-like structure. This effect seems not to be mediated by altered polymerization and dynamic of microtubules.

Discussion

More than a decade after the first description of DISC1 as a susceptibility gene for major psychiatric disorders, multiple studies attempted to trace its potential functions during brain development. It has become clear that DISC1 is crucial for the correct development of several telencephalic structures by regulation e.g. cell division, synapse formation or neuronal migration (reviewed in Brandon et al., 2009; Soares et al., ; Narayan et al., 2013). Using a variety of in vitro assays, in our previous study we found evidence that DISC1 knockdown by RNA intereference results in a migration defect of MGE-derived cortical interneurons (Steinecke et al., 2012). Here we first demonstrate that DISC1 is essential for the proper tangential migration of cortical interneurons in vivo. We then examined that underlying cellular and molecular mechanisms that lead to the migration defects of DISC1 deficent interneurons. Our results indicate that DISC1, via interactions with Akt and Girdin, influences network structure and stabilization of actin filaments. In addition, DISC1 also influences the stability of microtubles but had no effect microtubule polymerization.

DISC1 plays a critical role in tangential migration of cortical interneurons

Although some DISC1 models have been generated (Cash-Padgett and Jaaro-Peled, 2013), up to now there is no DISC1 knockout mouse available to study directly the potential of DISC1 during brain development. Therefore we used RNA interference techniques to study the impact of DISC1 on neuronal migration. By performing in utero electroporation we were able to reduce the level of DISC1 in cells of the ganglionic eminences at a defined time point during development. It has been claimed that it is possible to aim exclusively the MGE with this technique (Borrell et al., 2005). However, we and others were not able to restrict the transfection region to this part of the eminences, but rather almost always got many labeled cells in the basal as well as in the dorsal telencephalon after electroporation (Bai et al., 2008; Steinecke et al., 2012). To overcome this problem and to analyze only MGE-derived cells we used a marker that is expressed exclusively in $Lhx6^{+}$ cells, a transcription factor specific for postmitotic cortical interneurons generated in the MGE. This was confirmed by injecting plasmids in the MGE and cortex in brain slices. After electroporation, labeled neurons were only found in the MGE and not in the dorsal telencephalon. Using this approach, we found that after DISC1 knockdown fewer interneurons reached the cortex compared to control transfected cells. Similar experiments with a dominant-negative form of DISC1 also lead to migration defects, although they have been less pronounced compared to DISC1 knockdown.

Previous studies found abnormal migration after DISC1 knockdown in cortical projection neurons which migrate radially from the ventricular zone into the developing cortical plate (Kamiya et al., 2005; Kubo et al., 2010; Young-Pearse et al., 2010). Delayed migration has also been described for DISC1 deficient neurons in the developing hippocampus (Meyer and Morris, 2009; Tomita et al., 2011). Surprisingly, in the adult hippocampus, newly generated neurons over-migrated their target after DISC1 knockdown (Duan et al., 2007; Enomoto et al., 2009; Kim et al., 2009). Thus DISC1 seems to have opposite effects on neuronal migration of developing and adult hippocampal neurons. Finally, DISC1 seems to have no impact on postnatal neuroblast migration within the rostral migratory stream (Wang et al., 2011). Thus the influence of DISC1 on neuronal migration appears to be cell type specific and can also depend on the developmental stage of the neurons.

The loss of DISC1 binding partners can also lead to defects in neuronal migration. A prominent example is LIS1 that interacts with DISC1 at the centrosome (Kamiya et al., 2005). LIS1 deficient cortical projection neurons as well as cortical interneurons exhibit a delayed migration. This is caused by the reduction of the rate of nuclear movement, whereas process extension remains unaffected, resulting in prolonged and less branched leading processes

(McManus et al., 2004; Nasrallah et al., 2006; Gopal et al., 2010). These results are reminescent to effects observed after DISC1 knockdown (Steinecke et al., 2012, present results). The fact that impairment of LIS1 function results in migration defects and causes lissencephaly (Kato and Dobyns, 2003) underlines the relevance of DISC1 and its interacting partners during brain development.

DISC1 interferes with the actin cytoskeleton in migrating interneurons

Tangential migration of cortical interneurons differs from radial migration in several respects (reviewed in Nadarajah and Parnavelas, 2002; Marin and Rubenstein, 2003). Unlike cortical projection neurons, cortical interneurons originate in the ventral telencephalon and therefore have to migrate over much longer distances to reach their final destination in the developing cortex. As these cells have to find their way without guidance of glial fibers they exhibit a distinctive growth cone-like structure at the leading process tip which is thought to "explore" the environment and make steering decisions. (reviewed in Marin and Rubenstein, 2001). Its branched and highly dynamic appearance is crucial for interneuron migration (Bellion et al., 2005; Metin et al., 2006; Valiente and Martini, 2009; He et al., 2010). During tangential migration cortical interneurons consistently repeat two steps: (I) elongation of the leading process and (II) soma translocation combined with elongation stop and branching of the growth cone-like structure (Bellion et al., 2005). Accordingly, suppression of soma translocation by blocking the actin-associated motor protein nonmuscle myosin II or inhibition of ROCK, a Rho effector that regulates myosin II activity results in prolonged and very thin processes (Bellion et al., 2005; Shinohara et al., 2012). Likewise, in this study knockdown of DISC1 decreased the number of soma translocations on cortical feeder-layers and caused the elongation of leading processes.

Several previous studies indicate a crucial role of actin dynamics during soma translocation. It has been shown that nucleokinesis is associated with a precise pattern of actin condensations characterized by the initial formation of a cup-like structure at the rear nuclear pole. This is followed by a progressive actomyosin contraction that drives the nucleus forward and concludes with an actin spot at the base of the retracting trailing process (Bellion et al., 2005; Martini and Valdeolmillos, 2010; Shinohara et al., 2012). Furthermore, enriched F-actin signals have been observed in the leading process tip and also in front of the nucleus where they appear prior to translocation events and move to the direction of the leading process (Shinohara et al., 2012). It has been proposed that the soma is actively pulled forward by a F-actin flow along the leading process. This is based on results from migrating cerebellar granule cells where somal translocation is suppressed by severing the leading process tip and / or local disruption of F-actin along the leading process. Experiments using localized application of F-actin stabilizing and destabilizing agents revealed the necessity of actin dynamics especially within the growth cone-like structure at the leading process tip. In contrast, local perfusion of actin modulating agents at the cell soma has no effect on nucleokinesis (He et al., 2010). Using time-lapse video microscopy of actin-RFP expressing MGE cells we found evidence for both models of pushing and pulling the nucleus forward (Fig. 4C).

Given that actin remodeling is necessary for the correct interneuron migration we investigated the role of DISC1 on the actin cytoskeleton. For this we analyzed the leading process tips after DISC1 knockdown and found less F-actin in the growth cone-like structure compared to control cells. This result is comparable to the effect of a LIS1 knockdown in radially migrating neurons, which also display a migration defect as described above (Kholmanskikh et al., 2003). Thus DISC1 seems to have an impact on F-actin remodeling at the leading edge, which is essential for neuronal migration.

A binding partner of DISC1 involved in actin remodeling is Girdin (Camargo et al., 2007). After phosphorylation by Akt it detaches from the cell membrane and cross-links newly built actin filaments (Enomoto et al., 2006). Currently, there are two different hypothesis about the role of DISC1 and Girdin in newborn granule cells of the adult hippocampus. According to (Kim et al., 2009), DISC1 prevents the interaction of Girdin and Akt and therefor decreases the activation of Girdin by Akt. In contrast, another study points out the function of DISC1 to localize Girdin in growth cones thereby promoting Girdin activation at the axonal tip (Enomoto et al., 2009). Here we show that knockdown of DISC1 caused a reduction of F-actin, activated Girdin as well as activated Akt in the growth cone-like structure of cortical interneurons. Although it is not clear how DISC1 and Girdin interact, these results indicate a role of DISC1 in actin remodeling during tangential migration.

In summary, DISC1 is expressed in the leading process tip as well as in the cell soma mainly behind the nucleus of migrating MGE cells (Fig. 9, Steinecke et al., 2012) overlapping with phalloidin stained F-actin. Considering the growth cone-like structure at the end of the leading process reduced expression levels of DISC1 lead to less F-actin since the over all protein level of actin is not decreased. Additionally, reduction of active forms of the actin cross-linking protein Girdin and its activator Akt further confirm the disruption of actin remodeling caused by DISC1 deficiency. This points to the hypothesis that DISC1 knockdown impairs the actin cytoskeleton in tangentially migrating cortical interneurons, finally resulting in a restricted ability to perform soma translocations, as depicted in Fig. 9.

Knockdown of DISC1 interferes with microtubule stabilization in leading process tips of migrating interneurons

In addition to actin, the microtubule cytoskeleton plays an essential role during brain development. For glia-guided migrating cells it has been shown that microtubule organization is not solely responsible for the polarity, but also participates in migration. The centrosome as the main microtubule organization center is located in front of the nucleus. The correct localization of centrosome-associated proteins ensures its functionality and is necessary for the accurate radial migration of cortical neurons in the neocortex (Higginbotham and Gleeson, 2007). Microtubules spreading out of the centrosome built a cup-like structure surrounding the nucleus (Rivas and Hatten, 1995; Xie et al., 2003; Tsai et al., 2007) and reach into the leading process where they are linked to the cellular cortex (Asada and Sanada, 2010). It has been suggested that the DISC1 binding partner LIS1 activates the dynein motor complex, which is necessary for the coupling of the centrosome and the nucleus. During somal translocation the motor complex moves along microtubule fibers and pulls the nucleus forward. Loss of function of dynein and other components of the motor complex like LIS1 causes uncoupling of the centrosome from the nucleus and impairs neuronal migration and positioning (Shu et al., 2004).

In contrast, several studies found no impact of microtubule dynamics on nucleokinesis during tangential migration of cortical interneurons (He et al., 2010; Martini and Valdeolmillos, 2010). Thus the microtubule network is necessary basically to build the leading process and to act as a guide for the nucleus. However, also in tangentially migrating interneurons microtubule-dependent pulling forces in addition to actomyosin-dependent pushing forces have also been considered (Bellion et al., 2005). Thus, pulling as well as pushing mechanisms are possible in all migrating neurons whereupon the substrate that has a considerable impact on adhesion, is also crucial for the locomotion of cells (Bellion et al., 2005; He et al., 2010; Martini and Valdeolmillos, 2010; Luccardini et al., 2013). However, a recent study indicates an important role of microtubule dynamics in migrating MGE-derived cells. Prior nucleokinesis the centrosome moves forward along extracentrosomal microtubules into the leading process

where it docks to the cellular membrane and exposes a primary cilium at the cell surface. Nuclear translocation occurs along microtubule bundles comprising extracentrosomal microtubules followed by detaching of the centrosome from the cellular membrane suggesting involvement of the microtubule cytoskeleton in nucleokinesis too (Baudoin et al., 2012).

