Published in final edited form as:

Neurosci Res. 2016 November ; 112: 63–69. doi:10.1016/j.neures.2016.06.006.

Abnormal wake/sleep pattern in a novel gain-of-function model of DISC1

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Abstract

Sleep disturbances are common in psychiatric disorders, but the causal relationship between the two and the underlying genetic factors is unclear. The *DISC1* gene is strongly linked to mood disorders and schizophrenia in a Scottish pedigree. In an earlier study we found a sleep homeostasis disturbance in a *Drosophila* model overexpressing wild-type human *DISC1*. Here we aimed to explore the relationship between sleep and the *DISC1* gene in a mammalian model, a novel transgenic mouse model expressing full-length human *DISC1*. We assessed circadian rhythms by monitoring wheel running activity under normal 24-h light:dark conditions and in constant darkness and found the *DISC1* mice to have normal circadian photoentrainment and normal intrinsic circadian period. We also assessed sleep duration and quality in the *DISC1* mice and found that they were awake longer than wild-type controls at baseline with a tendency for lower rebound of delta activity during recovery from a short sleep deprivation. Thus we suggest that *DISC1* may be involved in sleep regulation.

Keywords

Non rapid eye movement sleep; rapid eye movement sleep; sleep homeostasis; circadian rhythms; *DISC1*; mouse model; behavior

INTRODUCTION

Sleep is an active process which is critical for our physical and mental health. Sleep is regulated by two processes: a homeostatic process which increases sleep drive as a function

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Conflict of Interest statement. None declared.

of the duration of wakefulness, and a circadian process which consolidates sleep to specific phases of the light/dark cycle (Borbely 1982). Various types of sleep disturbances in psychiatric disorders are well known and contribute to the distress of the patients (Wulff et al., 2010). Sleep disturbances are a core symptom of depression with insomnia afflicting a majority of the patients (Nutt et al., 2008). Sleep disturbances are also prominent in schizophrenia (Monti et al., 2013).

Sleep is hypothesized to result from the accumulation of homeostatic brain chemicals, nitric oxide and adenosine during wakefulness (Brown et al., 2012). These homeostatic sleep factors accumulate in the basal forebrain and cortex and inhibit wake-promoting neurons, such as orexinergic neurons (Sakurai 2007). In addition, involvement of cAMP response element binding protein (CREB) in sleep homeostasis has also been suggested (Hendricks et al., 2001; Graves et al., 2003). Sleep homeostasis is typically manipulated by sleep deprivation: sleep deprivation increases the homeostatic drive for sleep, increases the prevalence and power of delta activity and causes subsequent increased sleep once allowed to sleep (sleep rebound) (Brown et al. 2012). Nonetheless, the functional relationship between sleep disturbances and the pathology of major mental illness remains elusive. Rodent models are promising tools in this respect (Oliver et al., 2012; Phillips et al., 2012).

The *DISC1* gene was identified as the sole transcript with an open reading frame disrupted by an inherited chromosomal abnormality in a Scottish pedigree with familial mental illness (Blackwood et al., 2001). Although the genetic role of *DISC1* in sporadic cases of schizophrenia or mood disorders is under debate (Mathieson et al., 2012; Sullivan 2013; Porteous et al., 2014), neurobiology representing a loss of DISC1 function (e.g., RNAi interference) has indicated that the biological pathway involving DISC1 is likely to represent, at least in part, key pathophysiologies of major mental illness (Brandon and Sawa 2011; Tomoda et al., 2016). Nonetheless, the biological impact of DISC1 deficits in the Scottish pedigree is not yet perfectly understood. We have reported that transgenic *Drosophila* expressing wildtype (WT) human DISC1 in the mushroom body (a gain of function model) show several abnormalities, including disturbance in sleep homeostasis (Sawamura et al., 2008; Furukubo-Tokunaga et al., 2016). Here we generated transgenic mice that express WT human DISC1. The objective of this study was to explore, using a mammalian model, whether DISC1 is involved in sleep, which is frequently impaired in a wide variety of mental illnesses.

