HHS Public Access

Author manuscript

Neurobiol Aging. Author manuscript; available in PMC 2018 June 01.

Published in final edited form as:

Neurobiol Aging. 2017 June ; 54: 100–102. doi:10.1016/j.neurobiolaging.2017.02.020.

Effect of Androgen Supplementation on 24-Hour Activity-Rest Patterns of Aged Male Rhesus Macaques

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Abstract

Like elderly men, old male rhesus macaques show attenuated circulating levels of testosterone and dehydroepiandrosterone sulfate (DHEAS), and many of them also show reduced levels of daytime activity. It is unclear, however, if this age-associated behavioral change is causally related to the underlying decrease in circulating androgen levels. To test this possibility, old male rhesus macaques were given daily supplements of testosterone and DHEA for 6 months, designed to mimic the mean 24-hour circulating hormone patterns of young adults. Compared to the young adults, the old controls showed attenuated daytime activity levels. However, there was no difference between the androgen supplemented old animals and the aged-matched controls, even after 6 months of treatment. The data suggest that age-associated decreases in circulating androgen levels are unlikely to be a primary reason for altered activity-rest patterns in elderly men, and that androgen supplementation paradigms might not provide any obvious therapeutic benefit.

Keywords

Aging; Androgens; Dehydroepiandrosterone; Rhesus macaque; Testosterone

1. Introduction

The circulating concentrations of many steroid hormones decrease during aging, and may contribute to the etiology of age-related pathologies such a cognitive decline and immune senescence (Engleman et al., 2011; Kohama et al., 2016; Rapp et al, 2003a; 2003b). In women and female rhesus macaques the most dramatic hormonal changes occur after menopause, as a result of the precipitous decrease in synthesis and release of estradiol and

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Disclosure statement

The author declares no conflicts of interest.

progesterone from the ovaries, as well as a marked decrease in dehydroepiandrosterone sulfate (DHEAS) from the adrenal glands (Downs and Urbanski, 2006; Downs et al., 2008; Sorwell et al., 2012). Although men and male rhesus macaques show less dramatic age-related decreases in the circulating levels of DHEAS and testosterone (T), these androgens have well-defined circadian rhythms. Consequently, their decrease during aging contributes to attenuation of circadian signaling throughout the body, which in turn may play a key role in the etiology of perturbed activity-sleep patterns that are so prevalent in the elderly (Downs et al., 2007; 2008; Sitzmann et al., 2014; Sorwell and Urbanski, 2013; Urbanski and Sorwell, 2012; Urbanski et al., 2014).

The aim of the present study was to test whether providing old male rhesus macaques with daily supplements of testosterone and DHEA for 6 months would lead to improved sleep-wake patterns, defined as increased activity during the day and less disruption of rest during the night. The daily androgen supplementation paradigm that we employed was previously developed in the same old rhesus macaques, and was shown to recapitulate mean youthful circulating levels of DHEAS, estradiol, estrone, 5α-dihydrotestosterone and T, while preserving their characteristic circadian profiles but not the underlying pusatile patterns (Urbanski et al., 2014).

2. Materials and methods

2.1. Experimental animals

A total of six young adult (mean age at end of study = 12 years) and twelve old (mean age at end of study = 24 years) male rhesus macaques (*Macaca mulatta*) were used in this Institutional Animal Care and Use Committee approved study, 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*. They were housed indoors in a temperature controlled environment (24°C) under a 12L:12D photoperiod (lights on from 0700 h–1900 h). Daily meals at ~0800 h and ~1500 h (LabDiet High Protein Monkey Chow, St. Louis, MO, USA) were supplemented with fresh fruits or vegetables; fresh drinking water was available *ad libitum*.

2.2 Androgen supplementation

Based on their age, and mean morning plasma T and DHEAS concentrations, the old animals were separated into two equal groups. Half of the old animals (n=6) received daily androgen supplementation, as previously described (Urbanski et al., 2014), while the other half (n=6) served as age-matched controls. The androgen supplementation comprised oral T administration (12 mg/kg body weight, at 1900 h) and two oral DHEA administrations (0.04 mg/kg body weight, at 0700 and 1000 h). Both T and DHEA were obtained from Sigma-Aldrich Corp (St. Louis, MO, USA). Previously, it had been shown that when T is administered orally in oil, significant quantities bypass the liver, presumably because of reduced passage into the hepatic portal system and increased uptake by the lymphatic system, and elevate circulating T concentrations (Amory and Bremner, 2005). Consequently, we suspended the T in sesame oil at a concentration of 120 mg/ml and then mixed it with ~12 g of chocolate or placed it inside a 5-g cookie, based on the animal's preference.

Similarly, we suspended the DHEA in sesame oil (10 mg/ml) and mixed it with chocolate or placed it inside a cookie. The control animals did not receive control treats or vehicle but were otherwise exposed to the same diet and the same environmental conditions. At the end of the study, the mean (± SEM) body weights of the young (n=6), old controls (n=6), and old supplemented animals (n=6) were 9.5 ± 0.8 kg, 10.9 ± 0.6 kg and 12.1 ± 1.1 kg, respectively, and their mean testis weights were 26.1 ± 2.6, 28.4 ± 2.4 and 21.3 ± 2.5 g; there were no significant (p>0.05) between-group differences for either body weight or testis weight (Kuskal-Wallis 1-way ANOVA). As expected, mean (± SEM) plasma DHEAS concentrations were significantly (p<0.05) lower in the old controls (43.7 ± 12.7 ng/ml) than in the young animals (126.8 ± 18.1 ng/ml) (Urbanski et al., 2014), but significantly (p<0.01) elevated (359.2 ± 50.8 ng/ml) in the old animals ~3 hours after DHEA supplementation (Mann-Whitney U-tests).