DISC1 has been described previously to associate with microtubular organization and dynamics. Like LIS1 it is a part of the dynein motor complex and accumulates as well as stabilizes other centrosomal proteins at the centrosome and therefor up-regulates its function. Accordingly, overexpression of LIS1 as well as DISC1 leads to microtubule accumulation in the cell periphery and knockdown to a disorganized network in COS-7 cells (Smith et al., 2000; Kamiya et al., 2005). Additionally to its role in dynein-based microtubule-associated transport, DISC1 modulates the transport of organelles and components of the cytoskeleton through its direct interaction with kinesin-1 (Taya et al., 2007; Wang and Brandon, 2011). However, analyzing assembly and integrity of the microtubule cytoskeleton in DISC1 deficient NIH3T3 fibroblasts we could not observe any impact of DISC1 on microtubule organization. Cells with reduced expression levels of DISC1 displayed the same microtubule behaviour and EB3 dynamics as control cells indicating no effect on polymerization and depolymerization of microtubule fibers.

In axonal growth cones, branch formation is initiated by actin and then followed by microtubule invasion. *S*tabilized microtubule bundles extent into the growth cone where they interact with actin arcs and actin filaments. Explorative dynamic microtubule fibers reach into the periphery and respond to guidance cues from the environment. Since complete inhibition of microtubule dynamics prevents turning of the growth cone, localized stabilization induces turning (reviewed in Lowery and Van Vactor, 2009; Kalil and Dent, 2014). To identify possible effects of a DISC1 knockdown on microtubule-based functions of the growth cone-like structure we analyzed microtubule stabilization in leading processes of MGE cells. Reduced stabilization at the distal ends of the processes indicates an effect on the functionality of the steering apparatus as stabilized microtubule bundles do not reach into the growth cone-like structure. This was also observed in LIS1 deficient interneurons, further confirming the close relationship between these two proteins (Gopal et al., 2010). How this effect on the microtubule cytoskeleton is mediated and whether it also has an impact on the integration of guidance cues has to be examined in future studies.

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Figure captions

Fig. 1: DISC1 is necessary for the correct tangential migration of interneurons in vivo

(A), Schematic of the electroporation strategy. The reporter construct is controlled by the Lhx6 promotor and labels MGE-derived interneurons. The control and DISC1 miRNA constructs were co-electroporated with the reporter vector in a 4:1 ratio.

(B), Photomicrographs of slice cultures that were ex utero electroporated with the Lhx6 reporter vector that was co-injected with Alexa555 labeled control siRNA in the cortex as well as the MGE. The reporter is only activated in the MGE, but not the cortex. Scale bar: 200 µm.

(C), Inverted fluorescent micrographs of brain slices after in utero electroporation with control or DISC1 miRNA constructs as well as the Lhx6 reporter vector. Scale bars: 500 µm.

(C') Higher magnified parts of cortex and LGE demonstrate a different cell distribution after expression of control or DISC1 miRNA.

(D) Quantification of cells that migrated to the LGE and the cortex resulted in a decreased number of interneurons that reached the cortex in brains expressing DISC1 miRNA or short DISC1 compared to control transfected brains. Student`s t test: n.s. $p \geq 0.05$, *** $p < 0.001$. Error bars: SEM. "n" reflects the number of slices and "N" the number of brains.

Fig. 2: Knockdown of DISC1 leads to prolonged leading processes in vivo

(A) Photomicrographs of cells after in utero electroporation show alterations in process morpholgy.

(B) Quantification of the neurite length revealed prolonged leading processes after expression of DISC1 miRNA and short DISC1. Student`s t test: * $p < 0.05$, ** $p < 0.005$. Error bars: SEM. "n" reflects number of cells.

Fig. 3: DISC1 deficient cells perform less somal translocations in vitro

(A, A`) Time line of interneurons expressing control miRNA (A) or DISC1 miRNA (A') on a cortical feeder-layer. Arrow heads indicate swellings, arrows indicate newly built bifurcations. Time intervall 10.5 min. Overlays of the time points 0 min (green) and 40 min (red) demonstrate the movement of the cells. Scale bars: 20 µm.

(B) Graphic representation of the movement of cells mapped under (A) and (A`). Jumps in the graph indicate somal translocations. In contrast to the control, the DISC1 deficient cells performed no somal translocations within 40 min.

(C) Analysis of somal translocations per cell in 40 min resulted in a decreased number of translocation in DISC1 deficient cells compared to control cells.

(D) Anaylsis of the migration speed in 40 min shows a reduction after DISC1 miRNA expression.

(E) Analysis of soma translocation velocity revealed no difference.

Error bars: SEM. Student's t test: n.s. $p \geq 0.05$, *** $p < 0.001$, "n" reflects number of cells.

Fig. 4: DISC1 co-localizes with F-actin in migrating interneurons

(A) Fluorescent micrographs of interneurons stained with phalloidin and α-DISC1 antibodies. Arrows indicate an expression of DISC1 or F-actin behind the nucleus, arrowheads within the leading process tip. Scale bar: 10 µm.

(B) High magnification of a growth cone-like structure reveals the co-localization of DISC1 and F-actin. Scale bar: 5 µm.

(C) Time line of an actin-RFP expressing interneuron migrating on a cortical feeder-layer. Actin-RFP shows a consistent condensation in the leading process tip (arrowheads) and a dynamic condensation in the soma (arrows). During soma translocation actin-RFP spots move from the initial segment of the leading process to the cell rear. Scale bar: 10 µm.

Fig. 5: Knockdown of DISC1 affects the actin cytoskeleton in migrating interneurons
(A-D) Fluorescence micrographs of DISC1 miRNA expressing (green) and untransfected cells stained with phalloidin (A), α-actin (B), α-pGirdin (C), and α-pAkt (D) antibodies. Frames display the tips of the leading processes. TL: transmitted light. Scale bars: 10 µm.
(E) Quantification of the fluorescence intensities of control miRNA as well as DISC1 miRNA expressing cells. The ratio of the mean grey value (relative fluorescence intensity) of transfected and non-transfected cells was calculated. In their leading process tips DISC1 deficient cells displayed a weaker phalloidin, pAkt, and pGirdin, but not actin signal. Student's t test: n.s. $p \geq 0.05$, * $p < 0.05$, *** $p < 0.001$. Error bars: SEM. "n" reflects number of cells.

Fig. 6: DISC1 overlaps with microtubules in the growth cone-like structure
Fluorescent micrographs of a migrating interneuron stained with α-Tubulin as well as α-DISC1 antibodies. The leading process shaft is free of DISC1, but filled with tubulin fibers. TL: transmitted light. Scale bar: 5 µm.

Fig. 7: DISC1 deficient cells exhibit neither alterations in microtubule polymerization nor integrity
(A) Inverted fluorescent micrographs of NIH3T3 fibroblasts expressing control or DISC1 miRNA as well as Tubulin-GFP. Scale bar: 5 µm.
(B) Time line of NIH3T3 fibroblasts that expressed control or DISC1 miRNA as well as EB3-GFP. Arrowheads and arrows display EB3-GFP proteins ("shooting stars") that were followed over time.
(C) Analysis of the EB3 shooting star velocity showed no difference between control and DISC1 miRNA expressing NIH3T3 fibroblasts. Student's t test: n.s. $p \geq 0.05$. Error bars: SEM. "n" reflects number of shooting stars.

Fig. 8: Knockdown of DISC1 affects microtubule acetylation in the leading process of migrating interneurons
(A) Fluorescence micrographs of control and DISC1 miRNA expressing interneurons immunostained against acetylated Tubulin (acTub). Arrows display the end of the acTub expression in the leading processes. Scale bars: 5 µm.
(B) Analysis of the acTub signal in the leading process relative to the leading process length. DISC1 knockdown resulted in a decreased length of acTub compared to control cells. Student's t test: *** $p < 0.001$. Error bars: SEM. "n" reflects number of cells.

Fig. 9: Model how DISC1 orchestrates the tangential migration of cortical interneurons
(A) Soma translocation during tangential migration. DISC1 co-localizes with F-actin behind the nucleus and in the growth cone-like structure, where actin forces drive soma translocation.
(B-B') Interaction of DISC1 with Girdin provides a possible link between DISC1 and actin reorganization which is necessary for correct soma translocations. In wildtype interneurons (B), phosphorylated forms of Akt and Girdin lead to cross-linked F-actin. In the absence of DISC1 (B'), the amount of phosphorylated Akt as well as Girdin is decreased, an effect of DISC1 on the activation of these proteins. However, according to Kim et al. (2009) it is also possible that DISC1 is necessary for the proper localization of Girdin in the growth cone-like structure of migrating cortical interneurons. According to this scenario, a loss of DISC1 would also cause a reduction of non-phosphorylated Girdin. Although it is not completly clear how the three proteins DISC1, Akt and Girdin influence each other, reduced F-actin and increased G-actin levels indicate an impact of DISC1 on actin remodeling events resulting in less stabilized actin filaments.

