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Research Report

Unilateral cortical application of tumor necrosis factor α induces asymmetry in Fos- and interleukin-1β-immunoreactive cells within the corticothalamic projection

Lynn Churchill[a,*], Kyo Yasuda[a,b], Tadanobu Yasuda[a,c], Kelly A. Blindheim[a], Michael Falter[a], Fabio Garcia-Garcia[a,1], James M. Krueger[a]

[a]*Department of VCAPP, College of Veterinary Medicine, Washington State University, PO Box 646520, Pullman, WA 99164-6520, USA*
[b]*Department of Otorhinolaryngology, Hirosaki University School of Medicine, 5 Zaifu-Cho, Hirosaki 036-8216, Japan*
[c]*Department of Anesthesiology, Hirosaki University School of Medicine, 5 Zaifu-Cho, Hirosaki 036-8216, Japan*

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Abstract

A unilateral microinjection of tumor necrosis factor alpha (TNFα) (150 ng) onto the primary somatosensory cortex induces state-dependent asymmetries in electroencephalographic (EEG) slow wave activity during non-rapid eye movement sleep in rats [H. Yoshida, Z. Peterfi, F. Garcia-Garcia, R. Kirkpatrick, T. Yasuda, J.M. Krueger, State-specific asymmetries in EEG slow wave activity induced by local application of TNF alpha, *Brain Res.* 1009 (2004) 129–136]. In the current study, analogous TNFα injections were performed to determine Fos- and interleukin-1β (IL1β) immunoreactivity (IR). A unilateral microinjection of TNFα increased the number of Fos- and IL1β-IR cells in the primary somatosensory cortex relative to the contralateral side that received heat-inactivated TNFα. These asymmetric TNFα-induced increases in the number of Fos- and IL1β-IR cells were evident along the outside surface of the cortex (mainly layers II and III) in a restricted rostral to caudal zone. Asymmetrical increases in the number of Fos-IR cells were also observed in the subcortical region that receives the main cortical projection from the somatosensory cortex, the somatic region of the reticular nucleus of the thalamus (reticular thalamus). The IL1β-IR cells double-labeled with glial fibrillary acidic protein (GFAP), suggesting that many of the IL1β-IR cells were astrocytes. The number of the IL1β-IR cells in the reticular thalamus increased significantly ipsilateral to the TNFα injection. Current results indicated that Fos- and IL1β-IR may be utilized to study the functional neuroanatomy involved in the TNFα-mediated state-dependent enhancement of EEG slow wave activity.

Theme: Neural basis of behavior
Topic: Biological rhythms and sleep

Keywords: Somatosensory cortex; Reticular thalamus; Sleep; Cytokine; Glial–neuronal interaction; Rat brain

Abbreviations: DAB, diaminobenzidine tetrahydrochloride; EEG, electroencephalography; Fos-IR, Fos-like-IR; GFAP, glial fibrillary acidic protein; ic, internal capsule; IL1β, Interleukin1β; IL1β-IR, IL1β-like-IR; IR, like-immunoreactivity or like-immunoreactive; NGS, normal goat serum; NHS, normal horse serum; NREMS, non-rapid eye movement sleep; PBS, phosphate buffered saline; Ret, Ret thalamus, reticular thalamus, reticular nucleus of the thalamus; SSctx, somatosensory cortex; som, somatic region of the reticular thalamus; SRS, sleep regulatory substances; SWA, slow wave activity; TNFα, tumor necrosis factor α; VB, ventrobasal nucleus of the thalamus

* Corresponding author. Fax: +1 509 335 4650.
E-mail address: lnchurch@vetmed.wsu.edu (L. Churchill).
[1] Current address: Departamento de Fisiología, Facultad de Medicina, UNAM, A.P. 70-400, C.P. 04510, México DF, México.

