Photoperiodic modulation of adrenal gland function in the rhesus macaque: effect on 24-h plasma cortisol and dehydroepiandrosterone sulfate rhythms and adrenal gland gene expression

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Abstract

In temperate zones, day length changes markedly across the year, and in many mammals these photoperiodic variations are associated with physiological adaptations. However, the influence of this environmental variable on human behavior and physiology is less clear, and the potential underlying mechanisms are unknown. To address this issue, we examined the effect of changing photoperiods on adrenal gland function in ovariectomized female rhesus macaques (Macaca mulatta), both in terms of steroid hormone output and in terms of gene expression. The animals were sequentially exposed to the following lighting regimens, which were designed to simulate photoperiods associated with winter, spring/autumn and summer respectively: 8 h light:16 h darkness (short days), 12 h light:12 h darkness and 16 h light:8 h darkness (long days). Remote 24-h serial blood sampling failed to disclose any effect of photoperiod on mean or peak plasma levels of cortisol or dehydroepiandrosterone sulfate. However, there was a marked phase-advancement of both hormonal rhythms in short days, which was reflected as a similar phase-advancement of the daily motor activity rhythm. Gene microarray analysis of the adrenal gland transcriptome revealed photoperiod-induced differences in the expression of genes associated with homeostatic functions, including: development, lipid synthesis and metabolism, and immune function. Taken together, the results indicate that in primates, both circadian adrenal physiology and gene expression are influenced by seasonal changes in day length, which may have implications for adrenal-regulated physiology and behavior. Journal of Endocrinology (2009) 201, 275–285

Introduction

In mammals, many aspects of physiology and behavior are temporally regulated, showing circadian as well as circannual rhythms. Whereas, circadian rhythms reflect the daily organization of body functions, circannual rhythms represent an adaptation to the seasonal variations that occur in a natural environment throughout the year. It is well established that circadian rhythms are intrinsic to a wide range of body functions, including the sleep–wake cycle, metabolism, immune response, and reproduction (Hastings et al. 2007). On the other hand, circannual rhythms have been reported in metabolism, reproduction, and immune function in a number of mammalian species (Bilbo et al. 2002, Nakao et al. 2008). In humans, seasonal variations have been reported for blood pressure, immune response, birth rate, and sleep duration, as well as for behavioral traits associated with seasonal affective disorders, bulimia nervosa, anorexia, and suicide (Bronson 2004).

Although the underlying mechanism that regulates circannual rhythms is unclear, there is evidence that seasonal neuroendocrine changes are among the main causal agents. In particular, circannual oscillations in one of the major neuroendocrine structures, the hypothalamus–pituitary–adrenal (HPA) axis has been regarded as a potential mediator of seasonal changes, a view supported by the major role played by adrenal steroids in physiology and behavior. This hypothesis stems from the fact that the HPA axis is temporally regulated by the circadian pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus, which drives the rhythmic secretion of two major adrenal steroids, cortisol, and dehydroepiandrosterone sulfate (DHEAS; Hastings 1991, Urbanski et al. 2004, Downs et al. 2008). Furthermore, light itself exerts a remarkable effect on adrenal physiology; in mice, light exposure at night induces both gene expression and the secretion of corticosterone, through a pathway that involves the SCN and the sympathetic nervous system (Ishida et al. 2005).

Although circannual variations have also been reported for adrenal corticoids, the available data are largely inconclusive. In the case of cortisol, some human studies have reported seasonal differences (Van Cauter et al. 1981, Levine et al. 1994, Walker et al. 1997, King et al. 2000, Hansen et al. 2001), whereas others have failed to disclose such variations.
(Agrimonti et al. 1982, Wehr et al. 1993, Van Dongen et al. 1998, Lac & Chamoux 2006). Similarly, some studies have reported seasonal differences in DHEAS levels (Deslypere et al. 1983, Nicolau et al. 1984, Garde et al. 2000), whereas one study found no seasonal difference (Bjornerem et al. 2006).

In the present study, we used the rhesus macaque animal model to examine if circannual changes in day length can significantly influence adrenal gland function. Specifically, our first aim was to examine the effect of different photoperiods on plasma corticosteroid rhythms, both in terms of magnitude and in relation to motor activity rhythms. Our second aim was to explore potential photoperiod-induced gene expression changes within the adrenal gland using gene microarray. The results show that day length affects specific parameters of the 24-h plasma cortisol and DHEAS rhythms as well as the daily activity–rest cycle. Moreover, seasonal-like changes in day length influence the expression of genes involved in development, lipid synthesis and metabolism, and immune response.

