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Research article

REM sleep loss associated changes in orexin-A levels in discrete brain areas in rats

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HIGHLIGHTS

- REMS loss differentially increases Orx-A levels in discrete brain regions.
- Maximum increase in Orx-A levels was observed in the LC, the site of REM-OFF neurons.
- Orx-A levels remained unaffected in the PPT, the site of REM-ON neurons.
- REMSD activates and REMS rebound inhibits Orx-ergic system.

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ABSTRACT

Rapid eye movement sleep (REMS) serves house-keeping function of the brain and its loss affects several pathophysiological processes. Relative levels of neurotransmitters including orexin A (Orx-A) in various parts of the brain in health and diseases are among the key factors for modulation of behaviors, including REMS. The level of neurotransmitter in an area in the brain directly depends on number of projecting neurons and their firing rates. The locus coeruleus (LC), the site of REM-OFF neurons, receives densest, while the pedunculo-pontine area (PPT), the site of REM-ON neurons receives lesser projections from the Orx-ergic neurons. Further, the Orx-ergic neurons are active during waking and silent during REMS and NREMS. Therefore, the level of Orx-A in discrete regions of the brain is likely to be different during normal and altered states, which in turn is likely to be responsible for altered behaviors in health and diseases, including in relation to REMS. Therefore, in the present study, we estimated Orx-A level in LC, cortex, posterior hypothalamus (PH), hippocampus, and PPT after 96 h REMSD, in post-deprivation recovered rats and in control rats. This is the first report of estimation of Orx-A in different brain regions after prolonged REMSD. It was observed that after REMSD the Orx-A level increased significantly in LC, cortex and PH which returned to normal level after recovery; however, the level did not change in the hippocampus and PPT. The Orx-A induced modulation of REMS could be secondary to increased waking.

1. Introduction

Rapid eye movement sleep (REMS) is a unique reversible, physiological state expressed for varying duration; it repeats within the sleep state especially in the animals higher in evolutionary ladder. Normally it appears only after a period of non-REMS (NREMS) and barring in some diseased states (e.g. narcolepsy), REMS-like state does not appear during waking. Therefore, it was proposed that normally neurons responsible for REMS must be inhibited by the waking areas, while excited by the NREMS generating areas, which has been confirmed [1,2] and subsequently the complex neural connections for the regulation of REMS has recently been constructed [3]. It essentially suggests that as long as noradrenergic (NA)-ergic REM-OFF neurons in the locus coeruleus (LC) remain active, which of course are under the influence of varieties of neurotransmitter inputs, REMS is not expressed and those neurons must cease activity for the expression of REMS [3,4].

The levels of various neurotransmitters in different parts of the brain during REMS and REMS loss are likely to play significant role in REMS regulation during health and diseases. Orexin (Orx) is one of the many neurotransmitters involved in REMS regulation in health and diseases [5]. Orx synthesizing neurons are

Abbreviations: CSF, cerebrospinal fluid; ELISA, enzyme linked immunosorbent assay; FMC, free moving control; LC, locus coeruleus; PPT, pedunclo pontine tegmentum; LPC, large platform control; NREMS, non REMS; Orx-A, orexin-A; PeF, perifornical area; PH, posterior hypothalamus; REMS, rapid eye movement sleep; REMSD, REMS deprivation; REC, recovery.