MATERIALS AND METHODS

Mice

The procedures used were in accordance with EU Directive 2010/63/EU for animal experiments. Full length human DISC1 was inserted under α CaMKII promoter in pMM403 vector (Dr. Kida, Tokyo University of Agriculture). The purified insert was injected into oocytes of C57BL/6N mice at the Transgenic Core Laboratory of Johns Hopkins University. The mice were maintained by heterozygous $\times$ C57BL/6N matings and adult male heterozygous and WT littermate mice were compared in all the experiments.

Biochemistry

Expression of the transgene at the mRNA level was confirmed by reverse-transcriptase PCR with the following primers:

Sense: 5'-CTGTCTGCGAGGGCC-3'

Antisense: 5'-TCTCACAGAGGTCACAGTAGGGGCTGCTGCAC-3'

Expression of the transgene on the protein level was confirmed by immunoprecipitation with anti-FLAG M2 Affinity Gel (Sigma A2220) followed by immunoblot with rabbit anti human DISC1 exon 10 (amino acids 665–678) antibodies.

Wheel running activity

Mice were placed in cages with a 4.5-inch running wheel, and their activity was monitored with VitalView software (Mini Mitter, OR). Photoentrainment experiments were done under a 12 h light: 12 h dark cycle. Light intensity was provided by Philips Daylight deluxe fluorescent lamps. The circadian period in constant darkness was calculated with ClockLab (Actimetrics, IL). For phase-shifting experiments, each animal was exposed to a light pulse (1,000 lux; CT16) for 15 min.

Sleep recordings and analysis

Sleep recordings were done as previously described (Altimus et al., 2008). In brief, we affixed a 2 channel EEG and 1 channel EMG implant (Pinnacle Technology, Inc. Lawrence, KS) into the skull of mice between the ages of 4 and 8 months while under ketamine/xylazine induced anesthesia. Mice were allowed 10 days to recover in a 12-hour light: 12-hour dark cycle before being transferred to the sleep-recording cage. Mice were then tethered with a preamplifier and allowed 3 days to acclimate to new cage and tether before recordings were started. EEG and EMG were recorded at a frequency of 200 Hz. Both EEG and EMG signals were amplified 5,000× and digitized at 14 bits before being sent to the recording software. Signal acquisition was performed using the Sirena acquisition suite by Pinnacle Technology, Inc (Lawrence, KS). Sleep state was determined visually on 4-sec epochs by a researcher blind to genotype based on frequency and amplitude of EEG and EMG waves using Harmonie (Natus, CA) as detailed before (Franken et al., 1998; El Helou et al., 2013). Behavioral state was either determined to be wakefulness (low-voltage, high frequency EEG with high-amplitude EMG), NREM sleep (high-voltage, low frequency EEG with low-amplitude EMG), or REM sleep (prominent theta activity in EEG channels and minimal EMG). Sleep recordings were analyzed for 8 WT and 8 DISC1 mice. Durations of behavioral states were average per hour. EEG spectral analysis was performed on artifact-free NREM sleep epochs for a unipolar EEG signal using Fast Fourier transform (FFT) to calculate activity in the delta frequency range (1–4 Hz). Delta activity time course was calculated using averages per equal intervals as done previously (El Helou et al., 2013), and expressed in percent of the 24-h baseline mean for each mouse. The same analyses were repeated for recovery day after sleep deprivation.

Sleep Deprivation

Two hours of gentle handling were used to study the effects of sleep deprivation in mice. In summary, mice were placed in a cage and continuously monitored by researcher. If animal assumed sleep posture, gentle tactile stimulus was administered to prevent sleep. The 2 hour sleep deprivation occurred from ZT0-ZT2 which corresponds to when the lights were turned on (ZT0) for two continuous hours (ZT2). At ZT2 animals were placed back into sleep monitoring chambers and EEG/ EMG recordings resumed for the remainder of the experiment. All control days occurred prior to the sleep deprivation day under a 12 h light: 12 h dark light cycle and the comparable circadian time was analyzed for differences between groups. Sleep deprivation recordings were analyzed for 6 WT and 5 DISC1 mice.

