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Disturbed sleep/wake rhythms and neuronal cell loss in lateral hypothalamus and retina of mice with a spontaneous deletion in the ubiquitin carboxyl-terminal hydrolase L1 gene

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Abstract

Many neurodegenerative disorders including Parkinson's disease (PD) and Alzheimer's disease (AD) are associated with sleep disturbances with presumably multifactorial etiology. Ubiquitin C-terminal hydrolase L1 (UCH-L1) is involved in the pathophysiology of PD and AD. In the present study, we analyzed locomotor rhythms, orexin A-immunoreaction (Ir) in the lateral hypothalamus (LH) and melanopsin-Ir in the retina of gracile axonal dystrophy (*gad*) mice with a spontaneous deletion in the *Uch-l1* gene. In constant darkness, *gad* mice showed circadian rhythms in locomotor activity, indicating the integrity of the endogenous circadian rhythm generator. However, *gad* mice showed an increased activity during subjective day and a decreased number of orexin A-immunoreactive neurons in the LH compared with the wild type (WT). In addition, *gad* mice showed increased locomotor activity in the light period when kept in a standard photoperiod and entrainment to phase shifts was significantly slower than in WT. Moreover, melanopsin-Ir was significantly reduced in the retina of *gad* mice, suggesting an impairment of circadian light perception in *gad* mice.

Keywords: Alzheimer's disease; Circadian rhythms; Clock genes; *gad*; Hypocretin; Locomotor activity; Melanopsin; Neurodegenerative disorders; Orexin; Parkinson's disease; UCH-L1

Many neurodegenerative disorders including Parkinson's disease (PD) and Alzheimer's disease (AD) are associated with sleep disturbances (Dauvilliers, 2007). Sleep disturbances represent a major impairment of life quality for patients with neurodegenerative diseases and a physical and psychological burden for caregivers, which is often the reason for institutionalizing a patient (Hope et al., 1998). As many as 98% of patients with PD (Dhawan et al., 2006) suffer from sleep disturbances including excessive daytime sleepiness (EDS), nocturnal restlessness (Dhawan et al., 2006), sleep fragmentation (Factor et al., 1990), delayed sleep latency, and sleep maintenance-insomnia (Chaudhuri, 2003). Sleep disturbances such as poor nocturnal sleep and delayed sleep latency typically precede the motor symptoms and intensify with progression of PD (Abbott et al., 2005; Dhawan et al., 2006; Hobson et al., 2002). In addition, depression is often associated with PD and also affects sleep quality (Chaudhuri, 2003). Sleep disturbances affect up to 40% of patients with AD (Vitiello and Borson, 2001) and commonly manifest as severe night time sleep fragmentation and frequent daytime napping (Dauvilliers, 2007).

However, the pathophysiology of sleep disturbance in PD and AD is multifactorial and largely unknown (Braak et al., 2003; Dauvilliers, 2007; Dhawan et al., 2006). At the molecular level the deubiquitinating enzyme ubiquitin C-terminal hydrolase L1 (UCH-L1) which plays an important role in the labeling of abnormal proteins in the ubiquitin-

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proteasomal pathway and stabilizes monoubiquitin (Osaka et al., 2003) is downregulated in idiopathic AD and PD (Choi et al., 2004). Single nucleotide polymorphisms in the *UCH-L1* gene, which is also known as *PARK5*, are associated with sporadic AD (Xue and Jia, 2006) and PD (Leroy et al., 1998; Maraganore et al., 1999). Furthermore, overexpression of UCH-L1 rescues amyloid β-protein-induced decreases in synaptic plasticity and contextual memory in mice (Gong et al., 2006). Mice with a spontaneous deletion in the *Uch-l1* gene known as gracile axonal dystrophy (*gad*) mice exhibit severe sensory ataxia at an early stage, followed by motor paresis at a later stage (Kikuchi et al., 1990; Yamazaki et al., 1988). However, little is known about the role of UCH-L1 in brain in the control of sleep-wake cycle.