Materials and Methods

Animals
To avoid the confounding influence of changing sex-steroid concentrations across the menstrual cycle and across different photoperiods, the study used three long-term (> 3 months) ovariectomized adult female rhesus macaques (Macaca mulatta; age range: 8.5–12 years old). Each animal was fitted with an indwelling sub-clavian vein catheter, as previously described (Downs et al. 2008), which remained implanted for the duration of the study. The animals were caged singly in a temperature-controlled environment, with fixed light cycles that comprised 12 h of light per day (i.e. 12 h light:12 h darkness), and were cared for by the Oregon National Primate Research Center (ONPRC) Division of Animal Resources, in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals.

As part of a longitudinal experimental design, the animals were then sequentially exposed to the following three photoperiods: 8 h light:16 h darkness; 12 h light:12 h darkness and 16 h light:8 h darkness, for 10 weeks in each case. These three photoperiods were selected because they represent the winter, spring equinox, and summer respectively in Oregon. Note, in order to further facilitate the comparison of phases between the photoperiods, the time of lights on was fixed at 0700 h for each photoperiod. Primate chow (Purina Mills Inc., St Louis, MO, USA) was made available to the animals twice daily, at 0800 h and again at 1500 h. This diet was supplemented with fresh fruit and vegetables, which were provided at the time of the afternoon meal; drinking water was available at all times.

For the gene microarray analysis, nine ovariectomized adult (age range: 8.5–12 years old) female rhesus macaques were used. The animals (three per light regimen) were maintained under the photoperiods 12 h light:12 h darkness, 8 h light:16 h darkness and 16 h light:8 h darkness for 10 weeks. At the end of that period, the animals were anesthetized with ketamine (15–25 mg/kg i.m.) followed by pentobarbital sodium (25–30 mg/kg i.v.) and exsanguinated. This method of euthanasia is consistent with the recommendations of the American Veterinary Medical Association’s Panel on Euthanasia. In all cases, necropsies were performed within a narrow window of time (1000–1300 h). Various postmortem tissues were collected and made available to other investigators through the ONPRC Tissue Distribution Program. This research was approved by the Institutional Animal Care and Use Committee.

Activity recording and analysis

The activity–rest cycles of individual animals were continuously monitored using Actiwatch activity recorders (Mini Mitter Company Inc., Bend, OR, USA). Measure of gross motor activity by the accelerometers was digitally integrated into activity bouts of 5 min duration. Analysis of the activity bouts was performed using Sleepwatch software (Mini Mitter Company Inc). Total daily activity was estimated by calculating the average number of bouts per day, per animal, over time. Total photophase and scotophase activity were calculated by measuring the average number of bouts per animal, during photophase and scotophase respectively. Cosine correlation was used to estimate the phase of motor activity rhythm in each photoperiod.

Remote 24-h blood sampling

After ~10 weeks of exposure to each of the three photoperiods, blood samples were collected remotely from an adjacent room, via the indwelling vascular catheter and a swivel/tether-based sampling system (Downs et al. 2008). Beginning at 0700 h, hourly blood samples were collected from the undisturbed animals for an entire day, including their sleep period. The blood was immediately transferred into EDTA-coated borosilicate glass tubes, centrifuged at 4 °C, and the plasma supernatant was stored at −20 °C until assay.

Hormone assays

Plasma concentrations of cortisol and DHEAS were determined at the ONPRC Endocrine Services Laboratory as previously described (Downs et al. 2008). Briefly, cortisol levels were measured by electrochemiluminescence using an Elecsys 2010 Platform (Roche Diagnostics). DHEAS levels were determined by RIA, using an antibody raised against DHEAS-17-[(O-carboxymethyl)oxime-BSA, and [3H]DHEAS EAS (22 Ci/mmol). Intra-assay and inter-assay coefficients of variation were <10% for each assay. Assay detection limits were 3 ng/ml for both the steroids.
**Terminology and statistical analysis**

The terms photophase, and scotophase refer to the illuminated and the dark segment of a light–darkness cycle respectively. Rhythmic parameters, including amplitude and mesor were determined by Cosinor analysis. The acrophase measures the timing of a rhythm relative to a reference time point defined by the investigator, and is used for data which fits a mathematical model; for example, in a cosine curve fit the acrophase represents the rhythm’s peak. The mesor represents the adjusted mean of a cosine function fitted to the data. Statistical comparisons for motor activity, as well as hormone measurements, were performed using repeated measures ANOVA followed by the Bonferroni test.