Figures

Figure 1

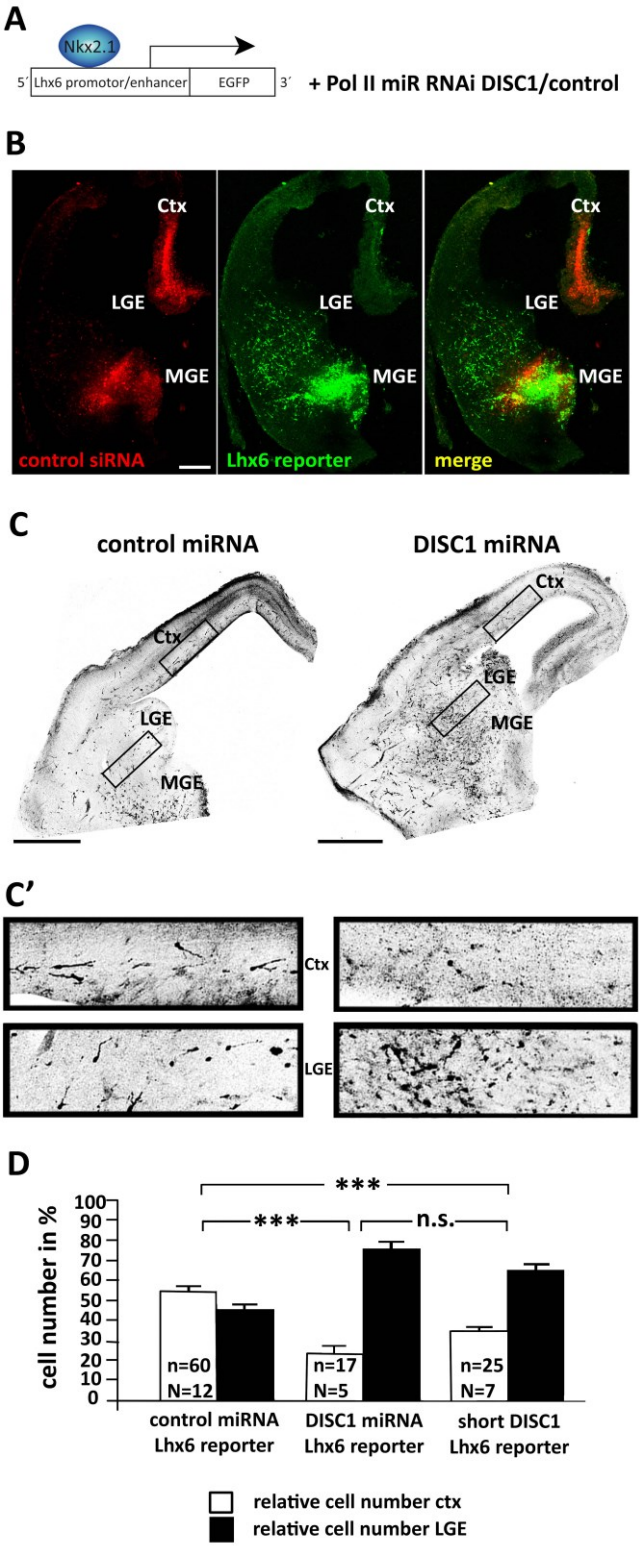
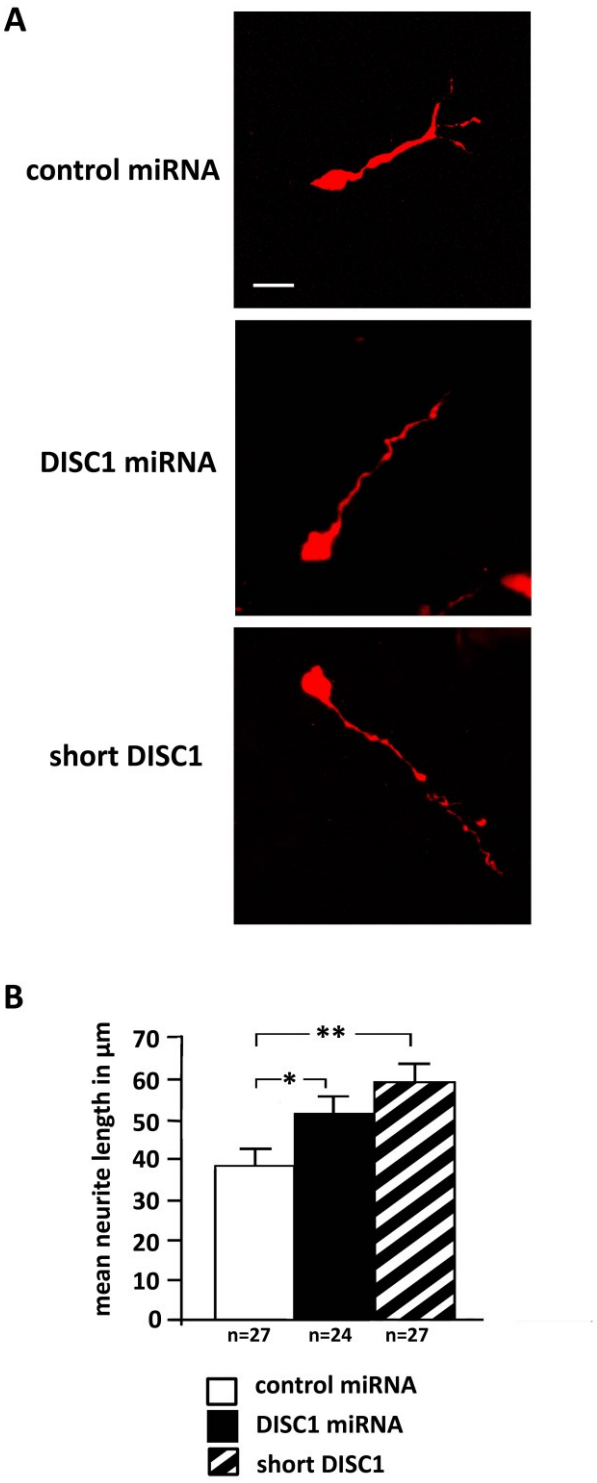


Figure 2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short DISC1
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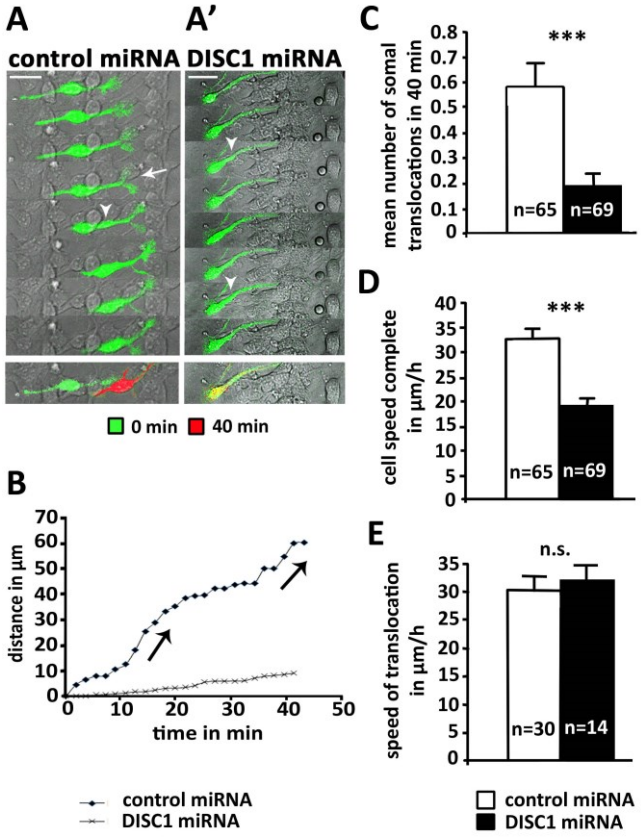
control miRNA
DISC1 miRNA
short DISC1

Figure 3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40 min
B
distance in µm
time in min
control miRNA
DISC1 miRNA
C
mean number of somal translocations in 40 min
***
n=65
n=69
D
cell speed complete in µm/h
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n.s.
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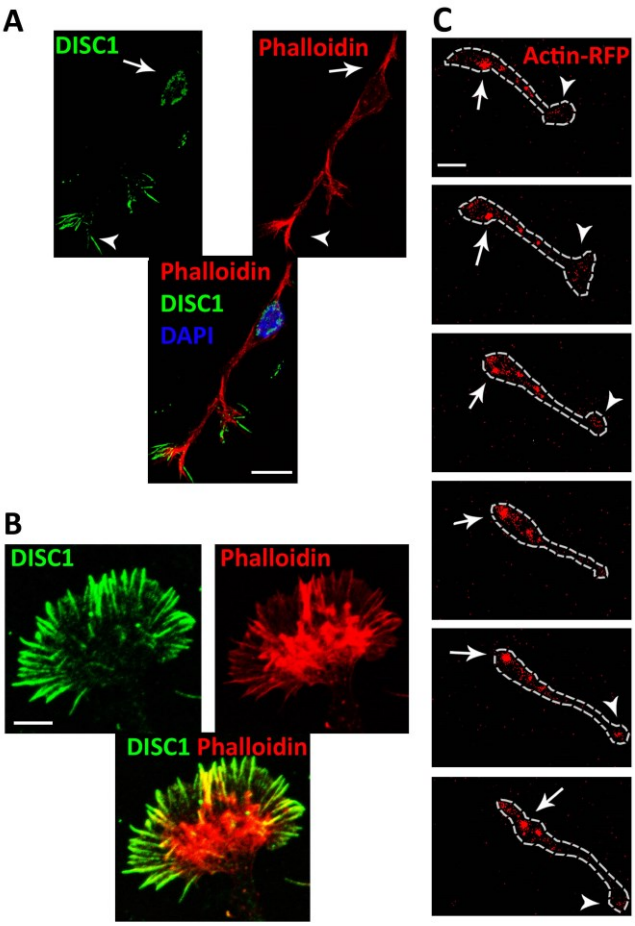
n=14
control miRNA
DISC1 miRNA

Figure 4


A
DISC1
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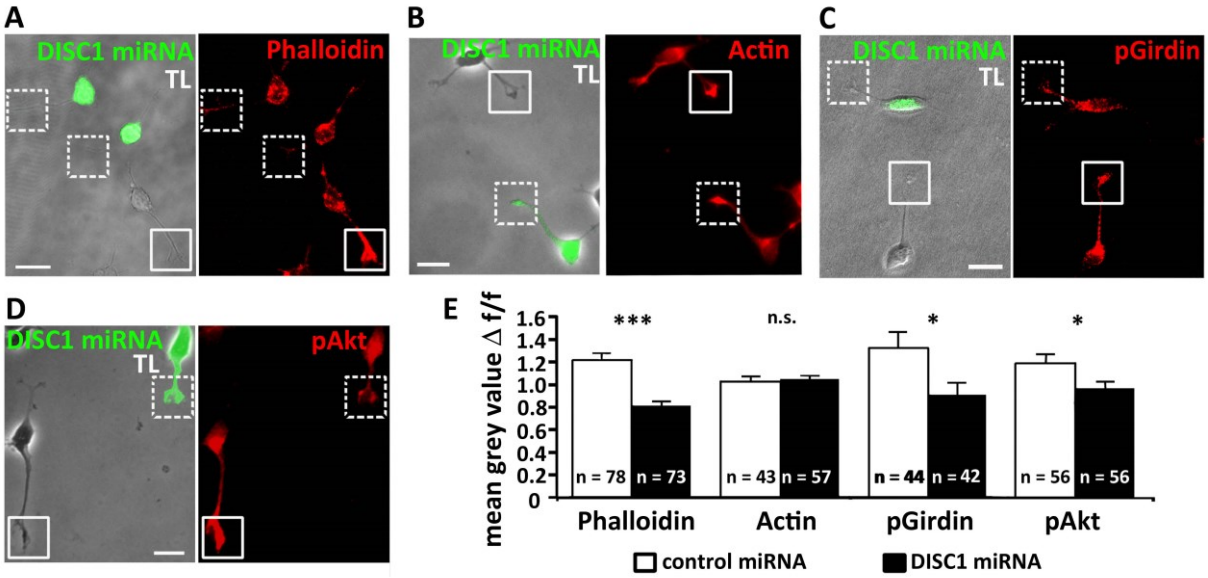
C
Actin-RFP

Figure 5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Phalloidin
B
DISC1 miRNA
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Actin
C
DISC1 miRNA
TL
pGirdin
D
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TL
pAkt
E
mean grey value Δ f/f
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
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n.s.
*
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Phalloidin
Actin
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control miRNA
DISC1 miRNA