Behavioral testing

Behavioral testing was conducted according to the published protocols from our lab (Hikida et al., 2007; Jaaro-Peled et al., 2013; Johnson et al., 2013). The assays started at the age of three months. In order to minimize inter-trial interference the tests were performed with about a week between the tests and from less stressful to more stressful trials in the following order: open field test, spontaneous alternations in the Y-maze, prepulse inhibition of acoustic startle, and forced swim test. The above series of tests was performed on 9 WT and 8 DISC1 mice. Contextual fear conditioning was performed on a separate cohort of 8 WT and 10 DISC1 mice, following a published protocol (Vecsey et al., 2009).

Statistics

Statistical analysis for the sleep experiment was performed using Statistica (StatSoft, OK). Wheel counts and sleep measurements were compared using two-way ANOVA followed by Bonferroni post-hoc test or planned comparisons to examine changes over time, as well as, genotype. Circadian period in constant darkness and phase shift were compared using a student's t test. Significance levels were adjusted for repeated-measures using Huynh-Feldt correction. Statistical analysis for the behavioral studies was done with student t-test except for prepulse inhibition, which was analyzed by repeated measures ANOVA followed by Bonferroni post-hoc analysis.

RESULTS

Based on the hypothesis that a gain of DISC1 function may interfere with cellular cascades involving sleep regulation, we generated a transgenic mouse expressing FLAG-tagged wild-type (WT) human DISC1 under expression control of the α CaMKII promoter (Fig. 1A). As expected for α CaMKII-driven expression, transgenic DISC1 mRNA was expressed in the forebrain (olfactory bulbs, frontal cortex, hippocampus and striatum), but not in the cerebellum, as shown by reverse-transcriptase-PCR (Fig. 1B). The expression level of the transgenic protein is relatively low (lower than that of the endogenous Disc1) as shown by immunoprecipitation with anti-FLAG followed by immunoblot with antibodies against DISC1 (Fig. 1C).

To address possible disturbance of sleep regulation in the DISC1 model, we are interested in circadian activity and sleep homeostasis. More concretely, in the present study, we addressed

the influence on the circadian locomotor activity rhythm and sleep amount and intensity. First, in order to assess circadian activity, the mice were placed in cages with a running wheel and their activity was monitored (Fig. 2A). The DISC1 mice were photoentrained to a 12 h light: 12 h dark cycle on days 25–40 of the experiment, and showed normal activity onset, offset and behavior except for being less active during the dark (active phase of the day) in comparison to the WT mice ($p < 0.05$) (Fig. 2B). For the rest of the experiment the mice were housed in constant darkness to measure the intrinsic circadian behavior. The intrinsic circadian period was normal in DISC1 mice (Fig. 2C). On day 65 the mice were exposed to a light pulse to induce a phase shift. The phase shift was also normal in DISC1 mice (Fig. 2D).

We next addressed possible changes in sleep amount. At baseline, DISC1 mice spent more time awake and less time asleep than WT controls during both the light and the dark periods, as assessed by EEG and electromyography (EMG) recordings (Fig. 3A, **Baseline**). Longer duration of wakefulness was generally found in DISC1 mice, compared to WT controls, between Zeitgeber time 5 (ZT5) and ZT12 as well as between ZT17 and ZT23. Meanwhile, the duration of NREM sleep was shorter in DISC1 mice, compared with that in WT controls. REM sleep duration was also shorter in DISC1 than in WT mice between ZT2 and ZT9. The response of DISC1 mice to a short two hour sleep deprivation by gentle handling (starting at ZT0, when lights were turned on and the mice would normally go to sleep) did not differ from that of WT control mice (Fig. 3A, **Recovery**). We also examined sleep intensity by analyzing delta activity during NREM sleep. At baseline DISC1 mice tended to have a higher amplitude of day-night variations, but the difference did not reach significance (Fig. 3B, **Baseline**). During recovery from the two hour sleep deprivation, DISC1 mice tended to have lower delta activity than WT controls (Fig. 3B, **Recovery**).

