Behavioural Brain Research 256 (2013) 108–118

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Topography of the sleep/wake states related EEG microstructure and transitions structure differentiates the functionally distinct cholinergic innervation disorders in rat

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HIGHLIGHTS

- We used the rat models of the cholinergic neuronal innervation disorders.
- Bilateral NB or PPT lesions were done by ibotenic acid microinfusion.
- We investigated the impact of lesions during sleep.
- NB and PPT lesions are differently expressed in sensorimotor vs. motor cortex.
- Differing EEG microstructure and transition structure are the hallmarks of lesions.

ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form 22 July 2013
Accepted 26 July 2013
Available online 6 August 2013

Keywords:
Nucleus basalis
Pedunculopontine tegmental nucleus
Ibotenic acid
Lesion
Cholinergic neurons

ABSTRACT

In order to identify the differences for the onset and progression of functionally distinct cholinergic innervation disorders, we investigated the effect of bilateral nucleus basalis (NB) and pedunculopontine tegmental nucleus (PPT) lesions on sleep/wake states and electroencephalographic (EEG) microstructure in rats, chronically implanted for sleep recording. Bilateral NB lesion transiently altered Wake/NREM duration within the sensorimotor cortex, and Wake/REM duration within the motor cortex, while there was no change in the sleep/wake states distributions following the bilateral PPT lesion. Bilateral PPT lesion sustainably increased the Wake/REM and REM/Wake transitions followed by inconsistent dysregulation of the NREM/REM and REM/NREM transitions in sensorimotor cortex, but oppositely by their increment throughout four weeks in motor cortex. Bilateral NB lesion sustainably decreased the NREM/REM and REM/NREM transitions during four weeks in the sensorimotor cortex, but oppositely increased them in the motor cortex. We have shown that the sustained beta and gamma augmentation within the sensorimotor and motor cortex, and across all sleep/wake states, simultaneously with Wake delta amplitude attenuation only within the sensorimotor cortex, were the underlying EEG microstructure for the sleep/wake states transitions structure disturbance following bilateral PPT lesion. In contrast, the bilateral NB lesion only augmented REM theta in sensorimotor cortex during three weeks.

We have shown that the NB and PPT lesions induced differing, structure-related EEG microstructure and transition structure disturbances particularly expressed in motor cortex during NREM and REM sleep. We evidenced for the first time the different topographical expression of the functionally distinct cholinergic neuronal innervation impairment in rat.

1. Introduction

Human development, maturation, healthy aging [1,2], and many neurological diseases [3] are associated with a variety of the sleep-related behavioral disorders. Neurodegenerative diseases, such as Alzheimer's (AD) and Parkinson's (PD) diseases, involve the selective loss of specific neuronal populations within the brain [4,5]. In addition, regarding the cholinergic neuronal loss, while AD involves selective loss of the basal forebrain cholinergic system, PD is related to the selective loss of the pontine cholinergic system [6,7]. Moreover, AD has been characterized as a "disorder of cortical cholinergic innervation" [8]. The REM sleep behavior disorder (RBD), reflecting an underlying synucleinopathy with ascending pattern of the neurodegeneration, precedes as a symptom the

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http://dx.doi.org/10.1016/j.bbr.2013.07.047

onset of motor and cognitive disturbances by years or decades [9]. Recent imaging studies in humans demonstrated the thalamic cholinergic denervation in the PDs in contrast to AD [7], due to the degeneration of PPT cholinergic neurons, and related it to RBD [6], and to gait and balance impairment including falls in PD [10,11]. Neuropathological studies in humans have reported degeneration of about 50% of the PPT cholinergic neurons in PD in contrast to AD, where no reductions in PPT cholinergic neurons were found [11].

It is well known that the majority of the ascending cholinergic projections to the cerebral cortex, originate in the basal forebrain nuclei [12] consisting of the nucleus basalis of Meynert (NB), the substantia innominata, and the horizontal limb of diagonal band. This cortical cholinergic input system has been categorized as the rostral component of the brain ascending arousal system [13] that together with pontine reticular formation forms the ascending reticular activating system [14]. Since the cholinergic NB projections to the cortex are not diffuse, but modality and region-specific – distinct populations of cholinergic neurons are capable of modulating different cortical regions [15].