**RNA extraction and gene microarray**

During necropsy, the adrenal glands were quickly frozen in liquid N\textsubscript{2} and total RNA was extracted using RNAeasy columns (Qiagen); the final concentration and purity were determined by spectrophotometry, and the integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technology, Palo Alto, CA, USA). Complementary cDNA synthesis, cRNA synthesis, hybridization, and array scanning were performed by the OHSU Affymetrix Microarray Core, as described in the Affymetrix GeneChip Expression Analysis Technical Manual. As in previous studies (Wang et al. 2004, Dillman & Phillips 2005), the Affymetrix human HG_U133A gene microarray platform was used. RNA samples from individual adrenal glands were hybridized separately on their own GeneChip arrays.

Raw scanner image files were analyzed using the Affymetrix MAS 5.0 absolute expression analysis comprised in the GeneSifter software (Greospiza Inc, Seattle, WA, USA). The primers and probes were designed based on human sequences, using PrimerExpress software (Applied Biosystems), and purchased from Invitrogen and Sigma Genosys respectively.

The sequences were: NCKAP1-F, CATTGGCACAAGAAGCATTAGAG; NCKAP1-R, GGTAAATCGGCAAGTGTA, NCKAP1 probe, 5′-6FAM-CCACATCCCTTTTCTTTGTAAGTTCTC-TAMRA-3′; FADS1-F, CCCCTGATGACTGTTGCTTGGTCTCTTGTGTTAT; FADS1 probe, 5′-6FAM-TCCAAGCCCTCTGCTGCAGC-TAMRA-3′; IL11-F, GGCTGACCTGACACTTGGACT; IL11-R, TGTTTCGCCCCAGTACTG; IL11 probe, 5′-6FAM-TGCTGTGAGAAGACTCGGCTGTGGAACCT-TAMRA-3′; ACSL1-F, CAAGGCCCTGGCCCCAGACAGC; ACSL1-R, TGGGCGAGGATGACTG TATTCT, ACSL1 probe, 5′-6FAM-CCACACTCATGACACACCTGAAACT; ACSL1 probe, 5′-6FAM-TGCTGTGGAACCT-TAMRA-3′; β-actin-F, CTGACATCCGCAAAAGAC; β-actin-R, GGGCGGTGATCTCCTTCTG; β-actin probe, 5′-VIC-TGCTGTGATGCGCGGACCACC-TAMRA-3′.

**Results**

**Effect of photoperiod on plasma cortisol and DHEAS rhythms in the rhesus macaque**

Mean 24-h plasma cortisol rhythms, obtained under each of the three photoperiods are depicted in Fig. 1A. No significant differences were detected in either the mesor or amplitude (Table 1). The acrophases under both the 12 h light:12 h darkness and the 16 h light:8 h darkness photoperiods were attained around the time when lights came on (i.e. at 0700 h), but a significant phase advancement was disclosed under 8 h light:16 h darkness (P<0.05 vs 12 h light:12 h darkness; P<0.01 vs 8 h light:16 h darkness); notably, plasma cortisol levels reached a maximum while the animals were still in the darkness phase of their daily photoperiodic cycle (Fig. 1A, Table 1). Although the ascending phase of the 24-h plasma cortisol rhythm appears to be shorter in 8 h light:16 h darkness, this change was not statistically significant (Table 1). Mean 24-h plasma DHEAS rhythms, obtained under each of the three photoperiods are depicted in Fig. 1B. No significant differences were detected in either the mesor or amplitude, although both parameters showed a tendency to decrease under the 8 h light:16 h darkness photoperiod (Table 2). Under both the 12 h light:12 h darkness and the 8 h light:16 h darkness photoperiods the acrophase of the DHEAS rhythm occurred ~3–4 h after the lights came on in the morning; by contrast, under 8 h light:16 h darkness the acrophase occurred significantly earlier (P<0.05 vs 12 h light:12 h darkness and 8 h light:16 h darkness), within an hour of the beginning of the light phase (Fig. 1B, Table 2). As with cortisol, the ascending phase of the 24-h plasma DHEAS rhythm appeared to be shorter in 8 h light:16 h darkness, but this change was not statistically significant (Table 2).
Effect of photoperiod on motor activity

