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Locomotor Activity and the Expression of Orexin A and Orexin B in Aged Female Rhesus Macaques

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Abstract

Reduced activity has been linked to age-associated physiological changes but the underlying root cause is unclear. The goal of the present study was to compare the orexin neuronal system of old (23–29 years) female rhesus macaques with either *active* or *sedentary* 24-hour locomotor activity patterns. Using immunohistochemistry we counted the number of orexin A and orexin B neurons in the lateral hypothalamic area (LHA) of each animal. Overall, we observed no difference in the distribution pattern or number of either orexin A or orexin B immune-positive neurons between animals in the two groups. Thus, reduced activity in the elderly is unlikely to stem from a loss of orexin neuronal perikarya in the LHA. This, however, does not rule out the possibility that the reduced activity stems from reduced orexin neuronal projections to arousal centers of the brain, such as the locus coeruleus, or from attenuated release of orexin.

Keywords

Aging; Hypocretin; cFOS; Lateral Hypothalamic Area (LHA); Orexin; Rhesus macaque

1. Introduction

Reduced daytime alertness and perturbed sleep are common complaints of the elderly, severely impacting quality of life, and possibly contributing to the etiology of other age-

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Disclosure statement

The authors declare no conflicts of interest.

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associated pathologies such as cognitive decline and immune senescence. Although the principal cause of reduced daytime alertness is unclear, it is plausible that the defect resides in the orexin neuroendocrine system. Orexin A and orexin B (also called hypocretin 1 and 2) are peptides produced exclusively by neurons of the lateral hypothalamic area (LHA) and parts of contiguous hypothalamic regions. Importantly, there is evidence from both human and animal studies showing how orexin neurons are involved in the regulation of wakefulness and sleep. (e.g., Chemelli et al., 1999; Nishino et al., 2000; Peyron et al., 2000; Thannickal et al., 2000). Therefore, disruption of the orexin system with advanced age may help to explain the aging-associated decline of several physiological functions, especially wake-sleep regulation and the maintenance of daytime vigilance. Like humans, aged rhesus macaques show an increased incidence of disrupted wake-sleep cycles, characterized by reduced daytime locomotor activity and also often with increased nighttime restlessness (Downs et al., 2007). Consequently, our goal was to examine the orexin neuronal system of old female rhesus macaques and to test the hypothesis that age-associated decline in daytime activity is associated with significant changes in the orexin system. Our prediction was that animals with more sedentary activity patterns would show fewer immune-positive orexin A and orexin B neurons in their LHA.

2. Materials and methods

2.1. Experimental animals

A total of fourteen female rhesus macaques (*Macaca mulatta*), ranging in age from 23 to 29 years, were used in this Institutional Animal Care and Use Committee approved study. They were housed in a temperature controlled environment (24°C) under a 12L:12D photoperiod (lights on from 0700 h – 1900 h) and were cared for by the Division of Comparative Medicine at the Oregon National Primate Research Center (ONPRC) in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*. Meals (Purina High Protein Monkey Chow No. 5045, Purina Mills, Inc., St. Louis, MO, USA), were provided daily at 0800 h and 1500 h, and were supplemented with fresh fruits or vegetables; fresh drinking water was available *ad libitum*.

2.2. Monitoring of locomotor activity

Actiwatch activity recorders were used to monitor 24-hour locomotor activity in each animal, as previously described (Urbanski, 2011; Urbanski et al., 2012; Supplementary Material). Seven of the old animals had activity profiles very similar to what we typically observe in young animals, with mean daytime levels above 175 units. These animals showed a mean daytime activity range of 192–309, and so were classified as being *active*. In contrast, seven of the old animals showed significantly attenuated daytime activity levels with a mean activity range of 63–163, and these were classified as being *sedentary* (Fig. 1). The mean ($\pm$ SEM) ages and body weights of the animals in the *active* and *sedentary* groups were 25.4 ± 1.0 years *versus* 26.3 ± 0.9 years, and 7.6 ± 0.3 kg *versus* 8.2 ± 0.3 kg, respectively, which were statistically not different (Student's *t*-test, $p > 0.05$). Five of the seven animals in each group had either gone through menopause or had been ovariectomized, and so they had very low circulating estradiol levels; two of the animals in each group had undergone estradiol hormone replacement therapy.