The sleep wake cycle is generated by a switch between sleep promoting and arousing brain regions under the control of the endogenous rhythm generator in the suprachiasmatic nucleus (SCN) (Saper et al., 2005a; Saper et al., 2005b). The molecular clockwork within the SCN consists of transcriptional/translational feedback loops of clock genes encoding for transcriptional regulators such as the basic-helix-loop-helix transcription factor brain and muscle ARNT-like protein 1 (BMAL1). The SCN receives light information from the eye through the retino-hypothalamic tract. Specialized ganglion cells projecting to the SCN respond directly to light and contain the photopigment melanopsin (Berson et al., 2002; Hattar et al., 2002). The sleep promoting region is located in the ventrolateral preoptic nucleus (VLPO) and the arousal system consists of different cell groups in the brainstem known as the ascending reticular activating system (Saper et al., 2005a; Saper et al., 2005b). Orexin A (hypocretin) -neurons in the lateral hypothalamus (LH) stabilize the switch and promote wakefulness (Saper et al., 2005a; Saper et al., 2005b) and loss of these neurons results in narcolepsy (Nishino et al., 2000). The relationship between the retina, the SCN, and the LH has been summarized in Fig. 1. Orexin A has a complex association with the dopaminergic system in the basal ganglia (Rye and Jankovic, 2002). An increasing loss of orexin A cells with progression of PD has been described (Fronczek et al., 2007; Thannickal et al., 2007; Thannickal et al., 2008).

In the present study we investigated the role of the UCH-L 1 enzyme in locomotor activity rhythm and in brain regions involved in the control of sleep-wake cycle using UCH-L 1-deficient *gad* mice, which is a spontaneous mutant with an in-frame deletion in exons 7 and 8 of the *Uch-l1* gene (Saigoh et al., 1999). We found that *gad* mice show higher subjective daytime activity and impaired entrainment to phase shifts of the photoperiod. At the cellular level, the number of orexin A-immunoreactive cells in the LH and the density of melanopsin-immunoreaction in the retina were significantly reduced compared with wild type littermates. In summary, our data show a destabilized circadian rhythm in locomotor activity and an impairment of circadian light perception in *gad* mice.

Fig. 1. Diagram to illustrate the relationship of the components of the circadian system analyzed in this study. The melanopsin-containing light responsive retinal ganglion cells are responsible for circadian light reception and project to the endogenous rhythm generator within the suprachiasmatic nucleus (SCN). Output from the SCN reaches the lateral hypothalamus (LH) via a multisynaptic pathway. Orexin A derived from the LH plays an important role in the stabilization of the sleep wake cycle (Adapted after Berson et al., 2002 and Saper et al., 2005b).

1. Methods

1.1. Animals

All experiments reported here were conducted in accordance with accepted standards of humane animal care and were consistent with Federal Guidelines and The European Communities Council Directive (89/609/EEC). Adult homozygous *gad* and wild-type (WT) mice were obtained by mating heterozygous males with heterozygous females. Genotypes were determined by PCR amplification of genomic DNA as previously described (Sakurai et al., 2008). Each experimental group consisted of six male mice of the same phenotype (n = 6). Analysis of locomotor activity was conducted with 16–25 week-old mice. Analysis of orexin A, melanopsin, and BMAL1 immunohistochemistry was conducted with younger mice at 8–14 weeks (without motor symptoms) and older mice at 16–25 weeks (with obvious motor symptoms) after birth (Yamazaki et al., 1992).

1.2. Analysis of locomotor activity rhythms

Mice were housed in individual cages equipped with infrared detectors (Mouse-e-motion, Hamburg, Germany) at constant room temperature and with food and water available *ad libitum*. Locomotor activity was recorded continuously in 6 min intervals. Acto grams, activity profiles, activity onset, and chi-square periodograms were calculated using Clocklab software (Actimetrics, Wilmette, IL). Activity profiles and chi-square periodograms were based on the data of 12 consecutive days. Automated onset-time and offset-time calculation provided by Clocklab software was used to determine the onset and offset of activity in each individual animal, respectively. Mice were entrained to a standard photoperiod of 12 h light (150 lux): 12 h darkness (dim red light > 680 nm, 2 lux) (LD) for at least 1 week before the experiments. Activity profiles plotting the average activity as a function of time were used to analyze subjective daytime activity in the interval between 2 h after activity offset and 2 h before activity onset (Experiment 1) or daytime activity in the interval between 2 h after lights on and 2 h before lights off (Experiment 2). The (subjective) daytime activity was expressed in percent of the daily (24 h) activity. Initial experiments revealed significant disturbances in circadian locomotor activity rhythms only in older mice (16–25 weeks after birth with obvious motor symptoms) but not in younger (8–14 weeks after birth without