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Research report

Diurnal rhythm and stress regulate dendritic architecture and spine density of pyramidal neurons in the rat infralimbic cortex

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ABSTRACT

The medial prefrontal cortex (mPFC) participates in several higher order cognitive functions and is involved in the regulation of the stress response. The infralimbic cortex (ILC), the most ventral part of the mPFC, receives a strong afferent input from the master circadian pacemaker, the suprachiasmatic nucleus. This fact raises the possibility that, similarly to stress, the diurnal rhythm may affect structural plasticity of neurons in the ILC. Here we investigated, whether diurnal changes in combination with immobilization stress have any impact on the dendritic morphology of layer III pyramidal neurons in the ILC. Prefrontal cortices were collected from control rats at two different time points of the diurnal cycle (12 h apart), and from rats exposed to 1-week of daily restraint stress either during their active or resting period. Dendritic architecture and spine density of Golgi–Cox stained neurons were digitally reconstructed and analyzed. We found that in control rats during the active period, the basilar dendrites were always longer and more complex, and had more spines than during the resting period. Similar although less pronounced diurnal differences exist in the apical dendrites. Stress affected dendritic architecture in a way that the diurnal differences either disappeared or became reduced in their magnitude. Our findings indicate that the diurnal rhythm has a unique impact on the structural plasticity of pyramidal cells in the ILC and that stress interferes with this form of neuroplasticity.

1. Introduction

The medial prefrontal cortex (mPFC) is implicated in a number of higher order functions including cognitive and executive operations such as working memory, selective attention, decision making and goal-directed behavior as well as processing emotions and regulation of stress responses [13,14,45]. Many of these higher order functions that are regulated by the PFC are subject to diurnal rhythmicity and exhibit typical diurnal patterning [24,28,39]. Disruption of the normal biological rhythms can affect both the cognitive functioning, such as short-term memory and attention [6,37], as well as regulation of mood [18]. These findings raise the possibility that a common underlying diurnal mechanism may affect these higher level brain functions. The suprachiasmatic nucleus (SCN) is the likely source of these diurnal rhythms, since it is the master circadian pacemaker in the brain [19]. Interestingly, the SCN has a pronounced multisynaptic pathway, projecting via the paraventricular thalamic nucleus (PVT) to the mPFC [42]. Furthermore, this SCN–mPFC pathway is specific to the infralimbic cortex (ILC), whereas the other mPFC subareas are not involved [42]. Although the functional role of this SCN–PVT–ILC pathway is yet unknown, it has been suggested that it may play a specific role in modulating some higher level brain functions which exhibit diurnal rhythms, such as mood and attention [42].

The mPFC in rats consists of anatomically and functionally distinct areas that are most commonly divided into three main subregions: the infralimbic (ILC), prelimbic (PLC), and anterior cingulate cortex (ACC) [11,20]. The mPFC, besides regulating higher order cognitive functions, has a significant role in modulating autonomic and endocrine responses to stress, as it provides negative feedback control to the hypothalamic–pituitary–adrenal (HPA) axis and regulates the stress responses of other structures [13,14]. It is generally accepted that the different mPFC subregions play functionally distinct roles in modulating the stress response [14]. Indeed a number of reports suggested that even neighboring areas such as

