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Negative results

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 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 2 groups. Thus, reduced activity in the elderly is unlikely to stem from a loss of orexin neuronal perikarya in the lateral hypothalamic area. 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.

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-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 14 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 7 AM–7 PM) and were cared for by the Division of Comparative Medicine at the Oregon National Primate Research Center in accordance with the National Research Council's

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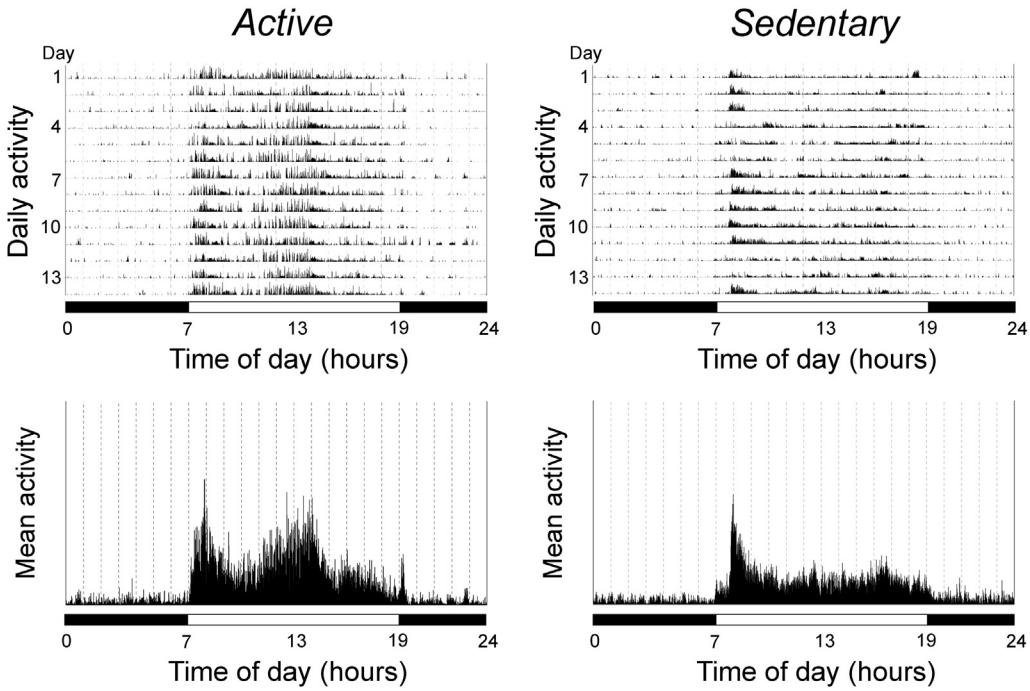

Fig. 1. Representative actograms from a representative *active* and a *sedentary* aged female rhesus macaques. In the upper panels, the height of the vertical lines within the actograms is indicative of the intensity of physical activity at any particular time of day; the mean 24-hour activity profiles 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.

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 hours and 1500 hours 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, 7 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 (±standard error of the mean) 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 (Students *t* test, $p > 0.05$). Five of the 7 animals in each group had either gone through menopause or had been ovariectomized, and so they had very low-circulating estradiol levels; 2 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 were processed for orexin A IHC, and a separate series was processed for orexin B IHC. Each series comprised 25-μm-thick sections collected at 300-μm 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 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-μm 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 with 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 2 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 and de Lecea, 2000), but very few studies of the orexin system have been conducted in aged humans (Fronczek