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Research paper

Hypocretinergic and cholinergic contributions to sleep-wake disturbances in a mouse model of traumatic brain injury

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ABSTRACT

Disorders of sleep and wakefulness occur in the majority of individuals who have experienced traumatic brain injury (TBI), with increased sleep need and excessive daytime sleepiness often reported. Behavioral and pharmacological therapies have limited efficacy, in part, because the etiology of post-TBI sleep disturbances is not well understood. Severity of injuries resulting from head trauma in humans is highly variable, and as a consequence so are their sequelae. Here, we use a controlled laboratory model to investigate the effects of TBI on sleep-wake behavior and on candidate neurotransmitter systems as potential mediators. We focus on hypocretin and melanin-concentrating hormone (MCH), hypothalamic neuropeptides important for regulating sleep and wakefulness, and two potential downstream effectors of hypocretin actions, histamine and acetylcholine. Adult male C57BL/6 mice (n=6–10/group) were implanted with EEG recording electrodes and baseline recordings were obtained. After baseline recordings, controlled cortical impact was used to induce mild or moderate TBI. EEG recordings were obtained from the same animals at 7 and 15 days post-surgery. Separate groups of animals (n=6–8/group) were used to determine effects of TBI on the numbers of hypocretin and MCH-producing neurons in the hypothalamus, histaminergic neurons in the tuberomammillary nucleus, and cholinergic neurons in the basal forebrain. At 15 days post-TBI, wakefulness was decreased and NREM sleep was increased during the dark period in moderately injured animals. There were no differences between groups in REM sleep time, nor were there differences between groups in sleep during the light period. TBI effects on hypocretin and cholinergic neurons were such that more severe injury resulted in fewer cells. Numbers of MCH neurons and histaminergic neurons were not altered under the conditions of this study. Thus, we conclude that moderate TBI in mice reduces wakefulness and increases NREM sleep during the dark period, effects that may be mediated by hypocretin-producing neurons and/or downstream cholinergic effectors in the basal forebrain.

1. Introduction

Traumatic brain injury (TBI) is a major public health problem that is considered a silent epidemic because of the long-term cognitive deficits and behavioral and medical complications experienced by survivors. In the United States alone, more than 5.3 million individuals currently suffer from a TBI-related disability (Chew and Zafonte, 2009). The neuropsychiatric consequences of TBI may include sleep disorders, mood disorders, personality changes, and cognitive impairment (Bhalerao et al., 2013). Chronic sleep-wake disturbance is highly prevalent, affecting the majority of individuals who have sustained a TBI (Kempf et al., 2010; Rao and Rollings, 2002). Many TBI patients report regular daytime napping and increased sleep need (Ponsford et al., 2013; Ponsford and Sinclair, 2014), and excessive daytime sleepiness (EDS) occurs in approximately 25–42% of individuals who have suffered TBI (Ponsford and Sinclair, 2014; Sommerauer et al., 2013). The alterations in sleep-wake behavior after TBI may be prolonged, and evident for years after the trauma (Kempf et al., 2010). Despite the debilitating effects of post-TBI sleep-wake disturbances, their etiology is not well understood. Furthermore, current behavioral and pharmacological therapies targeting post-TBI sleep-wake disturbances have only limited efficacy (Chew and Zafonte, 2009; Ouellet et al., 2015; Ponsford and Sinclair, 2014; Rue-Evans et al., 2013; Sheng et al., 2013).

Post-TBI sleep-wake disturbances may be due, in part, to altered neurotransmitter systems that regulate sleep and wakefulness.

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Neurotransmitter systems implicated in arousal include, among others, hypocretin (a.k.a. orexin) neurons of the lateral hypothalamus (Adamantidis et al., 2007; Brisbare-Roch et al., 2007), cholinergic neurons of the basal forebrain (Arrigoni et al., 2010; Irmak and de Lecea, 2014), and histaminergic neurons in the tuberomammillary nucleus (TMN) (Brown et al., 2001; Brown et al., 2012; Parmentier et al., 2002). Of importance to the etiology of post-TBI disturbances, hypocretin promotes wakefulness and stabilizes the sleep-wake cycle (Kilduff and Peyron, 2000; Krystal et al., 2013; Mochizuki et al., 2004; Taheri et al., 2002; Zeitzer et al., 2006). Activation of hypocretin neurons increases transitions from sleep to wakefulness (Adamantidis et al., 2007) and antagonizing hypocretin induces somnolence (Brisbare-Roch et al., 2007; Hoever et al., 2012; Morairty et al., 2014).

In contrast, melanin-concentrating hormone (MCH) neurons are sleep-promoting (Peyron et al., 2009): intracerebroventricular injection of MCH (Verret et al., 2003) or optogenetic stimulation of MCH neurons increases NREM sleep and REM sleep (Jego et al., 2013; Konadhode et al., 2013), whereas MCH deficient mice sleep less (Tsunematsu et al., 2014; Willie et al., 2008). MCH neurons are intermingled with hypocretin neurons in the lateral hypothalamus, and as such, damage to the hypocretin and/or MCH neurons of the lateral hypothalamus could alter sleep-wake behavior after TBI. Indeed, hypocretin is reduced in the hypothalamus of mice (Willie et al., 2012) and in cerebrospinal fluid of human patients (Baumann et al., 2005) after TBI. Furthermore, the number of hypocretin neurons are reduced in post-mortem brains of patients who died from TBI (Baumann et al., 2009). In cases of fatal TBI in humans, one study found a significant reduction in MCH neurons (Valko et al., 2015), whereas another found that MCH neurons were not affected (Baumann et al., 2009). To our knowledge, numbers of hypocretin or MCH neurons have not been investigated in cases of nonfatal TBI in humans.