Figure 6

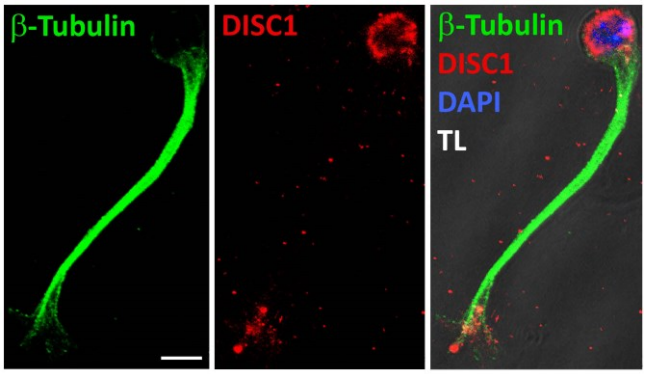
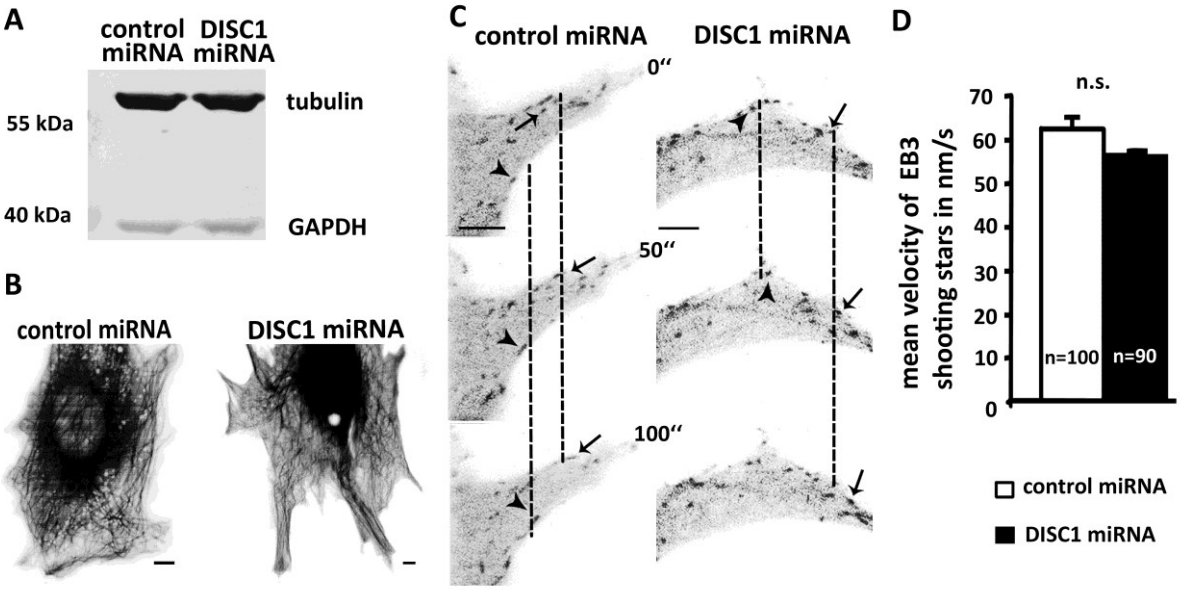


Figure 7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tubulin
GAPDH
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DISC1 miRNA
C
control miRNA
DISC1 miRNA
0“
50“
100“
D
n.s.
mean velocity of EB3 shooting stars in nm/s
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60
50
40
30
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10
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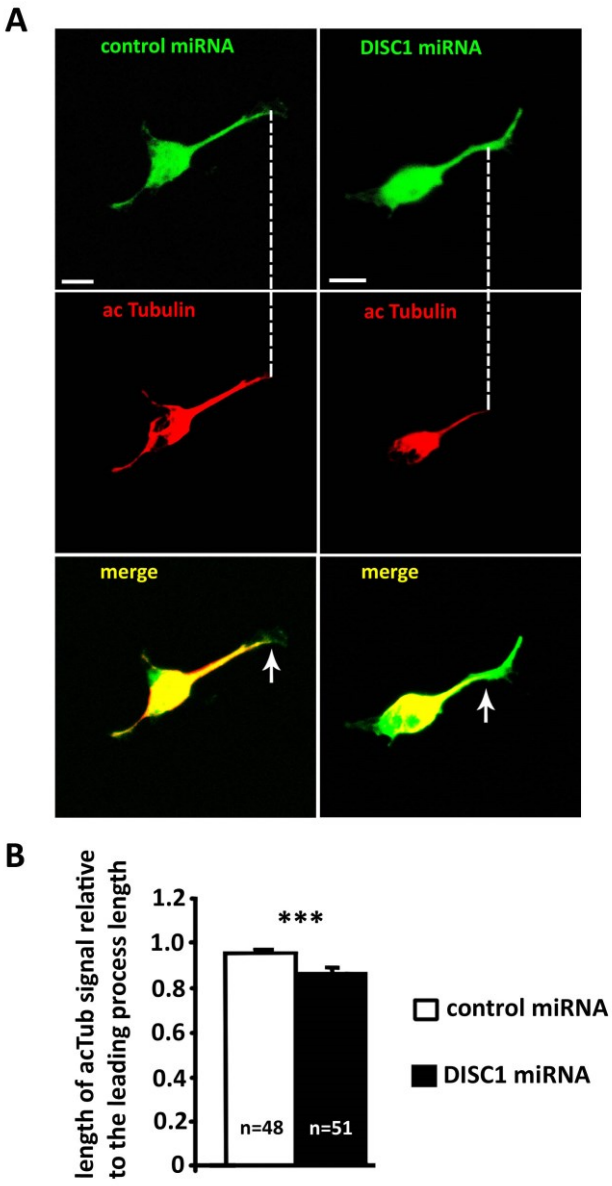
n=90
control miRNA
DISC1 miRNA

Figure 8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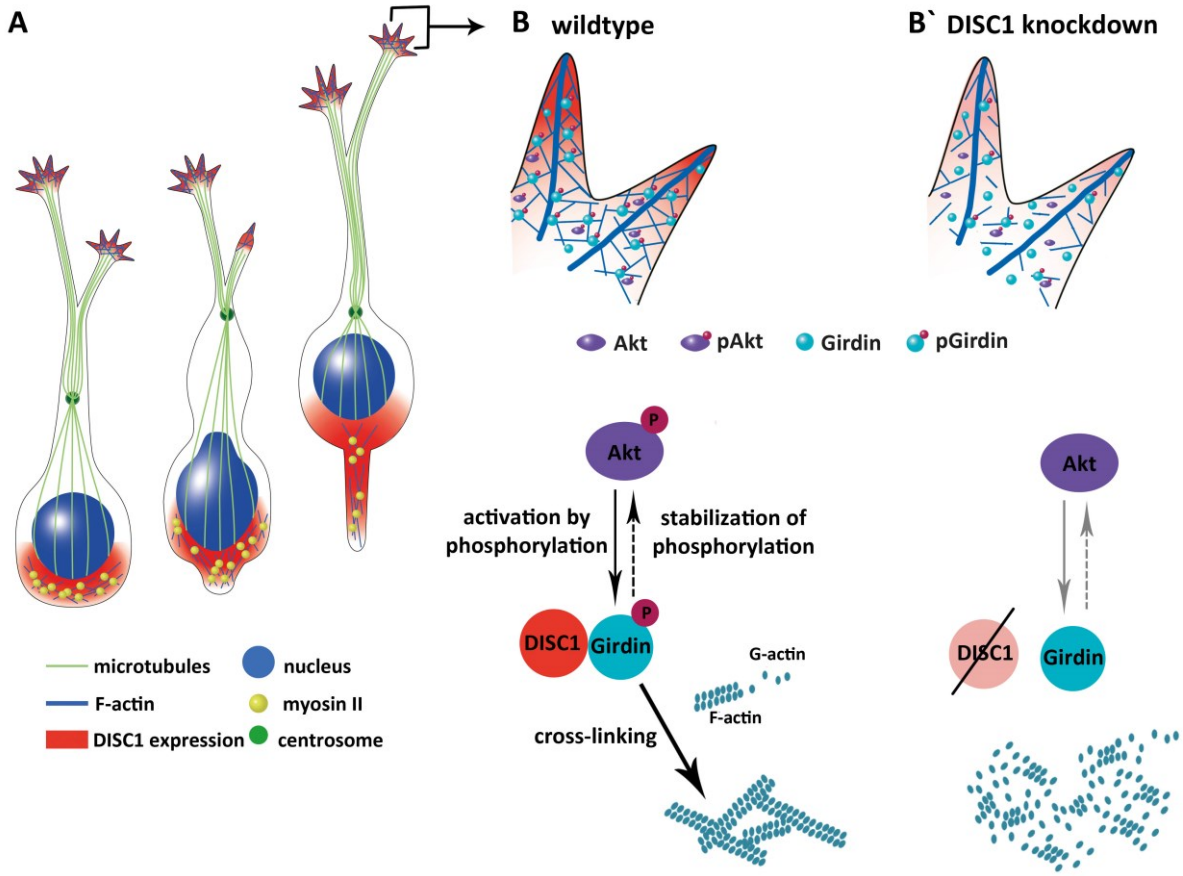
0.6
0.4
0.2
0
***
n=48
n=51
control miRNA
DISC1 miRNA

Figure 9


A
B wildtype
B` DISC1 knockdown
Akt
pAkt
Girdin
pGirdin
P
Akt
activation by phosphorylation
stabilization of phosphorylation
DISC1
Girdin
G-actin
F-actin
cross-linking
microtubules
nucleus
F-actin
myosin II
DISC1 expression
centrosome

4 Abschlussdiskussion

GABAerge cortikale Interneurone sind fundamental für die korrekte Funktionalität des Gehirns. Sie modulieren die Weitergabe von Informationen durch Projektionsneurone und synchronisieren deren Aktivität. Man unterscheidet eine Vielzahl verschiedener Interneuronensubtypen, die sich hinsichtlich Morphologie, elektrophysiologischem Verhalten und Genexpression unterscheiden. (Kepecs & Fishell 2014) Die Ausbildung des GABAergen Systems während der embryonalen und postnatalen Entwicklung beinhaltet die Neurogenese in den Proliferationszonen des basalen Großhirns, die Migration in den Cortex sowie die vollständige Differenzierung und Integration in das entstehende neuronale Netzwerk. Fehler bei der Entwicklung des GABAergen Systems sind eine mögliche Ursache für ein unausgeglichenes Verhältnis von Erregung und Hemmung. Solche Veränderungen in der Balance von Inhibition und Exzitation wurden bei psychischen Erkrankungen, wie beispielsweise Schizophrenie, nachgewiesen und liefern Erklärungsansätze für die Symptome der Patienten. Man geht diesbezüglich davon aus, dass der Grundstein für das Ausbilden solcher Erkrankungen bereits während der Entwicklung des Gehirns gelegt wird und spätere Einflüsse zu deren Ausbruch führen können. (Lewis & Levitt 2002; Marin 2012; Inan et al. 2013)

In der vorliegenden Arbeit wurde die Rolle von *DISC1*, einem Risikogen für Schizophrenie und ähnliche Erkrankungen (Porteous et al. 2006), bei der tangentialen Migration corticaler Interneurone, einem wesentlichen Schritt bei der Entwicklung des GABAergen Systems, untersucht. Es konnte gezeigt werden, dass eine verringerte Expression von DISC1 zu einem Defekt bei der Migration führt, welcher möglicherweise durch Veränderungen bei der Aktin-Reorganisation hervorgerufen wird.