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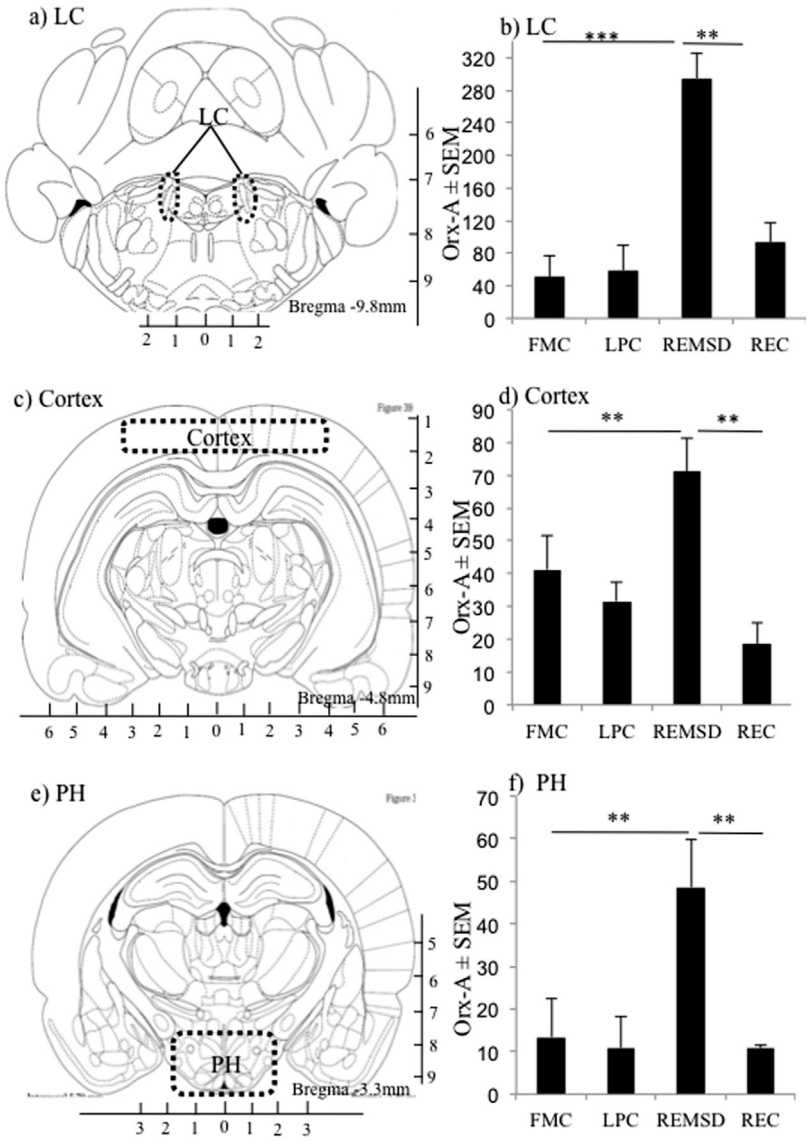

Fig. 1. Left panel shows coronal sections from the rat brain atlas [22]. The areas dissected out for estimation of Orx-A have been marked on the sections. The mean (±SEM) Orx-A level in the dissected portion ($n = 5$) has been shown in the corresponding histogram on the right panel. The Orx-A level increased in all the areas shown. Orx-A concentration is pg/g wet tissue weight. $^{***}p \leq 0.001$; $^{**}p \leq 0.01$; $^{*}p \leq 0.05$. Abbreviations are as in the text.

present in the perifornical (PeF) area, located at the lateral part of posterior hypothalamus (PH) and are generally active during waking, slow down and finally become quiescent during NREMS and REMS [6–8]. Activation of these neurons indeed increased waking and reduced REMS [9–11]. Recently, we have shown that Orx-ergic neurons indeed affect REMS by modulating the neurons in the LC [11]. Orx levels in the brain correspond to the activity of Orx-ergic neurons, i.e. higher during waking and REMS [12] and lower during NREMS. Interestingly, these Orx-ergic neurons project to many brain areas including the brainstem, LC, PH, which are classically known to be involved in the regulation of wakefulness-NREMS-REMS [11,13–15] and to hippocampus and cortex, among many other areas which are apparently not involved in sleep-waking-REMS regulation [16]. Also, Orx-ergic neurons in PeF have local self-collateral inputs [17].

All the neurons even in any one functional area in the brain do not behave and perform identically. Thus, it is obvious that the levels of the neurotransmitters in different projected regions of the brain are likely to be different during alteration in one function. The levels of neurotransmitters would contribute to pre-disposition of expressions of behavior and variations in quality and quantity of expressions of behavior including diseases and symptoms thereof. Therefore, it is essential to estimate the levels of a neurotransmitter in relation to changes in a particular function simultaneously in multiple areas of the brain where the neurons from one area project to. Thus, to address this issue, in the present study, we estimated Orx-A levels in control, after REMS deprivation (REMSD) and after recovery from REMSD in different brain areas known to be involved in REMS regulation and in the areas although not involved in regulation of REMS as such, but are known to modulate function(s) affected by REMS loss. As Orx is of two types Orx-A and Orx-B, in this study, we estimated levels of Orx-A. To the best of our knowledge, this is the first such study especially after long-term REMSD.

2. Materials and methods

2.1. Animals and REMSD

Experiments were conducted on inbred male Wistar rats (200–250 g; $n = 20$), maintained in 12:12 light/dark (L/D) cycle and