2.3. Immunohistochemistry (IHC) for orexin A and orexin B

For each animal a series of unilateral coronal hypothalamic sections was processed for orexin A IHC and a separate series was processed for orexin B IHC. Each series comprised 25- μ m-thick sections collected at 300- μ m intervals, and spanned the entire rostral-caudal length of the LHA. As previously described (Downs et al., 2007; Supplementary Material), the IHC was performed on free-floating hypothalamic sections using the Vectorstain ABC Standard Kit amplification procedure (Vector Laboratories Inc., Burlingame, CA, USA) and 3,3'-diaminobenzidine tetrachloride (DAB) as the chromogen. The primary antibodies (Santa Cruz Biotech, Inc., Santa Cruz, CA, USA) included goat polyclonal antibodies to orexin A (C-19 sc-8070) and orexin B (C-19 sc-8071). For each animal, 5 stained sections (300- μ m apart) were selected; these included the section with peak orexin neuronal density plus 2 adjacent rostral sections and 2 adjacent caudal sections.

3. Results

Representative examples of mean 24-hour locomotor activity patterns from *active* and *sedentary* old animals are depicted in Fig. 1. The intensity of 24-hour activity was significantly ($p < 0.05$) lower in the *sedentary* animals as compared to the *active* animals (Supplementary data Table S1). This was due to their significantly decreased activity during daylight hours ($p < 0.05$). As expected, activity during the night was much lower than in the daytime, but no significant difference in nocturnal activity was detected between the two groups. Animals from both the *active* and *sedentary* groups showed similar robust staining of orexin A and orexin B neuronal perikarya, which was qualitatively indistinguishable (Supplementary data Fig. S1). They also showed similar bell-shaped distribution patterns for orexin A and orexin B neurons in the LHA, and no significant difference in the total number of immune-positive neurons (Fig. 2).

4. Discussion

Numerous studies have already demonstrated that orexin neurons play a critical role in maintaining vigilance and wakefulness (e.g., Bourgin et al., 2000; Chemelli et al., 1999; Peyron et al., 1998; 2000; Sutcliffe et al., 1998), but very few studies of the orexin system have been conducted in aged humans (Fronczek et al., 2005; 2007; 2012; Hunt et al., 2015) and to the best of our knowledge only one previous orexin study has been performed in aged nonhuman primates (Downs et al., 2007). In the present study, we examined both the orexin A and orexin B neuronal populations in and around the LHA of aged female rhesus macaque, as this is the anatomical region where all of the orexin neurons are located (Downs et al., 2007). Furthermore, by centering our selection of LHA sections around the peak density of orexin neurons, we ensured that the analysis focused on comparable regions of the LHA of all the animals. Importantly, the distribution patterns and total number of orexin A and orexin B neurons in the LHA were similar in *active* and *sedentary* old animals, suggests that any orexin-mediated contribution to age-related changes in day-time activity do not reside at the perikaryal level and are unlikely to stem from differences in orexin synthesis.

Orexin neurons located in the lateral LHA and in the close vicinity send wide-spread projections to brain regions involved in the regulation of wake and sleepiness (Horvath et al.,

1999; Van Den Pol et al., 1999). Therefore, our failure to detect significant differences at the orexin perikaryal level between *active* and *sedentary* old animals, does not rule out the possibility that reduced daytime activity in the *sedentary* animals stems from decreased orexin neuronal projections to arousal centers of the brain, such as the locus coeruleus (LC), or from attenuated release of orexin. In support of this view we previously reported a significant age-related decrease in the density of orexin axonal projections in the LC of male rhesus macaques, as well as a decline in tyrosine hydroxylase gene expression in the LC, which could not be attributed to any orexin neuronal loss in the LHA (Downs et al., 2007). Consequently, similar changes may also underlie aging in females, especially in those showing reduced daytime activity levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Many old rhesus macaque monkeys show attenuated activity levels during the day
- Orexin A and orexin B immunoreactivity is highly expressed in the lateral hypothalamic area
- The number of orexin neurons is similar in active and sedentary old female macaques

Fig. 1.

Representative actograms from a representative *active* and a *sedentary* aged female rhesus macaques. In the upper panels, the height of the vertical lines within the actograms is indicative of the intensity of physical activity at any particular time of day; the mean 24-hour activity profiles across the ~2 weeks are depicted in the lower panels. The horizontal black and white bars correspond to the times of night and day, respectively.

Fig. 2.

Histograms comparing the distribution patterns of orexin A-immunoreactive neurons (A) and orexin B-immunoreactive neurons (B), from seven *active* and seven *sedentary* aged female rhesus macaques. For each animal, the number of immunoreactive orexin neurons was counted in five 25- μ m-thick LHA sections spaced 300 μ m apart. The total number of detected orexin A-immunoreactive and orexin B-immunoreactive neurons are also depicted, in C and D respectively. Data are expressed as mean $\pm$ SEM. No significant differences were observed between the distribution and number of LHA orexin neurons between the *active* and *sedentary* animals.