4.1 DISC1 ist in tangential migrierenden cortikalen Interneuronen exprimiert

In bisherigen Expressionstudien konnte nicht belegt werden, dass DISC1 während der Embryonalentwicklung im basalen Telencephalon vorhanden ist. Daher war es zunächst notwendig, die Expression in postmitotischen Zellen der MGE an E14,5, dem Höhepunkt der Interneuronmigration, nachzuweisen. In Anlehnung an vorherige Veröffentlichun-

gen diente der adulte Hippocampus dabei als Positivkontrolle (siehe Anhang A1 und A2; Austin et al. 2004; Schurov et al. 2004). Über RT-PCR unter Verwendung zweier unabhängiger Primer-Paare (siehe Anhang A3) sowie *In-Situ*-Hybridisierung an coronalen Hirnschnitten konnte die DISC1-mRNA im basalen Teil des embryonalen Großhirns aufgezeigt werden. Der Nachweis des DISC1-Proteins erfolgte daraufhin mittels Immunhisto- sowie Immuncytochemie. Doppelmarkierungen mit Lhx6 und Calbindin bestätigten dabei, dass es sich bei $DISC1^+$ Zellen tatsächlich um cortikale Interneurone handelt.

Da die subzelluläre Verteilung Hinweise auf die Funktion eines Proteins gibt, erfolgte eine Antikörperfärbung an vereinzelten Interneuronen der embryonalen MGE. Diese zeigt eine DISC1-Expression in den Spitzen des Führungsfortsatzes und im Zellsoma, wobei DISC1 vor allem hinter dem Zellkern lokalisiert ist. Dies deutet auf Interaktionen mit anderen cytosolischen Bestandteilen hin, so dass DISC1 durchaus Einfluss auf die Migration haben könnte.

Um die Funktion von DISC1 untersuchen zu können, wurde im Folgenden das Proteinlevel von DISC1 mittels RNA-Interferenz (RNAi) gesenkt und die daraus resultierenden Defekte analysiert. Grundlage der RNAi-Methode ist der Abbau von mRNA aufgrund komplementärer doppelsträngiger RNA. Dieser Mechanismus ermöglicht es Zellen, exogene RNA, die durch Pathogene eingebracht wurde, zu eliminieren und mittels endogener Micro-RNAs (miRNAs) die Genexpression während der Entwicklung und Differenzierung zu regulieren. (Kim & Rossi 2007) Für das Herunterregulieren der DISC1-Expression wurde ein Ausschnitt der DISC1-mRNA als miRNA-Sequenz gewählt, der bereits in einer anderen Studie Verwendung fand (Kamiya et al. 2005). Dieser wurde in einen kommerziellen Vektor eingefügt, der für eine effektive Prozessierung der miRNA optimiert wurde und zusätzlich für EGFP als Transfektionsmarker kodiert (Invitrogen). Als Kontrolle erfolgte die Transfektion mit einem Kontroll-miRNA-Vektor, dessen miRNA keiner bekannten mRNA ähnelt. Damit wurde sichergestellt, dass die Transfektion der Zellen oder die Expression von EGFP bei den anschließenden Experimenten nicht zu Effekten führte.

4.2 Interneurone mit vermindertem DISC1-Level zeigen einen Migrationsdefekt

Zahlreiche Studien haben den Einfluss von DISC1 auf die Migration neuronaler Zellen untersucht. Demnach führt das Herunterregulieren von DISC1 im embryonalen Cortex von Mäusen und Ratten zu einer verminderten radialen Migration cortikaler Projektionsneurone (Kamiya et al. 2005; Young-Pearse et al. 2010; Kubo et al. 2010). Auch im embryonalen Hippocampus führt ein Mangel an DISC1 zu Defiziten bei der Migration (Meyer & Morris 2009; Tomita et al. 2011). Gegensätzliche Effekte zeigen sich jedoch bei neugeborenen Neuronen im adulten Hippocampus. Unter DISC1-Mangel migrieren diese Zellen überraschenderweise weiter als unter Kontrollbedingungen (Duan et al. 2007; Enomoto et al. 2009; Kim et al. 2009). Dagegen scheint DISC1 bei der ebenfalls postnatal ablaufenden Neuroblasten-Migration auf dem rostralen Migrationsweg (Richtung olfaktorischen Bulbus) keine Rolle zu spielen (Wang et al. 2011). Diese Ergebnisse deuten auf unterschiedliche Einflüsse von DISC1 innerhalb verschiedener Zelltypen und Entwicklungsphasen hin. Allerdings ist es auch möglich, dass das Ausmaß der beobachteten Effekte davon abhängt, wie stark das DISC1-Proteinlevel verringert wurde. Dies wird bei Studien zur embryonalen Entwicklung des Hippocampus deutlich. Während zunächst ein Migrationsdefekt als Folge eines verminderten DISC1-Levels nur bei Körnerzellen nachgewiesen wurde (Meyer & Morris 2009), zeigten spätere Untersuchungen auch Effekte auf Pyramidenzellen. Dabei gehen die Autoren davon aus, dass die Effizienz des ursprünglich verwendeten RNAi-Konstruktes nicht ausreicht, um in diesen Zellen einen Effekt hervorzurufen. (Tomita et al. 2011) Dies deutet darauf hin, dass verschiedene Zelltypen unterschiedlich auf eine verminderte DISC1-Expression reagieren. So sind hippocampale Körnerzellen anfälliger für geringe Veränderungen in der DISC1-Expression, während die Pyramidenzellen erst bei größeren Protein-Schwankungen Störungen bei der Migration aufweisen.

In der vorliegenden Studie wurde erstmals ein Einfluss von DISC1 auf die tangentiale Migration cortikaler Interneurone gezeigt. Dafür wurde der Effekt einer verminderten DISC1-Expression zunächst *in vitro* an MGE-Explantaten untersucht, wobei signifikant mehr kontrolltransfizierte Zellen aus den Gewebeverbunden auswanderten als Interneurone, die DISC1-miRNA exprimierten. Um Effekte durch die Interaktion der DISC1-miRNA mit anderen Zellbestandteilen auszuschließen, wurde ein sogenanntes Rescue-Experiment durchgeführt. Hierzu wurden die MGE-Zellen zusätzlich mit einem Vektor

transfiziert, der für humanes DISC1 kodiert. Dessen mRNA wird aufgrund von Sequenzunterschieden zur murinen DISC1-mRNA trotz miRNA-Expression nicht abgebaut (Kamiya et al. 2005; Young-Pearse et al. 2010). So konnte die Anzahl derjenigen Zellen, die das Explantat verließen, wieder erhöht werden, wodurch die verringerte DISC1-Expression als Ursache für den Migrationsdefekt bestätigt wurde. Es kann demnach geschlussfolgert werden, dass DISC1 eine notwendige Komponente für die korrekte tangentiale Migration cortikaler Interneurone aus der MGE darstellt.

Auch in Schnittkulturen, die den Zellen eine physiologischere Migrationsumgebung bieten, zeigten Interneurone mit einem DISC1-Defizit einen Migrationsdefekt. Um artifizielle Einflüsse von *In-Vitro*-Versuchen ausschließen zu können, war jedoch auch der Nachweis in vivo notwendig. In diesem Zusammenhang kam die Methode der *In-Utero*-Elektroporation zum Einsatz, wobei lebenden Embryonen die Vektor-Lösung in die Ventrikel injiziert wird. Anschließend wird über Elektroden ein elektrisches Feld angelegt, wodurch die Zellen der Ventrikularzone Poren bilden. Aufgrund ihrer negativen Ladung werden die Vektoren in Richtung des positiven Pols beschleunigt und gelangen durch die geöffneten Poren in die Zellen. Nach Borrell et al. (2005) ist es dabei möglich, ausschließlich Zellen der MGE zu manipulieren, indem die Elektroden in einem bestimmten Winkel an den embryonalen Kopf gehalten werden. Anderen Arbeitsgruppen (vgl. Gelman et al. 2009) war dies jedoch nicht möglich. Um dennoch den Einfluss einer verminderten DISC1-Expression auf die tangentiale Migration von Interneuronen aus der MGE in vivo untersuchen zu können, wurde das EGFP aus den miRNA-Vektoren entfernt und stattdessen ein zusätzliches Reporter-Konstrukt in die Zellen eingebracht. Dieses wurde so konstruiert, dass aufgrund der Expression von EGFP unter dem Lhx6-Promotor ausschließlich Zellen aus der MGE markiert wurden (Du et al. 2008; Lavdas et al. 1999). Durch die Elektroporation der Ventrikularzone der MGE an E13,5 – also einen Tag vor dem Höhepunkt der Interneuronenmigration – konnte sichergestellt werden, dass keine migrierenden Neurone markiert wurden, welche die MGE bereits verlassen hatten. Wie bei den *In-Vitro*-Versuchen wiesen Interneurone mit verringertem DISC1-Proteinlevel nach drei Tagen *in utero* einen Migrationsdefekt auf. Im Vergleich zur Kontrolle erreichten weniger Zellen den Cortex und verblieben nach Verlassen der MGE in der LGE. Diese Experimente veranschaulichen, dass DISC1 auch unter physiologischen Bedingungen im lebenden, sich entwickelnden Embryo Einfluss auf die Migration cortikaler Interneurone der MGE hat. Die Wahl der Methode fiel dabei auf die *In-Utero*-Elektroporation, weil die eine zeit- und ortsspezifische Transfektion von Zellen im le-

benden Gehirn ermöglicht, ohne eine Immunantwort (z.B. durch Transfektion mittels Viren) oder eine veränderte Regulation zahlreicher weiterer Gene (bei transgenen Mauslinien) hervorzurufen.

Mit Hilfe von RNAi wurde die Expression von DISC1 verringert. Dies entspricht aber nicht den pathologischen Formen, die in Schizophreniepatienten bisher gefunden wurden. So wurde das DISC1-Gen in einer schottischen Familien aufgrund einer chromosomalen Translokation entdeckt. Infolge dieser Translokation wird eine verkürzte Form des DISC1-Proteins gebildet, welcher C-terminale Domänen fehlen. Aufgrund der verkürzten Aminosäuren-Abfolge ist die Funktionalität des Proteins eingeschränkt. Zusätzlich wirkt es sich dominant-negativ auf das DISC1-Protein aus, welches auf dem anderen Allel korrekt kodiert wird. Dies hat einen Defekt bei der Migration cortikaler Projektionsneurone zur Folge, der dem eines mäßig verminderten DISC1-Levels entspricht. (Kamiya et al. 2005) In der vorliegenden Studie konnte erstmals ein Effekt dieses verkürzten Proteins auf die Migration cortikaler Interneurone gezeigt werden. Auch hier war der Migrationsdefekt geringer ausgeprägt als unter Verwendung von RNAi. Doch nicht nur drastische Veränderungen am Protein, sogar einzelne Punktmutationen im *DISC1*-Gen können zu negativen Effekten führen. So zeigt ein Mausmodell mit einer DISC1-L100P-Mutation ebenfalls eine eingeschränkte tangentiale Migration während der Embryonalentwicklung (Lee et al. 2013). Zusammen mit den zuvor erwähnten Ergebnissen lässt dies darauf schließen, dass DISC1 eine essentielle Rolle bei der Migration cortikaler Interneurone spielt.

