Neuroscience 297 (2015) 211–218

CORTICAL NEURONAL ACTIVITY DOES NOT REGULATE SLEEP HOMEOSTASIS

M.-H. QIU,[a,b*] M. C. CHEN[b] AND J. LU[b*]

[a] *State Key Laboratory of Medical Neurobiology and Department of Neurobiology, School of Basic Medical Science, Fudan University, Shanghai 200032, China*

[b] *Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston 02115, USA*

Abstract—The neural substrate of sleep homeostasis is unclear, but both cortical and subcortical structures are thought to be involved in sleep regulation. To test whether prior neuronal activity in the cortex or in subcortical regions drives sleep rebound, we systemically administered atropine (100 mg/kg) to rats, producing a dissociated state with slow-wave cortical electroencephalogram (EEG) but waking behavior (e.g. locomotion). Atropine injections during the light period produced 6 h of slow-wave cortical EEG but also subcortical arousal. Afterward, rats showed a significant increase in non-rapid eye movement (NREM) sleep, compared to the same period on a baseline day. Consistent with the behavioral and cortical EEG state produced by systemic atropine, c-Fos expression was low in the cortex but high in multiple subcortical arousal systems. These data suggest that subcortical arousal and behavior are sufficient to drive sleep homeostasis, while a sleep-like pattern of cortical activity is not sufficient to satisfy sleep homeostasis.

Key words: cortex, arousal, atropine, c-Fos, homeostasis.

INTRODUCTION

Sleep homeostasis is a fundamental mechanism that regulates and maintains the quantity and quality of sleep. Extended wakefulness increases homeostatic sleep drive, or sleep pressure. This homeostatic drive manifests as an increase, or rebound, in the amount of sleep, as well as an increase in the delta power (0.5–4 Hz) of the cortical electroencephalogram (EEG). Delta EEG, along with total sleep time, is hypothesized to be the primary biomarker of the sleep homeostatic drive. Supporting this, recent studies have shown that local cortical activity during wakefulness increases cortical slow wave activity (SWA) during the subsequent non-rapid eye movement (NREM) sleep period (Vyazovskiy et al., 2011). However, others have argued that delta power is an epiphenomenon of sleep homeostasis (Davis et al., 2011). Complete midbrain transection in rat pups and adult cats show that the forebrain is necessary for sleep homeostasis (de Andres et al., 2003; Villablanca, 2004; Todd et al., 2010). Specifically, these and other studies (Gvilia et al., 2006a,6b, 2011) speculate that the hypothalamus may be the key neuronal substrate for sleep homeostasis. SWA is clearly a marker for local sleep homeostasis and may drive a local cortical homeostatic response, but it is unclear if SWA *per se* is sufficient to satisfy the global sleep homeostatic drive.

Systemic atropine administration in rodents slows the cortical EEG and reduces expression of the neuronal activity marker c-Fos throughout the cortex, but during this state rodents exhibit normal behavior (eating, drinking, grooming and walking) and a wake-like increase in electromyographic (EMG) activity (Vanderwolf, 1988, 1992; Qiu et al., 2014).Consistent with the animal's behavioral state, atropine induces c-Fos in arousal systems such as the tuberomammillary nucleus (TMN) and locus coeruleus (LC) but not in the sleep systems such as the ventrolateral preoptic nucleus (VLPO) (Qiu et al., 2014). Atropine thus creates a dissociated state, with cortical sleep-like EEG but wake-like subcortical arousal behavior (Irmis, 1971; Davis et al., 2011; Qiu et al., 2014). This dissociation allows us to examine whether sleep-like cortical activity can satisfy the sleep homeostatic drive. We administered atropine to rats during the light period, producing a 6-h dissociated state when rats would otherwise be sleeping. We then recorded sleep–wake behavior afterward to determine if induction of this sleep-like cortical activity was sufficient to satisfy homeostatic sleep need and prevent sleep rebound.

EXPERIMENTAL PROCEDURES

Animals

Pathogen-free adult male Sprague–Dawley rats (280–300 g, Harlan) were individually housed with *ad libitum* access to food and water. All animals were housed under light-controlled conditions (12-h light/12-h dark cycle, with lights on at 07:00 h; 100 lux) in an isolated

*Correspondence to: M.-H. Qiu, Rm 705, Zhi Dao Building, 138 YiXueYuan Road, Shanghai 200032, China. Tel: +86-21-54237657. J. Lu, CLS 709, 3 Blackfan Circle, Boston, MA 02115, USA. Tel: +1-6177353231.
E-mail addresses: mhqiu@shmu.edu.cn (M.-H. Qiu), jlu@bidmc.harvard.edu (J. Lu).

Abbreviations: EEG, electroencephalogram; EMG, electromyogram; LC, locus coeruleus; LDT, lateral dorsal tegmental nucleus; NREM, non-rapid eye movement; PBS, phosphate-buffered saline; PPT, pedunculopontine tegmental nucleus; REM, rapid eye movement; SWA, slow wave activity; SWE, slow wave energy; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic area.

http://dx.doi.org/10.1016/j.neuroscience.2015.03.070

ventilated chamber maintained at 20–22 °C. All protocols were approved by the Institutional Animal Care and Use Committees of Beth Israel Deaconess Medical Center, and these experiments were carried out in accordance with U.S. National Institutes of Health guidelines regarding the care and use of animals for experimental procedures. Every effort was made to minimize the number of animals used and any pain and discomfort experienced by the animals.

