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Cytotoxic chemotherapy increases sleep and sleep fragmentation in non-tumor-bearing mice

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ABSTRACT

Sleep disruption ranks among the most common complaints of breast cancer patients undergoing chemotherapy. Because of the complex interactions among cancer, treatment regimens, and life-history traits, studies to establish a causal link between chemotherapy and sleep disruption are uncommon. To investigate how chemotherapy acutely influences sleep, adult female c57bl/6 mice were ovariectomized and implanted with wireless biotelemetry units. EEG/EMG biopotentials were collected over the course of 3 days pre- and post-injection of 13.5 mg/kg doxorubicin and 135 mg/kg cyclophosphamide or the vehicle. We predicted that cyclophosphamide + doxorubicin would disrupt sleep and increase central proinflammatory cytokine expression in brain areas that govern vigilance states (i.e., hypothalamus and brainstem). The results largely support these predictions; a single chemotherapy injection increased NREM and REM sleep during subsequent active (dark) phases; this induced sleep was fragmented and of low quality. Mice displayed marked increases in low theta (5–7 Hz) to high theta (7–10 Hz) ratios following chemotherapy treatment, indicating elevated sleep propensity. The effect was strongest during the first dark phase following injection, but mice displayed disrupted sleep for the entire 3-day duration of post-injection sleep recording. Vigilance state timing was not influenced by treatment, suggesting that acute chemotherapy administration alters sleep homeostasis without altering sleep timing. qPCR analysis revealed that disrupted sleep was accompanied by increased IL-6 mRNA expression in the hypothalamus. Together, these data implicate neuroinflammation as a potential contributor to sleep disruption after chemotherapy.

1. Introduction

Substantial adverse effects are commonly reported following the use of chemotherapy for the treatment of cancer (Hassett et al., 2006; Lindley et al., 1999; Binkley et al., 2012). Among breast cancer patients, cognitive impairments, fatigue, and sleep problems rank among the most commonly reported side effects influencing quality of life (Vardy, 2009; Azim et al., 2011; Cella et al., 2001; Wood et al., 2006; Jereczek-Fossa et al., 2002). Estimates suggest that 75–96% of cancer patients suffer from chemotherapy-induced fatigue (Vardy, 2009; Stone et al., 1998, 2000, 2003; Knobf, 1986; Yellen et al., 1997), and fatigue and sleep disturbances are two of the most commonly recognized symptoms associated with chemotherapy (Downie et al., 2006; Ashbury et al., 1998; Bower et al., 2000; Byar et al., 2007; Liu et al., 2012). Sleep and fatigue, though related, have unique behavioral and physiological traits; disrupted sleep may contribute to fatigue or may occur independently in response to chemotherapy (Ancoli-Israel, 2001). Sleep is objectively assessed through actigraphy, which provides information regarding total sleep time and wakefulness after sleep onset, or polysomnography, which can provide additional information about sleep stages. In contrast, fatigue is a subjective measure that involves a sense of generalized tiredness, altered executive functioning, and limb heaviness. Due to mainly logistical reasons, studies dissociating fatigue, daytime sleepiness, and sleep disruption in chemotherapy patients have been sparse, and even less is known about the effects of chemotherapy on sleep architecture.

Despite the high prevalence of sleep problems associated with chemotherapy, the mechanism by which chemotherapeutics alter vigilance states is not known. One possible explanation is that chemotherapy-induced inflammation promotes sleep. Indeed, many chemotherapeutic regimens initiate or exacerbate inflammation (Sauter et al., 2011; van der Most et al., 2008; Mills et al., 2005; Tsavaris et al., 2002; Pusztai et al., 2004; Penson et al., 2000), and activation of p38 MAPK signaling by chemotherapeutics is hypothesized to lead to downstream cytokine production and

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chemotherapy related side effects (Elsea et al., 2008; Wood et al., 2006). Specifically, serum interleukin-6 (IL-6), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1) concentrations are elevated in patients following treatment with doxorubicin and cyclophosphamide (Janelsins et al., 2012), a common chemotherapy regimen for breast cancer. There are several established mechanisms through which the peripheral immune system can influence the brain and subsequent behavior, including via the direct transport of cytokines into the brain or communication of immune status to the brain via the vagus nerve (Wilson et al., 2002; Kelley et al., 2003; Ahles and Saykin, 2007). Many proinflammatory cytokines increase sleep and lethargy following peripheral immune activation (Krueger et al., 2001, 2011; Opp, 2005). Previous studies have demonstrated that mice receiving doxorubicin, cyclophosphamide, and 5-fluorouracil have increased central inflammation and display a 'fatigue' phenotype (reduced voluntary wheel running behavior) that progressively worsens with each chemotherapy treatment (Weymann et al., 2014; Smith et al., 2014). Because increased cytokine expression can exert direct effects on sleep, in addition to fatigue, we assessed the acute effects of chemotherapy on sleep and sleep propensity in the present study. We hypothesized that chemotherapy alters sleep parameters and central cytokine signaling. Because cancer patients frequently report sleep disruption, we predicted that mice receiving chemotherapy would display increased sleep that is of poor quality in conjunction with hypothalamic inflammation.