The PPT is postulated to have important functions relevant to the regulation of REM [16–18], arousal [19], and various motor control systems [20], and breathing control [21–25], and the direct projections from PPT reach both the basal forebrain and thalamus [26]. Therefore, the PPT presents the control relay nucleus for the integrated contributions of these two cholinergic systems to regulation of cortical activation [27,28].

It is also important to note that although sleep is classically considered as a global phenomenon, orchestrated by central specialized neuronal networks modulating whole-brain activity [29], recently it has been proposed that sleep is local in nature, or fundamental propriety of small neuronal groups [30]. A lot of experimental evidence in animals and humans suggest that: sleep and wakefulness might be simultaneously present in different cerebral regions; that the boundaries between these behavioral states are not strictly defined; and that brain-sleep state may be spatially non-uniform, as well as sleep and wakefulness may not be temporally discrete behavioral states [29].

In order to identify the sleep/wake state related EEG differences for the onset and progression of the functionally distinct cholinergic innervation disorders in rat, we investigated the effects of the bilateral NB and PPT lesions on the expression of the sleep/wake states distribution, EEG microstructure and transitions structure.

2. Materials and methods

We performed the experiments on 27 adult, male Wistar rats, chronically instrumented for sleep recording, and randomly assigned to one of three experimental groups: physiological controls ($n = 8$), bilaterally NB lesioned rats ($n = 12$), and bilaterally PPT lesioned rats ($n = 7$).

Prior to surgery and consistently throughout the experimental protocol, the animals were maintained on a 12-h light–dark cycle, and were housed at 25 °C with free access to food and water. All animal procedures were in compliance with the EEC Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes, and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Sinisa Stankovic", University of Belgrade (Approval No 2-21/10).

2.1. Surgical procedure

The surgical procedure details, employed for the chronic electrode implantation for sleep recording, have been previously described [31–33], and are outlined below. We implanted two epidural parietal stainless-steel screw electrodes for EEG cortical activity recording from motor (A/P: +1.0; R/L: 2), and sensorimotor (A/P: −3.0; R/L: 2) cortex [34] under ketamine/diazepam anesthesia (Zoletil 50, VIRBAC, France, 50 mg/kg; i.p). Bilateral electromyogram (EMG) wire electrodes were implanted into the dorsal nuchal musculature to assess skeletal muscle activity. The referential stainless-steel screw electrode was implanted in the nasal bone. All electrode leads were soldered to a miniature connector plug (39F1401, Newark Electronics, Schaumburg, IL, USA), and the assembly was fixed to the screw electrodes and skull using acrylic dental cement (Biocryl-RN, Galenika a.d. Beograd, Serbia).

During the operative procedure we performed the bilateral NB or PPT lesions induced by the stereotaxically guided microinfusion of 0.1 M IBO/0.1 M PBS into the NB (A/P: −1.4; R/L: 3; D/V: 7.0 from the brain surface), or into the PPT (A/P: −7.8; R/L:1.9; D/V: 7.0 from the brain surface), using a Digital Lab Standard Stereotaxic Instrument with a Quintessential Stereotaxic Injector (Stoelting Co, Wood Dale, IL, USA), and a Hamilton syringe (1 μl). The IBO (Sigma; pH = 7.4) concentration was chosen on the basis of previous studies [35]. The microinfusions were introduced at a volume of 100 nl, using a single, 60 s pulse [31,33]. Following microinfusion within the right NB or PPT the Hamilton syringe was left within the local brain tissue for 5 min, before removal from the brain, allowing the solution to diffuse within the NB or PPT. After removal from one brain side the Hamilton syringe was washed out by saline, and then it was stereotaxically positioned within the left NB or PPT for the next IBO microinfusion.