Activity–rest profiles from a representative animal, obtained under each of the three photoperiodic regimens are depicted in Fig. 2. One-way ANOVA analysis of total activity spectra from the three animals showed that there was no significant difference in average total activity, or average light-phase activity between the different photoperiods (Fig. 2B). Interestingly, the hourly activity during the light phase, although not significantly different, tended to be higher under 8 h light:16 h darkness than

![Graphs showing the effect of photoperiod on motor activity](image)

Figure 1 Effect of photoperiod on the 24-h circulating (A) cortisol and (B) DHEAS rhythms in female rhesus macaques. Plasma samples were collected from the same animals after 10 weeks of exposure to each of the three photoperiods. Values are expressed as means ± S.E.M. (N = 3). Note, the data are double plotted to aid visualization of circadian changes. Vertical dashed lines within each panel indicate the acrophases. The vertical dashed line across all panels indicates the beginning of the light phase. The horizontal white and black bars represent day and night respectively.

Table 1 Effect of photoperiod on plasma cortisol in rhesus macaques

<table>
<thead>
<tr>
<th>Photo period</th>
<th>Mesor (mean ± S.E.M.)</th>
<th>Amplitude (mean ± S.E.M.)</th>
<th>Acrophase (h, min)</th>
<th>Rising period (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 h light:16 h darkness</td>
<td>79·4±3·9</td>
<td>64·9±10·5</td>
<td>0448±0013</td>
<td>1100±0109</td>
</tr>
<tr>
<td>12 h light:12 h darkness</td>
<td>96·9±8·9</td>
<td>73·5±5·1</td>
<td>0724±0012*</td>
<td>1118±0118</td>
</tr>
<tr>
<td>16 h light:8 h darkness</td>
<td>76·1±11·9</td>
<td>61·0±5·9</td>
<td>0757±0014†</td>
<td>0918±0150</td>
</tr>
</tbody>
</table>

Statistical comparisons were made by repeated measures ANOVA, followed by Bonferroni test (*P < 0·05, †P < 0·01, vs 8 h light:16 h darkness). No significant differences were found for mesor, amplitude or rising period (P > 0·05).

Table 2 Effect of photoperiod on plasma dehydroepiandrosterone sulfate (DHEAS) in rhesus macaques

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Mesor (mean ± S.E.M.)</th>
<th>Amplitude (mean ± S.E.M.)</th>
<th>Acrophase (h, min)</th>
<th>Rising period (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 h light:16 h darkness</td>
<td>105.4 ± 24.0</td>
<td>55.8 ± 5.2</td>
<td>0744 ± 0020</td>
<td>1018 ± 0112</td>
</tr>
<tr>
<td>12 h light:12 h darkness</td>
<td>125.6 ± 32.8</td>
<td>63.7 ± 12.4</td>
<td>1012 ± 0012*</td>
<td>1000 ± 0034</td>
</tr>
<tr>
<td>16 h light:8 h darkness</td>
<td>91.2 ± 21.8</td>
<td>36.6 ± 5.0</td>
<td>1011 ± 0004*</td>
<td>0840 ± 0140</td>
</tr>
</tbody>
</table>

Statistical comparisons were made by repeated measures of ANOVA, followed by Bonferroni test (*P < 0.05, vs 8 h light:16 h darkness). No significant differences were found for mesor, amplitude or rising period (P > 0.05).

Figure 2 Effect of photoperiod on motor activity in ovariectomized female rhesus macaques. (A) Left panels: representative actograms from an individual animal that was exposed sequentially for 10 weeks to 8 h light:16 h darkness, 12 h light:12 h darkness and 16 h light:8 h darkness lighting regimens. Note, the activity data are double plotted to aid visualization of circadian changes. Right panels: representative mean activity during the 10-week periods. The arrow indicates the advancement of activity onset, and the vertical dashed line indicates the beginning of the diurnal phase. The horizontal white and black bars indicate day and night respectively. (B) Cosinor analysis was used to assess motor activity variables over each 10-week period. Comparisons were made using repeated-measures ANOVA followed by Bonferroni test. Values represent the means ± S.E.M. from all three animals. *P < 0.05, **P < 0.01.
12 h light:12 h darkness or 8 h light:16 h darkness (data not shown). On the other hand, average darkness-phase activity was clearly higher under 8 h light:16 h darkness than 12 h light:12 h darkness or 16 h light:8 h darkness (P < 0.05 vs 12 h light:12 h darkness; P < 0.01 vs 16 h light:8 h darkness; Fig. 2B).