4.3 Unter DISC1-Mangel vollziehen Interneurone weniger somale Translokationen

Neben einer verminderten Migration zeigen die Interneurone mit einem DISC1-Mangel sowohl *in vitro* als auch *in vivo* morphologische Veränderungen. Im Vergleich zu Zellen der Kontrollgruppe weisen sie einen längeren, aber weniger verzweigten Führungsfortsatz auf. Dieser stellt eine besondere Struktur dar, die während des Migrationsvorganges gebildet wird. Treten morphologische Veränderungen auf, weisen sie auf Abweichungen im Migrationsmodus hin. Nach Bellion et al. (2005) besteht die Migration cortikaler Interneurone aus zwei Schritten: der somalen Translokation und dem Auswachsen

des Führungsfortsatzes. Dementsprechend führt die Inhibition der somalen Translokation zu verlängerten Führungsfortsätzen (Shinohara et al. 2012). Die durch den DISC1-Mangel verursachten morphologischen Veränderungen könnten daher die Folge einer reduzierten Anzahl an Translokationen sein. Um dies zu belegen, wurde die Migration einzelner Interneurone in Co-Kulturen mit cortikalen Zellen beobachtet. Wenn sie DISC1-miRNA exprimierten, vollführten sie seltener somale Translokationen als unter Kontrollbedingungen. Obwohl Anschwellungen im Führungsfortsatz zusehen waren, erfolgte keine Nucleokinese. Demzufolge war in den meisten Fällen auch kein nachhängender Fortsatz erkennbar. Betrachtet man allerdings die Geschwindigkeit, welche die Zellen für eine somale Translokation benötigten, so zeigt sich kein Unterschied. Diese Ergebnisse weisen darauf hin, dass der Migrationsdefekt, der bei einem DISC1-Mangel beobachtet werden kann, aufgrund einer verminderten Anzahl an somalen Translokationen zustande kommt. In Anlehnung an die zuvor erwähnten Veröffentlichungen, könnten die verlängerte Führungsfortsätze eine Folge davon sein.

Die Bedeutung von DISC1 bei der Migration wird durch Effekte, die infolge des Verlusts von DISC1-Interaktionspartnern entstehen, verdeutlicht. Das prominenteste Beispiel hierfür ist das Protein LIS1 (Lissencephaly1). Wie bei DISC1-Mangel führt eine verminderte Expression von LIS1 zu einem Migrationsdefekt bei cortikalen Projektions- und Interneuronen, welcher durch eine verringerte Anzahl somaler Translokationen hervorgerufen wird. Auch in diesem Fall weisen die Zellen längere und wenig verzweigte Führungsfortsätze auf. (McManus et al. 2001; Nasrallah et al. 2006; Gopal et al. 2010)

4.4 Der Einfluss von DISC1 auf das Aktin-Cytoskelett

Zellen bewegen sich aufgrund von Polymerisations- und Depolymerisationsprozessen einzelner Cytoskelettkomponenten sowie mit Hilfe von Cytoskelett-assoziierten Motorproteinen. So realisiert das Cytoskelett auch die Nucleokinese in migrierenden Interneuronen. Es wird davon ausgegangen, dass die Polymerisation von Aktin und das Ansetzen von Motorproteinen an Aktinfilamenten für das Aufbringen der erforderlichen Kraft zuständig ist. In diesem Zusammenhang werden verschiedene Möglichkeiten diskutiert, an welcher Stelle die Kraft zur Bewegung des Zellkern aufgewendet wird. Diverse Studien beschreiben das Ausbilden eines Aktin-Netzwerkes, welches sich vor der Nucloekinese

hinter dem Kern formiert. Eine Aktin-Kontraktion, die über das Motorprotein MyosinII realisiert wird, führt anschließend dazu, dass der Kern nach vorn geschoben wird. Blockiert man die MyosinII-Aktivität, kommt es zu einem Migrationsstopp. (Bellion et al. 2005; Martini & Valdeolmillos 2010)

Aktin-Kondensationen wurden aber auch vor dem Kern beobachtet (Shinohara et al. 2012). Nach He et al. (2010) sind diese entscheidend für die Nucleokinese. Im Gegensatz zu anderen Studien untersuchte diese Gruppe die Migration von Körnerzellen des Kleinhirns, indem sie Aktin-modulierende Chemikalien direkt an bestimmte Zellstrukturen applizierte. Dabei stellte sich heraus, dass Veränderungen des Aktin-Cytoskeletts innerhalb des Führungsfortsatzes zum Abbruch der Migration führen. Die Applikation der Chemikalien am Zellsoma stoppte die Bewegung der Zellen dagegen nicht. Folglich gehen die Autoren davon aus, dass die Nucleokinese durch einen vorwärts gerichteten Aktin-Fluss am Führungsfortsatz ermöglicht wird.

In der vorliegenden Studie sollte aus diesem Grund ein besonderes Augenmerk auf Aktin-Kondensationen innerhalb migrierender Interneurone gelegt werden. Mit Hilfe von Fluoreszenz-markiertem Aktin konnte gezeigt werden, dass diese in der Zelle sehr dynamisch verteilt und sowohl vor als auch hinter dem Zellkern zu finden sind. Ebenso weist die Spitze des Führungsfortsatzes ein starkes Aktin-Signal auf. Diese Ergebnisse deuten darauf hin, dass ein Kraftaufwand zur Bewegung des Zellkerns sowohl vor als auch hinter dem Kern – möglicherweise gleichzeitig – zustande kommt.

Neben dem konstanten Aktin-Signal zeichnet sich die Struktur am Ende des Führungsfortsatzes, die mit axonalen Wachstumskegeln vergleichbar ist, durch eine starke DISC1-Expression aus. Daher wurde sie gewählt, um daran den Einfluss eines DISC1-Mangels auf das Aktin-Cytoskelett zu untersuchen. Während das Aktin-Proteinlevel unverändert blieb, nahm die Menge an filamentösem Aktin ab. Dies deutet darauf hin, dass DISC1 keinen Einfluss auf die Expression von Aktin, aber dessen Organisation hat. Es konnte damit gezeigt werden, dass eine verminderte DISC1-Expression das Aktin-Cytoskelett beeinträchtigt. Diese Beobachtung deckt einen potentiellen Mechanismus auf, wie ein Mangel an DISC1 zu dem beobachteten Migrationsdefekt führen kann.

Ein möglicher Mediator dieser Funktion von DISC1 auf die Aktin-Organisation ist Girdin. Dieser Bindungspartner von DISC1 liegt zunächst an die Zellmembran gebunden vor. Nach der Aktivierung durch das Protein Akt löst es sich aus der Membran und vernetzt Aktin-Filamente. Der funktionelle Zusammenhang der drei Faktoren DISC1, Girdin und Akt wird bisweilen konträr diskutiert. Demnach könnte DISC1 entweder für die Lo-

kalisation von Girdin zuständig sein und somit die Vernetzung von F-Aktin fördern (Enomoto et al. 2009) oder die Aktivierung von Girdin durch Akt und damit die Vernetzung von Aktin-Filamenten verhindern (Kim et al. 2009). In cortikalen Interneuronen führt eine verminderte DISC1-Expression zum Rückgang beider aktivierten Formen, also sowohl von Girdin als auch von Akt, in den Spitzen der Führungsfortsätze. Damit scheint DISC1 eher eine aktivierende Funktion auf Girdin und folglich auf die Stabilität neugebildeter Aktin-Filamente zu haben. Auch wenn noch nicht geklärt ist, auf welche Weise die drei Proteine miteinander interagieren, bekräftigen diese Ergebnisse die Erkenntnis, dass DISC1 das Aktin-Cytoskelett beeinflusst. Somit wurde ein möglicher Mechanismus aufgedeckt, über den DISC1 Einfluss auf die tangentiale Migration cortikaler Interneurone ausüben könnte.

4.5 Der Einfluss von DISC1 auf das Mikrotubuli-Cytoskelett

Bisher wurde DISC1 hauptsächlich im Zusammenhang mit dem Mikrotubuli-Cytoskelett beschrieben. So moduliert es als Interaktionspartner von Kinesin-I den Transport von Organellen und Cytoskelettkomponenten (Taya et al. 2007). Als Bestandteil des Centrosom-assoziierten Dynein-Motor-Komplexes spielt es eine Schlüsselrolle bei dessen Organisation und Dynamik. Es stabilisiert den Komplex durch Rekrutierung verschiedener Proteine (u.a. LIS1) und trägt auf diese Weise zu seiner korrekten Funktion bei. Entsprechend führt die Überexpression von DISC1 in COS-7-Zellen zu einer Akkumulation von Mikrotubuli in der Zellperipherie. Umgekehrt weisen Zellen, die eine dominant-negative Form von DISC1 exprimieren, ein vergleichsweise ungeordnetes Netzwerk auf. (Kamiya et al. 2005; Wang & Brandon 2011) Dagegen zeigte die Expression von DISC1-miRNA in NIH3T3-Fibroblasten keinen Effekt auf das Mikrotubuli-Netzwerk. Mit Hilfe von fluoreszenzmarkiertem Tubulin und EB3 wurde das Mikrotubuli-Cytoskelett hinsichtlich der Polymerisation und Dynamik analysiert. Das verminderte DISC1-Level führte zu keinerlei Veränderungen gegenüber der Kontrollgruppe. Bildung und Integrität des Mikrotubuli-Netzwerkes wurden durch den DISC1-Mangel nicht beeinflusst.

NIH3T3-Fibroblasten wurden für diese Arbeit mehrfach als Modell herangezogen. Obwohl sie sich morphologisch stark von migrierenden Interneuronen unterscheiden, wei-

sen sie eine vergleichbare DISC1-Verteilung innerhalb der Zelle auf. Dabei ist die Expression in den Filopodien und Lamellopodien besonders auffällig (siehe Anhang A4). Dennoch muss darauf hingewiesen werden, dass es sich um ein Modell-System handelt. Aufgrund unterschiedlicher Arten der Fortbewegung, sind NIH3T3-Fibroblasten nur eingeschränkt mit migrierenden Interneuronen vergleichbar.

Wie bereits beschrieben, ist der Führungsfortsatz migrierender Neurone eine besondere Struktur. Die dynamische Spitze, deren Verzweigung für die Migration notwendig ist (Martini et al. 2009; Valiente and Martini 2009; Yanagida et al. 2012), wird mit dem Wachstumskegel eines auswachsenden Axons verglichen. Die stabilisierten Mikrotubuli des Fortsatzschaftes reichen demnach in die Wachstumskegel-artige Struktur hinein, während einzelne dynamische Tubulinfasern weiter in die Peripherie hinauslaufen und auf Lenkungsfaktoren reagieren. Die Stabilisierung der Mikrotubuli ist dann entscheidend an der Reaktion der Wachstumskegel-artigen Struktur auf die Faktoren der Umwelt beteiligt. Dabei wird durch lokal begrenzte Stabilisierung bestimmt, in welche Richtung sich die Fortsatzspitze bewegt. Eine komplette Stabilisierung bewirkt dagegen ein gerades Auswachsen des Führungsfortsatzes. (Lowery and Van Vector 2009; Kalil and Dent 2014) Nach dem Herunterregulieren der DISC1-Expression in Interneuronen zeigt sich eine verringerte Stabilisierung von Mikrotubuli-Fasern am distalen Ende der Führungsfortsätze, wie sie auch bei einem Mangel an LIS1 auftritt (Gopal et al. 2010). Wie dieser Effekt vermittelt wird, muss in anschließenden Studien untersucht werden. Eine Ursache könnte das Zusammenspiel von DISC1 mit anderen Mikrotubuli-modulierenden Proteinen in der Wachstumskegel-artigen Struktur sein. Ein möglicher Kandidat ist das Protein MAP1A. Dabei handelt es sich laut Bindungsstudien um einen möglichen Interaktionspartner von DISC1 (Morris et al. 2003). Es bindet sowohl an Aktin als auch an Mikrotubuli und kontrolliert die Stabilität von Mikrotubuli in Neuronen. Der beobachtete Effekt muss jedoch nicht direkt durch einen Bindungspartner von DISC1 vermittelt werden. So wird auch Akt, dessen aktive Form bei einem DISC1-Mangel reduziert ist, mit der Stabilisierung von Mikrotubuli in migrierenden Zellen in Verbindung gebracht (Onishi et al. 2007).