2.3. Monitoring of activity patterns

As previously described (Urbanski, 2011; Urbanski et al., 2012), each animal was fitted with an Actiwatch activity monitor (Philips-Respironics, Bend, OR, USA), worn inside a protective case that was attached to a lightweight loose-fitting aluminum collar (Primate Products, Inc., Immokalee, FL, USA). The animals were caged individually during the activity monitoring in a room that housed up to 16 animals. At the start of the study, continuous activity recordings were made for ~2 weeks and a characteristic baseline pattern was established for each animal. Approximately 6 months later, activity was again monitored for ~2 weeks. The actograms were subsequently analyzed using Actiware-Sleep (version 3.4) software (Cambridge Neurotechnology Ltd, Cambridge, UK). The mean total daily activity (defined as the average 24-hour activity) was calculated for each animal, as was the mean daytime activity (defined as activity during the period between 0700 h and 1900 h) and mean night-time activity (activity between 1900 h and 0700 h). Differences between treatment groups were compared using the Mann-Whitney U-test.

3. Results

Figure 1 shows representative mean 24-hour activity patterns from a young adult, an old control and an old androgen-supplemented animal after 6 months of study. All of the animals showed intense activity during the day and very little activity during the night (Table 1). The activity levels in the old controls and old androgen-supplemented animals did not change significantly between the start and the end of the study (Supplementary data Fig. S1). In both of these groups, however, the overall mean activity levels and mean daytime activity levels at the end of the study were significantly lower than in the young adults ($p < 0.01$). Importantly, there was no significant (p>0.05) difference in daytime or night-time activity levels between the old supplemented animals and the age-matched controls.

4. Discussion

As expected, circulating androgen levels and daytime activity were significantly lower in the old controls than in the young animals. Despite 6 months of daily androgen supplementation, however, the daytime activity levels of the old treated animals were significantly lower and no different than the activity levels observed in the old controls.

These data demonstrate that androgen supplementation in the elderly has no obvious beneficial effect on 24-hour activity levels, even when administered in a relatively physiological circadian manner and for an extended period. They are therefore consistent with a recent report that 1 year of testosterone supplementation in older men offered no obvious benefit with respect to vitality or walking distance (Snyder et al., 2016).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grants: AG-036670, OD-010426 and OD-011092. Daily care of the animals was performed by Jamie Garten, Vasilios Garyfallou, Katie Moore, Kevin Mueller, Krystina Sorwell, Alison Weiss and Brandee Winstead, with additional help from the ONPRC Division of Comparative Medicine.

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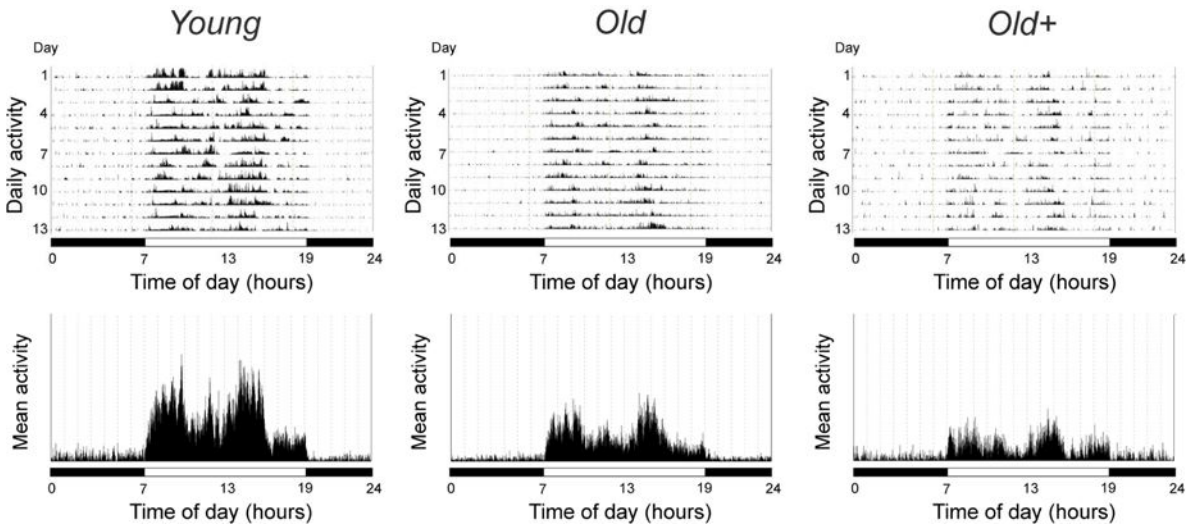

Fig. 1.

Representative actograms from a young adult and an old male rhesus macaque, as well as from an old male that received daily androgen supplementation (+) for 6 months. 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.

Table 1

Characterization of 24-hour activity patterns in male rhesus macaques

	YOUNG	*OLD*	*OLD+*
Average 24-hour activity	120.0 ± 8.2	83.0 ± 12.9**	88.6 ± 29.7**
Average daytime activity	215.3 ± 15.3	147.6 ± 24.2**	159.7 ± 57.9**
Average nighttime activity	24.6 ± 1.9	18.3 ± 3.0	17.5 ± 3.0

Values represent means ± SEM (n=6 animals per group).

OLD+ = 6 months of daily androgen supplementation.

** $P<0.01$, versus young animals (Mann-Whitney U-test).