Polygraphic recording and analysis

Under anesthesia (ketamine 100 mg/kg and xylazine 10 mg/kg, i.p.), eight rats were implanted with electrodes for recording EEG and EMG as described previously (Lu et al., 2000; Qiu et al., 2010) and were allowed to recover for 7–10 days. After recovery, animals were transferred to the recording room and habituated to the recording cables and room for 2 days. Following this habituation period, 48 h EEG/EMG activity (AM Systems, Sequim, WA, USA) from the beginning of the light period (07:00) was recorded from all the rats. The cortical EEG and EMG signal were amplified, digitized at a sampling rate of 256 Hz, and recorded using VitalRecorder (Kissei Comtec, Nagano, Japan). The behavior of the animals was recorded simultaneously with time-locked video recordings. EEG/EMG were filtered (EEG, 0.5–40 Hz band-pass; EMG 10 Hz high-pass) and automatically scored offline in 10-s epochs as wake, non-rapid eye movement (NREM) sleep, or rapid eye movement (REM) sleep in SleepSign (Kissei Comtec, Nagano, Japan) using established criteria (Lu et al., 2000; Lu et al., 2001). After automatic scoring, sleep–wake stages were examined and manually corrected. The data collected during the first 24-h period served as the baseline data for comparison to the experimental day (second 24-h period). The amount of time spent in wake, NREM sleep and REM sleep was determined from the scored EEG/EMG data. EEG power spectra for wake epochs were analyzed offline using Fast Fourier Transformation (512 point, Hanning window, 0–24.5 Hz with 0.5 Hz resolution using SleepSign).

Atropine injections

Atropine sulfate (100 mg/kg, Sigma) in saline was injected by i.p. at 2:00 and 4:00 PM in order to maintain a 6-h effect on the experimental day. For baseline data, rats were injected i.p. with saline vehicle (2 ml/kg) at 2:00 and 4 PM.

Perfusion and immunohistochemistry

After completing all experiments, rats were returned to their normal housing for another week before perfusion. For perfusion, rats were given an atropine administration, then 2 h later were deeply anesthetized with 10% chloral hydrate and perfused via the heart with saline followed by neutral phosphate buffered formalin (Fischer Scientific Co., Pittsburgh, PA, USA). The brains were harvested, post-fixed, and cryoprotected in 20% sucrose in phosphate-buffered saline (PBS) overnight, then sectioned in the coronal plane on a freezing microtome into four series of 40 um sections. Immunohistochemistry was performed in accordance with the free floating method described previously (Qiu et al., 2010). Briefly, sections were incubated with 0.3% H_2O_2 for 15 min to quench endogenous peroxidase activity. After washing in 0.1 M PBS (pH 7.4), the sections were incubated with a rabbit polyclonal primary antibody against c-Fos (Ab5, Cat# PC38, Oncogene Research Products, La Jolla, CA, USA) at a 1:10,000 dilution in PBS containing 0.25% Triton X-100 for 24 h at room temperature. On the second day, the sections were washed in PBS and incubated in biotinylated donkey anti-rabbit secondary antiserum (Jackson ImmunoResearch Laboratories, West Grove, PA, USA; 1:1000 dilution) for 1 h, followed by a 1:1000 dilution of avidin–biotin-peroxidase (Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature. The peroxidase reaction was visualized with 0.05% 3,3-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) in PBS and 0.01% H_2O_2 and strengthened with 0.002% Ni, 0.001% $CoCl_2$. After staining, sections were washed, mounted, dehydrated and coverslipped. As controls, adjacent sections were incubated without the primary antibody to confirm that no non-specific staining had occurred.

Statistical analysis

The quantitative data are presented as the mean ± standard error of mean (SEM). Statistical significance was assessed with the paired *t*-test, with $p < 0.05$ taken as the threshold of significance.

RESULTS

Two atropine injections at 2 and 4 PM induced a 6-h waking state with slow-wave EEG (2:00–8:00 PM). In agreement with a previous series of studies (Vanderwolf and Baker, 1986; Vanderwolf, 1988, 1992), rats under the influence of atropine walked, groomed, ate, and drank during this 6-h period. These ‘‘automatic’’ behaviors were strikingly similar to behavior during normal wakefulness. Occasionally, rats displayed a typical sleep posture, which we defined as ‘‘NREM sleep.’’ EEG/EMG recordings and the corresponding hypnograms and delta power spectra of a rat in Fig. 1 illustrate the distinctive dissociated state produced by atropine: high amplitude, slow-wave, sleep-like EEG with wake-like high EMG and movements (confirmed by time-locked video). Atropine-induced EEG delta power was much higher than that of the baseline period prior to the injection (Fig. 1A lower panel; B and C). Average slow-wave-activity (SWA, 0.5–4 HZ) during per hour showed that both SWA of the wake and NREM sleep by atropine were significantly increased, compared to that of the vehicle controls (Fig. 1B). The total amount of SWA (slow wave energy, SWE) for the 6 h of atropine effects was significantly increased compared to the corresponding vehicle period (Fig. 1C). Compared to the waking EEG during the same period of the baseline day, atropine significantly increased slow-wave EEG components (Fig. 1A lower panel; D and E). In the atropine-induced state, the EEG power spectrum contained prominent slow-wave 1.0-Hz oscillations but suppression of other EEG