2.2. Recording procedure

At the end of the surgical procedure, the scalp wounds were sutured and the rats were given a recovery period of 2 weeks before adapting to the recording cable and plexiglass chamber (30 cm × 30 cm × 30 cm) for one day. EEG and EMG activities were carried from the connector plug on the rat head by cable, and passed through the sealed port of the recording box. They were displayed on a computer monitor, and stored on disk for further off-line analysis. After conventional amplification and filtering (0.3–100 Hz band pass; A-M System Inc. Model 3600, Carlborg, WA, USA), the analog data were digitized (at a sampling frequency of 256/s), and recorded for 6 h, during the normal inactive circadian phase for rats (from 9 a.m. to 3 p.m.), using DataWave SciWorks Experimenter Verson 7.2 (Datawave Technologies, Longmont, CO, USA). Each rat was recorded weekly, for 4 weeks.

2.3. Tissue processing and histochemistry

At the end of the recording sessions the lesions were identified by NADPH–diaphorase histochemistry [36], and the NB or PPT cholinergic neuronal loss was quantified within the overall NB or PPT rostro-caudal dimension using Image J 1.46 software [31,33]. All statistical analyses for the cholinergic cells number changes were done using nonparametric single factor Kruskal–Wallis ANOVA with post-hoc Mann–Whitney U two-tailed tests.

2.4. Data analysis

In this study we included in data analysis the signals recorded from the control rats and all rats with positively identified bilateral NB and PPT lesions. Analysis of the recorded signals was conducted with software we developed using MATLAB 6.5 [31,33]. We applied Fourier analysis to signals acquired throughout each 6 h recording (2160 10 s Fourier epochs), and each 10 s epoch was differentiated as Wake, NREM or REM state for further analysis of the Wake, NREM and REM related EEG amplitudes of all the conventional frequency bands ($\delta = 0.3$–4 Hz; $\theta = 4.1$–8 Hz; $\sigma = 10.1$–15 Hz; $\beta = 15.1$–30 Hz; $\gamma = 30.1$–50 Hz) of the control and lesioned rats. First, we extracted all the 10 s Wake epochs from each 6 h recording, based on the product of sigma and theta frequency power on the *y*-axis, and the EMG power on the *x*-axis. Further, the differentiation of NREM and REM 10 s epochs was done using the EMG power on the *y*-axis, and the delta/theta power ratio on the *x*-axis [31]. Differentiation of the Wake epochs from sleep epochs, and further differentiation of the NREM and REM epochs was achieved using the two cluster *K* means algorithm. We improved these differentiation results by using the logarithmic values of quantities on both axes (Fig. 1).

To analyze the sleep/wake state related EEG amplitude changes we calculated group probability density distributions of all the Wake, NREM and REM conventional EEG frequency bands relative amplitudes over 6 h, of each experimental group, using the Probability Density Estimate (PDE) routine supplied with MATLAB 6.5. In order to eliminate any influence from absolute signal amplitude variations on the recordings, we computed the relative Fourier amplitudes:

$$(RA)_b = \frac{\sum_b Amp}{\sum_{tot} Amp} \quad b = \{\delta, \theta, \sigma, \beta, \gamma\}$$

For each sleep/wake state and each frequency band PDE analysis was performed on the ensembles of relative amplitudes by pooling measured values $(RA)_b$ from all animals belonging to a specific group.

More precisely, let the number of recorded animals be Nc (control), Nb1 (bilateral NB lesion) and Nb2 (bilateral PPT lesion). Further, let $n_{c,j(W)}$, $n_{c,j(NREM)}$ and $n_{c,j(REM)}$ denote the number of identified Wake, NREM and REM epochs, respectively, for the *j*th control animal. Group ensemble processing for the Wake state PDE analysis was formed by pooling $n_{c,1(W)}$, $n_{c,2(W)}$, . . ., $n_{c,Nc(W)}$; for the NREM state by pooling $n_{c,1(NREM)}$, $n_{c,2(NREM)}$, . . ., $n_{c,Nc(NREM)}$, and for the REM state by pooling $n_{c,1(REM)}$, $n_{c,2(REM)}$, . . ., $n_{c,Nc(REM)}$ relative amplitudes for each band. Analogous groups were formed for Nb1 and Nb2.

We also analyzed the mean number of all transition states (Wake/NREM, NREM/Wake; Wake/REM, REM/Wake; NREM/REM, REM/NREM) during 6 h of the sleep recordings in all the experimental groups. For each animal and each experimental condition the sleep/wake related state (Wake, NREM and REM) was