Note that DISC1 mice did not show differences from WT mice in activity level as measured by total horizontal locomotion in a novel open field over 2 h (Fig. 4A, **left**) or number of arm entries during 5 min of spontaneous alternations in the Y-maze (Fig. 4B, **left**). DISC1 mice also did not seem to be more anxious than WT mice as assessed by % center activity during the first 5 minutes in a novel open field (Fig. 4A, **right**). DISC1 mice showed no deficits in spontaneous alternation in the Y-maze (Fig. 4B, **right**), startle response (Fig. 4C, **left**) prepulse inhibition (Fig. 4C, **right**), and the forced swim test (Fig. 4D). Finally, in the contextual fear conditioning paradigm, DISC1 mice froze normally in the trained context, indicating normal long term memory 24 h after training. DISC1 mice froze less than WT in the altered context resulting in higher context-specific freezing (Fig. 4E).

DISCUSSION

Here we report involvement of DISC1 in sleep regulation in mammals for the first time: we found that transgenic mice overexpressing wild-type DISC1 were awake longer than wild-type controls at baseline with a tendency for lower rebound of delta activity during recovery from a short sleep deprivation.

Although the DISC1 transgenic mice were awake more, they ran less on the wheel during the dark phase and did not differ from WT controls in locomotion activity in a novel open

field. DISC1 mice do not seem to have an altered stress response, as they behaved normally in a set of behavioral tests we employed, with the only detected behavioral change being less freezing in an altered context, suggesting better context discrimination.

Our results suggest that DISC1 affects signaling cascades associated with sleep regulation, especially those involved in wakefulness and arousal, such as the hypocretin and monoamine systems. DISC1 affects the dopamine system (Niwa et al., 2010; Jaaro-Peled et al. 2013; Su et al., 2014), which controls sleep/wake states via the D2 dopamine receptor (Dzirasa et al., 2006). Indeed, a rodent model similar to the one presented here - a rat model expressing the full length human DISC1, shows dopaminergic abnormalities (Trossbach et al., 2016). Given that nitric oxide, adenosine, and CREB have been underscored as molecules associated with sleep regulation, study with these molecules in this model may be a useful next step. We reported that modulation of DISC1 signaling affects redox signaling (Johnson et al. 2013). Thus, we may find an alteration of nitric oxide and related cascades in this model. DISC1 also interacts with cAMP-sensitive phosphodiesterase-4 (PDE4) and CREB family members (Millar et al., 2005; Sawamura et al. 2008; Soda et al., 2013), making it likely that DISC1 affects sleep by affecting cAMP signaling. Testing of a *Disc1* loss of function mouse model (Kuroda et al., 2011; Seshadri et al., 2015; Shahani et al., 2015; Tomoda et al. 2016) will further facilitate understanding the role of DISC1 in sleep regulation.

Acknowledgments

We thank Dr. Satoshi Kida for the pMM403 vector, Mr. Gatan Poirier for processing of EEG/EMG recordings, and Ms. Yukiko Lema for organizing the figures.

FUNDING

This work was supported by NARSAD Young Investigator Award to [H.J.-P]; National Institute of Health [MH-084018, MH-094268 Silvo O. Conte center, MH-069853, MH-085226, MH-088753, MH-092443, MH105660], as well as grants from Stanley, S-R, RUSK, NARSAD, MSCRF to [A.S.], salary awards from CIHR and FRQS to [V.M.], and R01-GM076430-09 to [S.H.].