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1. Introduction

The issue of how the brain is organized to produce sleep is central to neurobiology. For instance, because sleep involves regulated unconsciousness, an understanding of this process may help provide a scientific explanation of consciousness. von Economo [66] presented evidence, based on brain lesions in humans, suggesting that sleep is an active process as opposed to the idea that was dominant at the time, that sleep results from the withdrawal of sensory input [28,52]. In the intervening century, many investigations have supported the idea that sleep is regulated by specific brain circuits, e.g., the anterior hypothalamus [24,25,34,49]. Perhaps the best evidence for this paradigm with regard to non-rapid eye movement sleep (NREMS) is that electrical, chemical, or thermal stimulation of the anterior hypothalamus induces NREMS [4,21,32,55,75]. However, this paradigm fails to address the issue of sleep homeostasis. Thus, the brain keeps track of its prior sleep/wake history over long periods of time; e.g., prolonged wakefulness results in increased sleep propensity [5]. The transfer of cerebrospinal fluid from a sleep-deprived animal (but not a control animal) into a fully rested recipient enhances sleep in the recipient. This finding suggests that the homeostatic processes for sleep involve sleep regulatory substances (SRSs) [7,42]. Several SRSs thought to play a role in those experimental findings have now been identified including molecules such as tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL1β) [38].

The literature concerning sleep regulatory circuits also fails to adequately explain a number of findings suggesting that sleep can be targeted to brain areas disproportionately activated during prior wakefulness. For instance, unilateral stimulation of the somatosensory cortex in awake humans produces asymmetry in the electroencephalogram (EEG) slow wave (1/2–4 Hz) activity during subsequent NREMS [26]. Similar results were obtained from rats and mice [67,68]. Furthermore, dolphins that only show EEG slow wave sleep on one side of the brain at a time [36] show sleep rebound only on the side that was sleep-deprived [40]. More recently, Huber et al. [22] showed that after a specific learning task, the subsequent EEG slow wave activity during sleep was enhanced in the area activated by the learning task. Others have also concluded that EEG slow wave activity of specific brain areas is, in part, dependent upon prior activity within that region [16,33].

A more recent view of brain organization of sleep posits that sleep is initiated within neuronal assemblies as a function of prior cellular activity and this provides the mechanism for the localization and targeting of sleep as well as for sleep homeostasis [29]. In this view, for example, TNFα, adenosine, nerve growth factor (NGF), and other SRSs are produced and released in response to neuronal and glial activity [10,38]. These substances in turn are posited to be made and act locally in autocrine, paracrine, and juxtacrine ways to alter the input–output relationships of neuronal assemblies (e.g., cortical columns, barrels, or a cortical–thalamic–cortical loop) and thereby induce a functionally different state in these assemblies. Indeed, expression of NGF within somatosensory pyramidal neurons is activity- and sleep-dependent [8]. Cortical TNFα levels also increase during prolonged wakefulness and during seizures; two conditions thought to be associated with enhanced cellular activity [18,45,48,63–65]. Also, activity-dependent increases of TNFα appear to be important for the developing nervous system [41]. Other investigators have also supported the view that sleep may be important in synaptic homeostasis and specific types of learning [5,6,27,56,62,69,70].

Individual auditory cortical columns oscillate between two functional states as defined by the amplitude of auditory-induced evoked response potentials [9,47]. One of the functional states, called the “S” state, usually occurs simultaneously with whole animal sleep. The “S” state is dependent upon its prior history in the sense that the probability of its occurrence is higher if, for the 15-min period prior to the measurement, it had been in the other functionally defined state. If such changes in the neuronal-group functional state are dependent upon activity-dependent SRSs, then one would predict that direct application of an SRS such as TNFα onto the surface of the cortex would drive the affected neuronal groups to simultaneously express the same functional state. Indeed, when TNFα is applied in this manner, EEG power is enhanced unilaterally [74]. We extend these observations; hereby reporting that the application of TNFα to the cortex enhances Fos expression within large nuclei present in the surrounding cortical area and the reticular nucleus of the thalamus (reticular thalamus), presumably from activation of the cortical projection to this area. We also report enhanced IL1β immunoreactivity (IR) within astrocytes in the cortex and reticular thalamus, suggesting that activation of the corticothalamic projection can control synthesis of IL1β in nearby astrocytes.

2. Methods

2.1. Agents

Rat recombinant TNFα was purchased from R&D, Inc. (Minneapolis, MN). TNFα was dissolved in pyrogen-free saline (PFS) and microinjected at 150 ng/2 μl.

2.2. Animals

Male Sprague–Dawley rats weighing 300–400 g were obtained from Taconic Farm, Inc. (Germantown, NY). The use of rats in these experiments was in accordance with Washington State University guidelines and was approved by the Animal Care and Use Committee. The rats were adapted to a 12:12-h light–dark cycle (lights on at 0200) at