Although post-TBI alterations in sleep may be mediated, in part, by direct actions of hypocretin, these changes in arousal state could also be due to actions of modulatory systems downstream of hypocretin. Hypocretinergic neurons project to many brain regions. For example, the histaminergic neurons of the TMN and the cholinergic neurons of the basal forebrain are both strong promoters of wakefulness (Haas et al., 2008; Han et al., 2014), and these brain regions are densely innervated by hypocretinergic projections [reviewed in Arrigoni et al. (2010), Sundvik and Panula (2015)]. Importantly, these systems may also be perturbed by TBI. Fatal TBI in humans causes a dramatic reduction in numbers of histaminergic neurons (Valko et al., 2015) and a reduction in activity and immunoreactivity of choline acetyltransferase (ChAT), an enzyme essential for acetylcholine synthesis (Dewar and Graham, 1996; Murdoch et al., 1998, 2002). To our knowledge, histaminergic and cholinergic neuronal populations have not been studied within the context of sleep-wake disturbance after experimental TBI.

The primary goal of the present study was to determine the effects of TBI on sleep-wake behavior and hypocretin/MCH cell numbers and their downstream targets in mice. We used the controlled cortical impact (CCI) model to induce mild or moderate TBI and determined the time course of effects on these and other outcome measures. We report that sleep is altered and hypocretin and basal forebrain cholinergic cell numbers are reduced in an injury severity-dependent manner. Cell counts for MCH and histamine neurons were not altered by TBI under the conditions of this study. Collectively, these data suggest that the effects of TBI on sleep may be mediated by hypocretinergic and cholinergic mechanisms.

2. Methods

2.1. Animals

Adult male C57BL/6J mice (~3–4 months old at time of use; Jackson Laboratory, Bar Harbor, ME) were group housed until baseline testing or surgery, after which they were single housed. Mice were housed under a 12:12 light:dark cycle at 29 ± 1 °C with food and water provided ad libitum. All procedures involving the use of animals were approved by the University of Washington IACUC in accordance with the US Department of Agriculture Animal Welfare Act and the National Institutes of Health policy on Humane Care and Use of Laboratory Animals.

2.2. Recording apparatus

Sleep-wake behavior of mice was determined based on the electroencephalogram (EEG) and cage activity patterns. EEG signals were amplified, filtered, and recorded for offline processing using custom software written in LabView for Windows (ICELUS, M. Opp, University of Washington; National Instruments, Austin, TX) as previously described (Baracchi and Opp, 2008; Ingiosi et al., 2015). EEG and cage activity records were visually scored in 10-s epochs. Raw EEG signals were subjected to fast Fourier transformation, yielding power spectra between 0.5 and 30 Hz in 0.5-Hz frequency bins. Arousal states were determined as previously described and classified as non-rapid eye movement (NREM) sleep, rapid eye movement (REM) sleep, or wakefulness (WAKE) based upon published criteria [e.g., Baracchi and Opp, 2008; Ingiosi et al., 2015; Sutton and Opp, 2014].

2.3. Experimental design

A schematic of the protocols used in Experiments 1–3 is presented in Fig. 1.

2.3.1. Experiment 1: effects of TBI on mouse sleep-wake behavior

For Experiment 1, EEG electrodes were surgically implanted into the skull under isoflurane anesthesia. The leads from the screw electrodes were soldered to the pins of a plastic connector (Digi-Key, ED85100-ND) to allow coupling to the recording system. Dental acrylic (Integrity Caulk, Dentsply) covered the electrodes and formed a headpiece to which the flexible recording tether could be connected. The section of the skull over the left parietal cortex was not covered with dental acrylic at this time. The incision was closed with sutures, and a subcutaneous injection of an analgesic (0.5 mg/kg buprenorphine) was given at the end of the surgery. Mice were allowed 7 days to recover before they were attached to a flexible tether for habituation to the recording system. After 3 days of habituation to the tether and recording environment, 48-h undisturbed baseline recordings were obtained.

After the 48-h baseline recordings, mice were randomized to sham surgeries ($n=7$; control mice), or to controlled cortical impact (CCI; $n=16$) to induce TBI as previously described (Febinger et al., 2015). In both groups, a 5-mm diameter craniotomy using a trephine was made over the left parietal cortex, approximately -2 mm relative to bregma and 2.5 mm lateral to the midline. A unilateral impact between lambda and bregma is routinely used in protocols using CCI to induce TBI (Boulet et al., 2013; Boychuk et al., 2016; Febinger et al., 2015; Miller et al., 2014). The skull fragment was removed without disrupting the underlying dura, and TBI was induced in the experimental group. Mice in the experimental group were subjected to CCI using the Leica Impact One system (Richmond, IL) equipped with an electrically-driven 3-mm diameter metal piston controlled by a linear velocity displacement transducer. CCI parameters were: 5.0 m/s

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