Neuroscience Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

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

Hanna Jaaro-Peled[a,*], Cara Altimus[b], Tara LeGates[b], Tyler Cash-Padgett[a], Sandra Zoubovsky[a], Takatoshi Hikida[a], Koko Ishizuka[a], Samer Hattar[b,**], Valérie Mongrain[c], Akira Sawa[a,***]

[a] Department of Psychiatry, Johns Hopkins University School of Medicine, United States
[b] Department of Biology, Johns Hopkins University, United States
[c] Department of Neurosciences, Université de Montréal, Canada

ARTICLE INFO

Article history:
Received 19 November 2014
Received in revised form 16 June 2016
Accepted 20 June 2016
Available online xxx

Keywords:
Non rapid eye movement sleep
Rapid eye movement sleep
Sleep homeostasis
Circadian rhythms
DISC1
Mouse model
Behavior

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.

1. 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 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

* Corresponding author. Tel.: +1 410 955 4726.
** Corresponding author. Tel.: +1 443 287 1868.
*** Corresponding author. Tel.: +1 410 516 4231.
E-mail addresses: hjaarop1@jhmi.edu (H. Jaaro-Peled), shattar@jhu.edu (S. Hattar), asawa1@jhmi.edu (A. Sawa).

http://dx.doi.org/10.1016/j.neures.2016.06.006
0168-0102/

is not yet perfectly understood. We have reported that transgenic Drosophila expressing wild-type (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.

2. Materials and methods

2.1. Mice

The procedures used were in accordance with EU Directive 2010/63/EU for animal experiments. Full length human DISC1 was inserted under αCaMKII 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 × C57BL/6N matings and adult male heterozygous and WT littermate mice were compared in all the experiments.

2.2. 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.

2.3. 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 (1000 lux; CT16) for 15 min.

2.4. 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-h light:12-h 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 5,000x 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-s 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.

2.5. 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

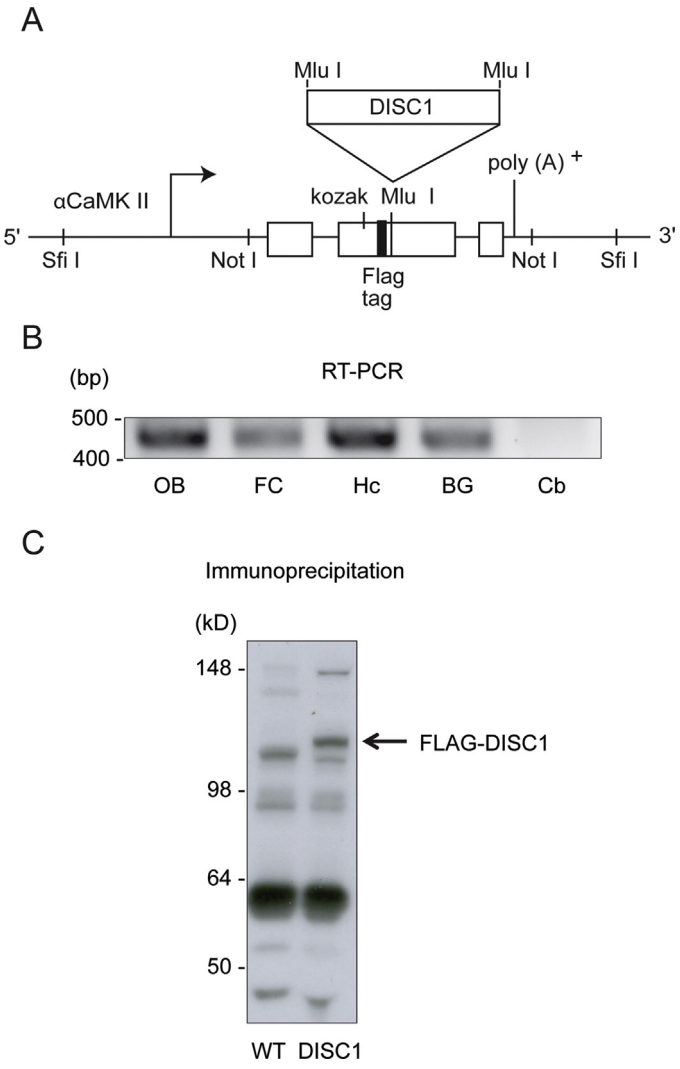

Fig. 1. Generation of a transgenic model expressing full-length human DISC1 under control of the αCaMKII-promoter. (A) Transgene construct: cDNA of full-length human DISC1 was inserted into a modified pMM403 vector under the αCaMKII promoter and a FLAG tag. (B) DISC1 transgene mRNA expression was assessed 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.

Please cite this article in press as: Jaaro-Peled, H., et al., Abnormal wake/sleep pattern in a novel gain-of-function model of DISC1. Neurosci. Res. (2016), http://dx.doi.org/10.1016/j.neures.2016.06.006