In addition, exposure to the 8 h light:16 h darkness photoperiod was associated with earlier activity onset (Fig. 2A), which occurred during the dark phase, while the lights were still off, as well as a significant advancement of the activity acrophase, compared with the 16 h light:8 h darkness photoperiod (P < 0.05, Fig. 2B).

**Effect of photoperiod on adrenal gland gene expression**

Rhesus macaque gene microarrays (Affymetrix, Santa Clara, CA, USA) were used to study the effect of photoperiod on the adrenal transcriptome. Gene expression profiles were obtained from long-term ovariectomized animals that had been maintained for 10 weeks in either 8 h light:16 h darkness, 12 h light:12 h darkness or 16 h light:8 h darkness photoperiods, with three animals per group. Comparisons were made between 12 h light:12 h darkness versus 8 h light:16 h darkness, and 12 h light:12 h darkness versus 16 h light:8 h darkness, with fold changes higher than 1.8 or lower than 1.8 being considered significant. A gene annotation tool was used to obtain gene abbreviations and descriptions. The data analysis revealed three main functional clusters: 1) development, 2) lipid synthesis and metabolism, and 3) immune function.

**Genes identified as being differentially expressed after exposure to short photoperiods**

Genes that were differentially regulated in 8 h light:16 h darkness compared with 12 h light:12 h darkness are depicted in Fig. 3. The set of genes associated with development included genes that play functional roles in morphogenesis (HOXD11, ALX3, WNT2), cytoskeletal regulation (NCKAP1), axonal growth (SEMA6B, ROBO1), and neural cell development (NPTX1). The set of genes associated with lipid synthesis and metabolism included genes that play functional roles in the activation of long-chain fatty acids (ACSL1), cytoskeletal regulation (ALX3), axonal growth (ROBO1), and neural cell development (NPTX1). The set of genes associated with immune response included genes that encode chemokine ligands (XCL2, CXCL4), and genes that are involved in peripheral inflammation (TACR1), virus response (IRF4, IFNA10), and early activation of T cells (CD69), among others. Differences in the expression of NCKAP1, IL-11, and FADS1 between the long (16 h light:8 h darkness) and medium (12 h light:12 h darkness) photoperiods were corroborated using qRT-PCR (Fig. 5).

**Discussion**

Day/night cycles can exert a profound effect on physiology and behavior, as well as gene expression (Hastings et al. 2003). On the other hand, it is unclear how seasonal changes in environmental conditions can influence these parameters, especially in humans and non-human primates. To address this issue, we examined the impact of photoperiodic manipulation on circadian functions and adrenal gene expression in a diurnal non-human primate, the rhesus macaque.

In their native habitat, in Northern India and China, these monkeys restrict their breeding activity to autumn and winter. This biological adaptation ensures that their offspring are born when environmental conditions are more favorable for survival, in the late spring and summer. The well-defined breeding season of rhesus macaques is especially evident at more extreme latitudes (e.g., Oregon at ~45° North), where the most likely proximate factor (Baker 1938) synchronizing it to the environment is the annual change in photoperiod (Urbanski 1995). Although less obvious, a seasonal reproductive rhythm also occurs in humans, with presumed conception rates peaking around the time of the vernal equinox (Roenneberg & Aschoff 1990, Bronson 1995, 2004). Consequently, both rhesus macaques and humans may show some seasonal fluctuation in circulating sex-steroid concentrations, which would be partially masked by the marked concentration changes that occur across the menstrual cycle. In female rhesus macaques and women, the menstrual
cycle is remarkably similar, with a peak of circulating estradiol concentrations occurring during the late follicular phase and a peak of progesterone concentrations occurring during the mid-luteal phase (Urbanski 1995, Downs & Urbanski 2006). It was unclear to us, whether seasonal and monthly fluctuating sex-steroid concentrations could affect adrenal gland function (Fonseca et al. 2001, Stavisky et al. 2003), and so as to avoid this potentially confounding issue, we performed our study using long-term ovariectomized rhesus macaques, exclusively.