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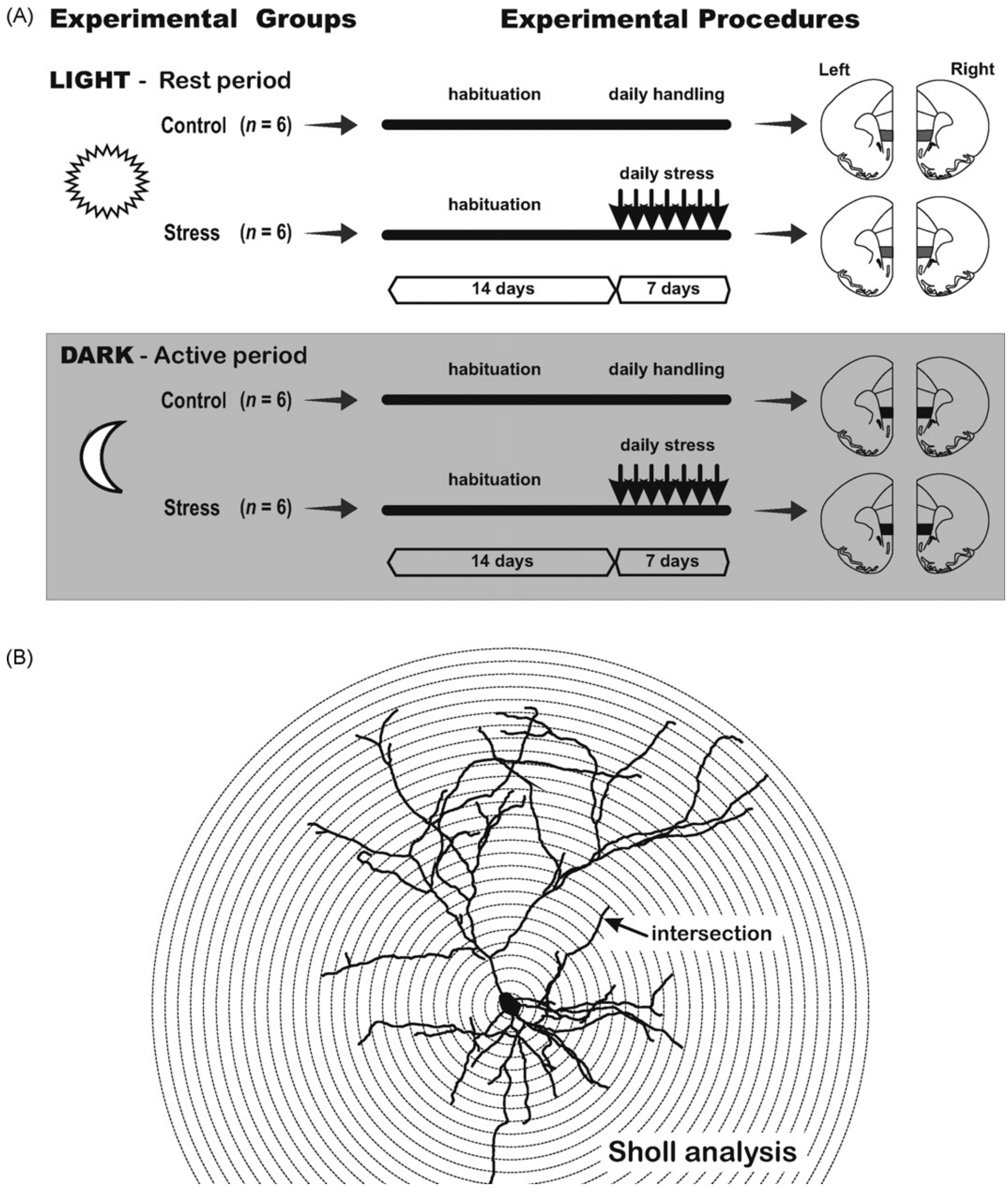

Fig. 1. (A) Experimental design and groups of experimental animals (for details, see Section 2). (B) Schematic illustrating reconstruction of the complete dendritic tree of a Golgi–Cox stained pyramidal neuron in layer III of the infralimbic cortex together with the Sholl circles that were used for numerical analysis. The points where dendrites cross the virtual circles are called intersections.

the ILC and PLC may have somewhat opposing modulatory effects on the stress response [34,43]. These findings highlight the importance of distinguishing between the different regions when one aims to study the mPFC.

In the present study, we investigated whether the dendritic architecture of layer III pyramidal neurons in the rat infralimbic cortex changes with the diurnal phases of resting and activity. Prefrontal cortices were collected at two different time points of the diurnal cycle (12 h apart); the samples were stained using the Golgi–Cox method and pyramidal neurons in the ILC were three-dimensionally reconstructed. Spines were counted and a Sholl analysis was performed to determine the length and complexity of the dendrites. Furthermore, with the same method, we examined the effect of 1-week of daily restraint stress that was applied either during the active or rest period of the rats.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Harlan-Winkelmann, Borchen, Germany) were housed in groups of three animals per cage with food and water ad libitum in temperature-controlled rooms ($21 \pm 1\,^{\circ}$C). All animal experiments were conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/EEC) and the US National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Lower Saxony Federal State Office for Consumer Protection and Food Safety, Germany. We used the minimum number of animals required to obtain consistent data.

At the beginning of the experiment (habituation phase), rats weighed 150–170 g, which according to Harlan-Winkelmann corresponds to an age of about 6–7 weeks. To investigate the impact of the light cycle on dendritic architecture, one set of rats was kept on a normal light cycle (lights on at 0700, lights off at 1900), and another set was kept on an inverse light cycle (lights off at 0700, lights on at 1900). Both sets of rats contained a control and a stressed group, resulting in four experimental groups: (1) control rats on a normal light cycle, (2) stressed rats on a normal light