Da Aktin-Kräfte zum Realisieren der Nucleokinese als notwendig erachtet werden, kann durch den Einfluss von DISC1 auf das Aktin-Cytoskelett der beobachtete Migrationsdefekt bei einem DISC1-Mangel erklärt werden. Es ist allerdings nicht ausgeschlos-

sen, dass auch der aufgezeigte Effekt auf das Mikrotubuli-Cytoskelett einen Einfluss auf die tangentiale Migration ausübt.

4.6 DISC1 stellt ein Risikogen für Schizophrenie dar

Bei Schizophrenie handelt es sich um eine multikausale Erkrankung, über deren Pathophysiologie und Pathogenes verschiedene Theorien existieren. Hierzu zählen die Glutamat-, Dopamin- und Immunhypothese. (Stone et al. 2007; Lang et al. 2007) Auch das GABAerge System wird vermehrt mit Schizophrenie in Verbindung gebracht. So fand man in Gehirnen von Schizophreniepatienten u.a. Hinweise auf Veränderungen der Anzahl bestimmter Interneuron-Subtypen. Außerdem konnten synaptische Defizite festgestellt werden. Diese können den Input auf die Interneurone (z.B. durch eine NMDA-Rezeptor-Unterfunktion) oder den Output auf andere Neurone (z.B. über einen Mangel am GABA-produzierenden Protein GAD67) betreffen. (Lewis et al. 2005; Marin 2012; Inan et al. 2013) Diese Ereignisse führen zu einer veränderten neuronalen Oszillation, was die Synchronität der neuronalen Aktivität im Gehirn verringert. Das wiederum hat Einfluss auf die Wahrnehmung und das Verhalten der Patienten. (Lewis et al. 2005; Gonzalez-Burgos und Lewis 2008; Lynall et al. 2010; Uhlhaas und Singer 2010)

Dass Veränderungen der Expression des Risikogens *DISC1* zu schizophrenierelevanten Phänotypen führen, konnte in Mausmodellen gezeigt werden. Dabei weisen Mäuse, die eine dominant-negative Form von DISC1 exprimieren oder deren DISC1-Gen eine Punktmutation enthält, sowohl morphologische Veränderungen als auch Verhaltensauffälligkeiten auf, die mit den Befunden von Schizophrenie-Patienten vergleichbar sind. Dazu zählen zum einen vergrößerte laterale Ventrikel, eine verringerte Cortex-Dicke und ein reduziertes Parvalbumin-Immunsignal. Zum anderen zeigten sich Defizite bezüglich der Aufmerksamkeit und Informationsverarbeitung, erhöhte Immobilität, verminderte Kommunikation unter Stressbedingungen und Verhaltensweisen, die mit depressivem Verhalten beim Menschen verglichen werden. (Hikida et al. 2007; Clapcote et al. 2007, Shen et al. 2008; Lee et al. 2013)

Die Ergebnisse dieser Arbeit verdeutlichen, dass DISC1 in tangential migrierenden Interneuronen der MGE exprimiert ist und ein Mangel an DISC1 zu einem Migrationsdefekt führt, der möglicherweise über Veränderungen bei der Aktin-Reorganisation hervorgerufen wird. Damit wird ein neuer Mechanismus aufgezeigt, über den DISC1 die

Entwicklung des GABAergen Systems beeinflusst und so zur Pathogenese von Schizophrenie beitragen kann. Gleichzeitig stärkt dies die These, dass Schizophrenie eine entwicklungsbiologische Erkrankung darstellt (Di Cristo 2007; Jaaro-Peled et al. 2009; Ayhan et al. 2011).

5 Zusammenfassung / Summary

5.1 Zusammenfassung

Das Gen *Disrupted-in-Schizophrenia 1 (DISC1)* wurde in einer schottischen Familie entdeckt, deren Familienmitglieder eine erhöhte Prävalenz für Schizophrenie aufweisen. Aufgrund einer chromosomalen Translokation kommt es zu einer Verkürzung des Gens und damit zu einem eingeschränkt funktionsfähigen Protein. Mittlerweile gilt *DISC1* als Risikogen für verschiedene psychische Erkrankungen, wie Schizophrenie oder Depression. Um die Entstehung dieser Krankheiten zu verstehen und mögliche Therapieansätze zu finden, rückte DISC1 in den Mittelpunkt zahlreicher Studien, welche die Funktion dieses Proteins im Gehirn untersuchen. Als ein sogenanntes Gerüstprotein zur zellulären Vermittlung von Proteinkomplexen ist es an diversen Entwicklungsprozessen, wie beispielsweise Proliferation und Migration neuronaler Zellen, im embryonalen sowie adulten Gehirn beteiligt.

In dieser Arbeit wird die Rolle von DISC1 bei der tangentialen Migration cortikaler GABAerger Interneurone dargelegt. Diese Zellen werden im basalen Telencephalon geboren und wandern anschließend in den sich entwickelnden Cortex ein. Obwohl sie nur etwa 20% der neuronalen Zellen im Cortex ausmachen, stellen sie eine immense Vielfalt an physiologisch, morphologisch sowie genetisch unterschiedlichen Zellen dar und sind entscheidend an der Funktionalität des Gehirn beteiligt. Es konnte gezeigt werden, dass migrierende Interneurone DISC1 exprimieren und ein Mangel an DISC1 sowohl *in vitro* als auch *in vivo* zu Defekten bei der tangentialen Migration führt. Weitere Untersuchungen konzentrierten sich auf das Cytoskelett, durch welches die Migration realisiert wird. Während kaum ein Einfluss auf das Mikrotubuli-Cytoskelett belegt werden konnte, führt ein Mangel an DISC1 zu Veränderungen bei der Aktin-Reorganisation. Damit zeigt die vorliegende Arbeit einen möglichen Mechanismus auf, wie DISC1 auf die tangentiale Migration cortikaler Interneurone Einfluss nimmt.

Diese Ergebnisse stärken die Annahme, dass Schizophrenie mit Störungen des GABAergen Systems einhergeht, und weisen darauf hin, dass es sich um eine entwicklungsbiologische Erkrankung handelt, deren Grundstein bereits während der Embryonalentwicklung gelegt wird.

5.2 Summary

The gene *Disrupted-in-Schizophrenia 1 (DISC1)* was found in a Scottish pedigree with an increased prevalence for schizophrenia. In this family a chromosomal translocation resulted in a shortened version of the DISC1 gene and led to a limited functional protein. Today, DISC1 is an accepted risk gene for several psychiatric diseases like schizophrenia or depression. To understand the pathogenesis of these diseases and to discover new therapeutic approaches numerous studies focused on the function of DISC1 in the brain. As a scaffold protein that mediates the formation of protein complexes in the cell it is involved in various developmental processes, such as proliferation, migration, and differentiation of neuronal cells.

The present study provides new insights into the role of DISC1 during tangential migration of GABAergic cortical interneurons. These inhibitory cells are born in the basal telencephalon and migrate into the developing cortex. Although they only account for approximately 20% of the neuronal cells in the cortex, they are composed of a variety of physiologically, morphologically, and genetically diverse cells that are crucial for the correct functionality of the brain. It has been shown that migrating interneurons express DISC1 and the loss of DISC1 leads to migration defects during tangential migration in vitro and in vivo. Further experiments focused on the cytoskeleton which accomplishes cell migration. Whereas a deficit of DISC1 rarely evokes effects on the microtubule cytoskeleton, the actin reorganisation is altered. Thereby this study depicts a possible mechanism how DISC1 exerts influence on the tangential migration of cortical interneurons.

These results lead to the assumption that schizophrenia could be associated with disturbances in the GABAergic system and indicate a developmental background within psychiatric diseases that originates from defects during embryonic development.

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7 Angaben zum Eigenanteil

Veröffentlichung 1: Steinecke A, Gampe C, Valkova C, Kaether C, Bolz J (2012) **Disrupted-in-Schizophrenia 1 (DISC1) is necessary for the correct migration of cortical interneurons**. J Neurosci 32:738-745.

In-Situ-Hybridisierung gegen DISC1-mRNA	Klonierung sonden-kodierender Vektor Sonden-Synthese In-Situ-Hybridisierung Mikroskopie
Test der DISC1-Antikörper-Spezifität an NIH3T3	Klonierung DISC1-RFP-kodierender Vektor Transfektion von NIH3T3 Immuncytochemie Mikroskopie Auswertung
miRNA und Test auf Effizienz	Klonierung miRNA-kodierende Vektoren Transfektion von NIH3T3 Herstellung von Proteinlysaten SDS-Page Western Blot Auswertung
Auswertung Morphologie DISC1-defizienter Interneurone in Plasmaclot und Schnittkulturen	Mikroskopie Auswertung
In-Utero-Elektroporation	Durchführung In-Utero-Elektroporation (50%) Anfertigung von Kryostatschnitten Immunhistochemie Mikroskopie Auswertung

Veröffentlichung 2: Steinecke A, Gampe C, Nitzsche F, Bolz J **DISC1 knockdown impairs the tangential migration of cortical interneurons by affecting the actin cytoskeleton**. (angenommen zur Veröffentlichung in Front Cell Neurosci am 20.06.2014)

In-Utero-Elektroporation	Klonierung GFP-freier miRNA-Vektoren Durchführung In-Utero-Elektroporation (50%) Auswertung der Morphologie
Aktin-Dynamik in MGE-Zellen	Klonierung Aktin-RFP/GFP-kodierender Vektor
Acetyliertes Tubulin in DISC1-defizienten Zellen der MGE	Immuncytochemie Mikroskopie Auswertung
Einfluss von DISC1 auf das Tubulin-Level in NIH3T3	Herstellung von Proteinlysaten SDS-Page Western Blot Auswertung
Tubulin-Dynamik in DISC1-defizienten NIH3T3 (Tubulin, EB3)	Klonierung Tubulin-GFP-kodierender Vektor Transfektion von NIH3T3 Mikroskopie Auswertung

8 Eigenständigkeitserklärung

Hiermit erkläre ich, dass mir die Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena bekannt ist und ich die vorliegende Dissertation selbst angefertigt habe. Dabei wurden keinerlei Textabschnitte Dritter oder eigener Prüfungsarbeiten ohne deren Kennzeichnung übernommen und keinerlei Hilfsmittel oder persönliche Mitteilungen ohne Angabe deren Quellen benutzt. Die Hilfe von Promotionsberatern oder Dritter, welche mittelbar oder unmittelbar geldwerte Leistungen für Arbeiten erhielten, die im Zusammenhang mit dem Inhalt der vorliegenden Dissertation stehen, wurde nicht in Anspruch genommen. Die vorliegende Dissertation ist zuvor nicht bereits als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht worden. Ich versichere bisher keinerlei Dissertation an einer Hochschule eingereicht zu haben.