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Highlights

- We generated transgenic mice expressing full-length DISC1 under the α CaMKII promoter
- DISC1 mice show normal circadian rhythm
- DISC1 mice show abnormal wake/sleep patterns

Figure 1. Generation of a transgenic model expressing full-length human DISC1 under control of the α CaMKII-promoter

(A) Transgene construct: cDNA of full-length human DISC1 was inserted into a modified pMM403 vector under the α CaMKII promoter and a FLAG tag.

(B) DISC1 transgene mRNA expression was assessed by RT-PCR. It was expressed in olfactory bulbs (OB), frontal cortex (FC), hippocampus (Hc), and basal ganglia (BG), but not in the cerebellum (Cb).

(C) DISC1 transgene protein expression was confirmed by immunoprecipitation with FLAG followed by immunoblot with DISC1 antibodies.

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Figure 2. The DISC1 mice show normal circadian activity

(A) Example actograms over 55 days of wheel running activity are shown for WT and DISC1 mice (x-axis: time in hours; y-axis: days). Days 25–40 animals were housed under a 12 h light: 12 h dark cycle. The remainder of the experiment was in constant darkness to measure the intrinsic circadian period of the animals and phase shift light pulses were given on day 65.

- (B)** The mean wheel counts during 12 h light: 12 h dark cycle indicates similar activity onset, offset and behavior, except lower activity of the DISC1 mice during dark phase (interaction $F_{143,1430}=3.18$, *** $p<0.0001$).
- (C)** Circadian period measured during constant darkness is not different between the groups.
- (D)** The response to a phase shift light pulse (day 65) also does not differ between groups.

Figure 3. DISC1 mice show abnormal wake/sleep patterns

(A) DISC1 mice show abnormal distribution of wakefulness and sleep. (**left panels, Baseline**) The hourly distribution of wakefulness, NREM sleep and REM sleep during baseline was significantly affected in DISC1 mice (interaction $F_{23,322} = 1.9$, $p = 0.02$). Significant genotype differences ($p < 0.05$) at specific hours are highlighted by red symbols. (**right panels, Recovery**) Following 2 h of sleep deprivation (SD, indicated by black rectangles), DISC1 mice no longer significantly differ from WT mice in the distribution or amount of wakefulness and NREM sleep (interaction $F_{23,207} < 0.7$, $p > 0.6$). DISC1 mice

show a tendency for decreased REM sleep during the light period compared to WT (interaction $F_{23,207} = 1.7$, $p = 0.07$), similar to baseline. White background indicates light periods, and grey background indicates dark periods.

(B) DISC1 mice show nonsignificant changes in the time course of delta activity during NREM sleep. **(left panel)** The time course of delta activity during baseline showed a tendency to be affected by genotype (interaction $F_{17,221} = 1.9$, $p = 0.08$), with DISC1 mice tending to have a higher amplitude of day-night variations. White background indicates the light period, and grey background the dark period. **(right panel)** The time course of delta activity during recovery after 2 h of sleep deprivation (SD, black rectangle) did not differ between genotypes (interaction $F_{10,80} = 1.2$, $p = 0.3$), but DISC1 mice tended to show a decreased overall delta level compared to WT during recovery (genotype effect $F_{1,8} = 3.7$, $p = 0.09$).

Figure 4. Behavioral characterization of the DISC1 mice

(A) DISC1 mice did not differ from WT littermates in locomotion over 2 h (left, $p=0.16$) and % center activity during the first 5 min (right, $p=0.56$) in a novel open field.

(B) DISC1 mice did not differ from WT mice in activity (left, $p=0.45$) or short term memory (right, $p=0.11$) as assessed by spontaneous alternations in the Y-maze.

(C) DISC1 mice showed normal startle response (left, $p=0.30$) and prepulse inhibition [right, $F(1,10)=0.70$, $p=0.42$].

(D) DISC1 mice did not differ from WT mice in the % immobility during the 4 last minutes of the forced swim test ($p=0.91$).

(E) Although DISC1 mice froze normally in the trained context after fear conditioning, they froze less in an altered context ($p<0.05$) resulting in higher context specific freezing ($p<0.001$).

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