2. Materials and methods

2.1. Animals

Adult (>8 weeks) female C57BL/6 mice were used in the present study. Upon arrival to our laboratory, mice were group housed and allowed to acclimate to a 14:10 light/dark schedule (Lights on: 0400 (ZT 0), Lights off: 1800 (ZT 14)). After one week of acclimation, mice were ovariectomized and implanted with wireless telemeters (described below), and then individually housed in polypropylene cages ($27.8 \times 7.5 \times 13$ cm) in sound-attenuating ventilated cabinets maintained at 22 ± 2 °C for the duration of the study. Body mass was measured immediately prior to transmitter implantation, one day prior to injection, and at the conclusion of the study. Post-surgery body masses were adjusted to account for the added weight of the transmitter. Food, (Harlan–Teklad No. 8640; Harlan Laboratories, Indianapolis, IN, USA), tap water, and cotton nesting material were supplied *ad libitum*. This study was conducted under approval of The Ohio State University Institutional Animal Care Committee and procedures followed the National Institutes of Health *Guide for the Use and Care of Laboratory Animals* and international ethical standards (Portaluppi et al., 2010).

2.2. Ovariectomy and telemeter implantation

Following acclimation, mice were deeply anesthetized with isoflurane vapors (3% induction, 1.5% maintenance) and ovariectomized and implanted with PhysioTel F20-EET biotelemetry transmitters (Data Sciences International [DSI], St. Paul, MN, USA) as described previously (Borniger et al., 2013; Ashley et al., 2012) and according to manufacturer instructions (DSI EET Device Surgery Manual). Briefly, following immobilization in a stereotaxic apparatus, a midline incision from the posterior margin of the eyes to midway between the scapulae was made. The skull was exposed and cleaned, and two stainless steel screws (00−96 × 1/16; Plastics One, Roanoke VA, USA) that would serve as cortical electrodes were inserted through the skull to make contact with the dura mater. One screw was placed 1 mm lateral to the sagittal suture and 1 mm anterior to bregma while the second screw was placed contralaterally 2 mm from the sagittal suture and 2 mm posterior to Bregma. The transmitter itself was inserted into a subcutaneous pocket along the back with biopotential leads oriented cranially. A set of leads was attached to the screws and secured with dental cement, while another set was inserted into the cervical trapezius muscles for EMG measurement. The surgical procedures were performed by an experienced surgeon using aseptic technique; buprenorphine (0.05 mg/kg, SC) was administered to provide analgesia and supplemental warmth was provided until the mice were mobile following emergence from anesthesia. Baseline sleep recordings began one week after transmitter implantation.

Following surgical implantation of the transmitter, each mouse cage was placed on top of receiver boards (RPC-1; DSI) in their original ventilated cabinets. These boards relay telemetered data to a data exchange matrix (DSI) and a computer running Dataquest A.R.T. software (version 4.1; DSI, St. Paul, MN, USA).

2.3. Doxorubicin and cyclophosphamide administration

Following baseline recording, mice were individually brought into an adjacent procedure room, positioned in conical restraint tubes, and injected intravenously (tail vein) with a cocktail of 13.5 mg/kg doxorubicin and 135 mg/kg cyclophosphamide in saline (hereafter referred to as 'chemo') or saline vehicle. This adjuvant drug cocktail is commonly used for the treatment of breast cancer. Chemotherapeutic dosing was derived from 75% of the human equivalent dose using a body surface area equation (Reagan-Shaw et al., 2007). A chemical hygiene plan, approved by OSU Environmental Health and Safety, was implemented to minimize the risk of human exposure to the chemotherapy drugs and their metabolites.

2.4. Experimental time-course

Baseline EEG and EMG signals were recorded for 3 days prior to chemotherapy administration. At ZT 7.5 on the third day of baseline recording, mice were individually brought into an adjacent procedure room, restrained in conical tubes, and administered a cocktail of intravenous (tail vein) doxorubicin/cyclophosphamide. Immediately following administration, mice were monitored for signs of distress, then returned to their home cage and placed back onto receiver boards. All mice received injections within a 20 min window, and biopotentials were subsequently recorded over the course of the following three days.

Approximately 72 h after the chemo or vehicle injections, mice were again individually brought into the adjacent procedure room, deeply anesthetized under isoflurane anesthesia, and rapidly decapitated. Trunk blood was collected and the brain was removed and placed on ice (and then at 4 °C) in RNA-Later (Qiagen) until specific regions were dissected 1 week later. The entire hypothalamus and brainstem were dissected and placed in −80 °C until RNA extraction.

2.5. Quantitative polymerase chain reaction (qPCR)

RNA was extracted from brain section homogenates in TriZol reagent (Life Technologies, Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. RNA pellets were resuspended in 30 μL sterile RNase-free water and quality and quantity were determined using a spectrophotometer (NanoDrop, Thermo Fischer Scientific Inc.). All RNA yields were above 200 ng/μL and had 260/280 and 260/230 ratios between 1.8 and 2.3. cDNA was synthesized using M-MLV reverse transcription and diluted 1:20 for subsequent PCR. Inventoried primer/probe pairs from Applied

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