Christin Gampe

9 Lebenslauf

Name Christin Gampe

Geburtstag 16/07/1985 - Jena

Privatadresse

Closewitzer Str. 2c, 07743 Jena, Deutschland

E-mail: chrgampe@gmx.de

Telefon: 0175/3422828

Berufliche Adresse

Friedrich-Schiller-Universität Jena

Institut für Allgemeine Zoologie und Tierphysiologie

Erbertstrasse 1, 07743 Jena, Deutschland

Telefon: 03641/949118

E-mail: chrgampe@gmx.de

Schulische Ausbildung

1996 – 2004 Abitur

Carl-Zeiss-Gymnasium Jena

Akademische Ausbildung

2004 – 2009

Studium der Ernährungswissenschaften mit Abschluss Diplom

Friedrich-Schiller-Universität Jena

Seit 2009

Promotion am Institut für Allgemeine Zoologie und Tierphysiologie

Friedrich-Schiller-Universität Jena

Reisestipendium *ProChance* zur Teilnahme am 41. Meeting der SfN Washington D.C. 2011

Publikationen

Erst-Autorenschaften

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The Journal of Neuroscience, 2012, 32:738-745

Co-Autorenschaften

Steinecke A, Zimmer G, Gampe C, Rudolph J, and Bolz J
EphA/ephrin-A Reverse Signaling Promotes the Migration of MGE-derived Cortical Interneurons
Development, 2014, 141(2):460-71

Zimmer G, Rudolph J, Landmann J, Gerstmann K, Steinecke A, Gampe C, and Bolz J
Bidirectional ephrinB3/EphA4 signaling mediates the segregation of MGE- and POA-derived interneurons in the deep and superficial migratory stream
The Journal of Neuroscience, 2011, 31:18364-18380

Posterpräsentationen

Nitzsche F, Gampe C, Steinecke A, and Bolz J
DISC1 expression pattern in the developing mouse brain
42nd annual meeting of the society for neuroscience, New Orleans 2012

Gampe C, Steinecke A, and Bolz J
Disrupted in Schizophrenia 1 (DISC1) is necessary for the correct migration of cortical interneurons
41st annual meeting of the society for neuroscience, Washington D.C. 2011

Steinecke A, Gampe C, Pensold D, and Bolz J
The role of Disc1 during interneuron migration
9th annual meeting of the german neuroscience society, Goettingen 2011

10 Anhang

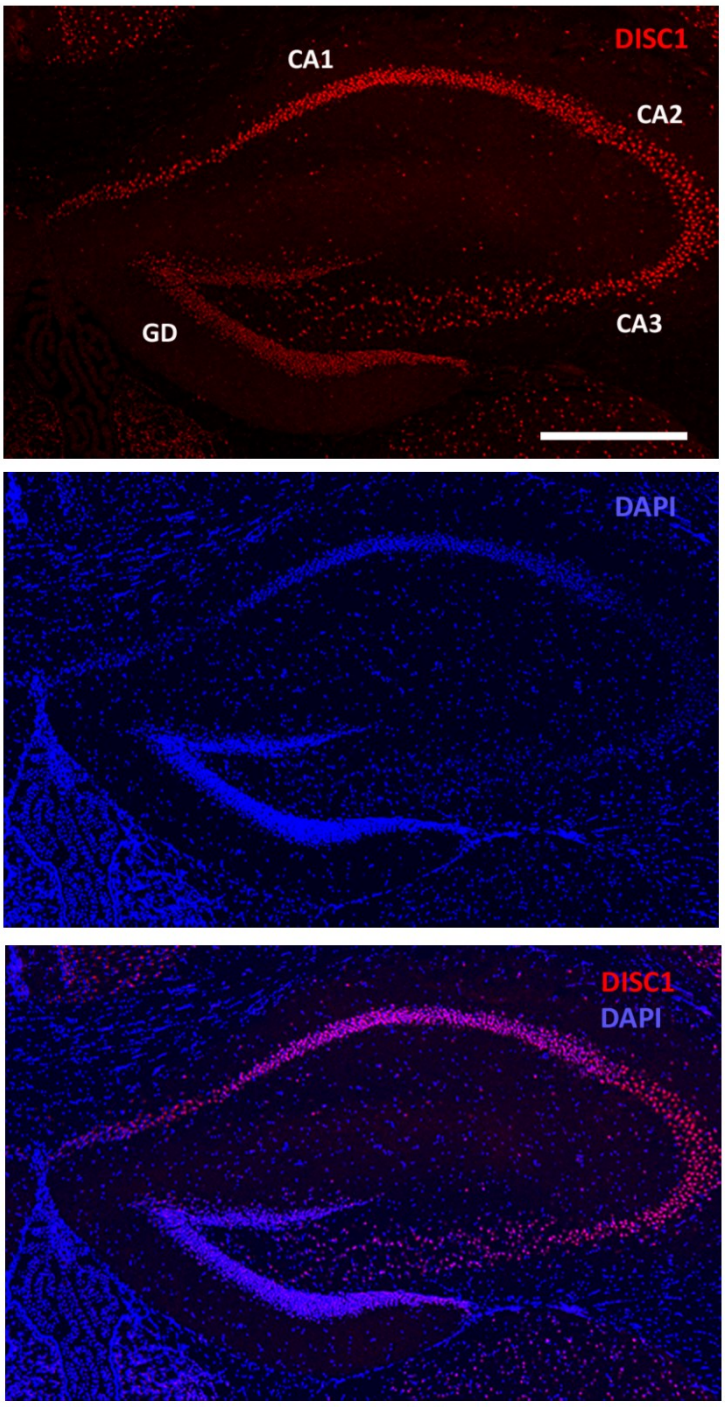

Abbildung A1: Antikörperfärbung gegen DISC1 am adulten Hippocampus zum Nachweis der Antikörperspezifität

Erkennbar ist die Markierung von Zellen der Corni ammoni (CA) 1-3 sowie des Gyrus dentatus (GD; vgl. Schurov et al. 2004, Austin et al. 2004; Skalenbalken: 200 µm). (geändert nach Steinecke 2013)

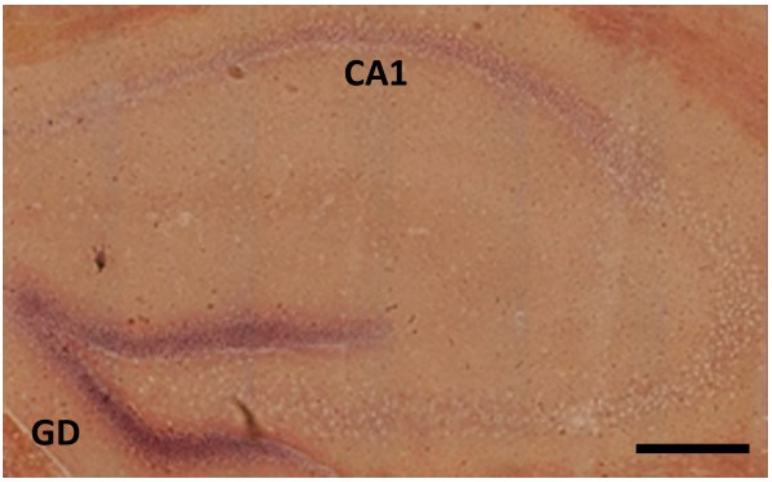

Abbildung A2: In-Situ-Hybridisierung gegen DISC1 am adulten Hippocampus zum Nachweis der Sondenspezifität

Es erfolgte eine Markierung von Zellen der Corni ammoni, v.a. von Neuronen in CA1, sowie des Gyrus dentatus (GD). Hier ist das Signal am stärksten, da die Neurone sehr dicht gepackt vorliegen (vgl. Schurov et al. 2004, Austin et al. 2004; Skalenbalken: 200 µm).

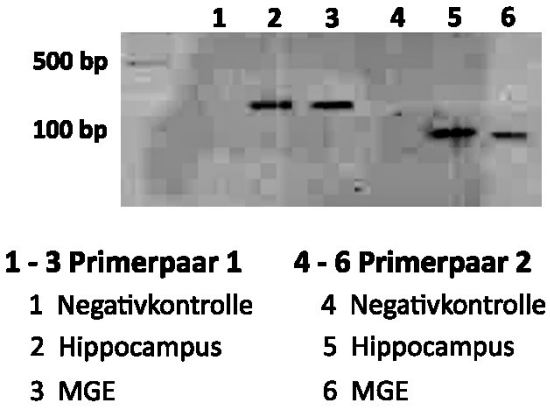

Abbildung A3: RT-PCR zum Nachweis von DISC1-mRNA im adulten Hippocampus sowie der embryonalen MGE

Es wurden zwei unabhängige Primerpaare zur Amplifizierung zweier unterschiedlicher Ausschnitte der DISC1-cDNA verwendet. Als Positivkontrolle erfolgte der Nachweis der DISC1-Expression im adulten Hippocampus.

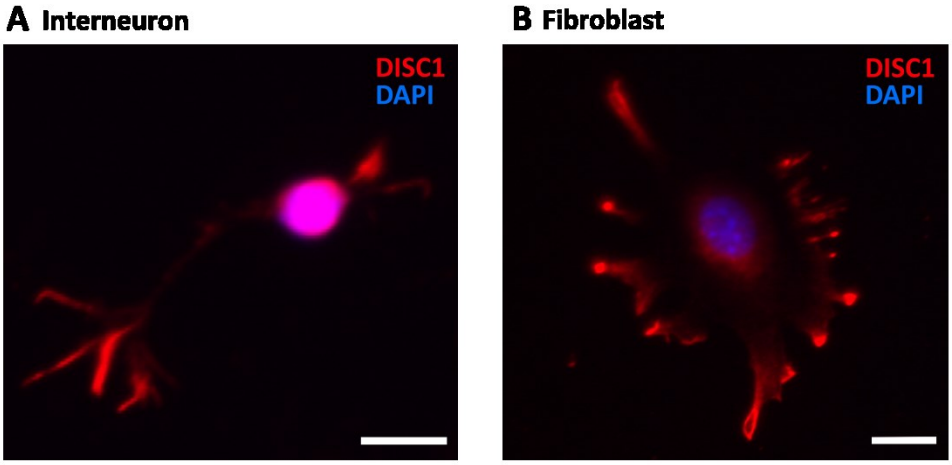

Abbildung A4: Vergleich der DISC1-Antikörperfärbung an Interneuronen und Fibroblasten

Sowohl bei Interneuronen (A) als auch Fibroblasten (B) ist DISC1 im Zellsoma lokalisiert. Besonders auffällig ist die Färbung in den Fortsatzspitzen: bei Interneuronen in der Wachstumskegel-artigen Struktur des Führungsfortsatzes, bei Fibroblasten in den Spitzen der Filopodien. (Skalenbalken: 10 µm)

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