The animals were chronically maintained under each of the three photoperiods, which were selected because they resemble the natural photoperiods that occur in temperate zones during the winter, spring/autumn, and summer. Also, the use of a moderately long 16 h light:8 h darkness photoperiod to mimic summer was expected to induce a larger effect on clock-mediated gene expression, than the use of a very long photoperiod (> 20 h of light per day; Wagner et al. 2007). Although natural photoperiods increase and decrease gradually across the seasons, we chose to use more abrupt photoperiodic transitions. This ensured that the animals had a full 10 weeks to stabilize their physiological rhythms to each new photoperiod. Equally important, by limiting the exposure to only 10 weeks, we reduced the chances of developing photorefractoriness. This condition is characterized by a spontaneous reversion in physiology to that

Figure 3 Effect of short days on adrenal gland gene expression. The gene microarrays were analyzed using the algorithm MAS 5.0. (A) Histogram depicting functional clustering of genes differentially regulated after exposure to short photoperiod. (B) Table of genes involved in development, lipid synthesis and metabolism, and immune response, differentially expressed in 8 h light:16 h darkness versus 12 h light:12 h darkness (P < 0.05). Statistical comparisons were made by Students t-test.

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of the previous photoperiodic condition, and has been observed in other species, such as sheep, when they are exposed to constant photoperiods for extended periods (Lincoln et al. 2005). At the end of each photoperiodic treatment, the serial collection of blood samples at multiple time points across the day and night, enabled changes in plasma hormone rhythms to be determined more precisely.

The results indicate that exposing the animals longitudinally to the different photoperiods had no obvious impact on either plasma cortisol mean levels or amplitude. On the other hand, these photoperiodic manipulations did affect some circadian aspects of the hormonal rhythm, especially the acrophase and rising kinetics. Also, the nocturnal periods of rising cortisol levels changed in response to the duration of the scotoperiod. These findings are in agreement with the clinical results of Wehr et al. (1993), which showed adaptive changes in the rhythm of cortisol when human subjects were chronically maintained under controlled experimental conditions. They also support the idea that the rhythm-generating component of the cortisol secretion mechanism undergoes adjustments in response to length of day, or duration of scotophase. In the present study, a significant

Figure 4 Effect of long days on adrenal gland gene expression. The gene microarrays were analyzed using the algorithm MAS 5.0. (A) Histogram depicting functional clustering of genes differentially regulated after exposure to short photoperiod. (B) Table of genes involved in development, lipid synthesis and metabolism, and immune response, differentially expressed in 16 h light:8 h darkness versus 12 h light:12 h darkness (P<0.05). Statistical comparisons were made by Students t-test.
monkey activity data are in complete agreement with those of perspective (i.e. relative to mid-day rather than dawn), our ANOVA, followed by Bonferroni test * up at 1300 h). Under 16 h light:8 h darkness, however, they woke up at 0700 h, which was 8 h before the middle of the day (i.e. at 1500 h). When viewed from this alternative phase-advancement of ~2.5 h was evident for the cortisol rhythm under short photoperiods, reaching the acrophase in the dark phase.

In addition, we observed that the phase of the motor activity rhythm adjusts to photoperiodic changes. An advance in both the onset and phase of the activity rhythm were observed under short days, resulting in higher levels of activity during the scotophase. Since the phase-advancement in cortisol rhythm parallels that of the activity rhythm a causal relationship may exist between the two rhythms. It could be hypothesized that, in view of its physiological functions, an earlier peak of cortisol helps an individual to achieve a state of arousal earlier in the day, thus facilitating an earlier awakening relative to dawn. In natural environments, short photoperiods are generally associated with the onset of unfavorably low temperatures and a scarce food supply. In this context, the advancement of the activity rhythm and other physiological functions in the winter would help to optimize the use of a shorter light phase. Another way of interpreting these data is that the animals centralize their daily activity around the middle of the day, regardless of photoperiod. This means that in short winter photoperiods, their daily activity onset occurs several hours before dawn. Note that in our study, we kept dawn fixed for each of the photoperiods, at 0700 h. Thus, under 8 h light:16 h darkness the animals woke up at ~0500 h, 6 h before the middle of the day (i.e. at 1100 h). Similarly, under 12 h light:12 h darkness they woke up at ~0700 h, again 6 h before the middle of the day (i.e. at 1300 h). Under 16 h light:8 h darkness, however, they woke up at ~0700 h, which was 8 h before the middle of the day (i.e. at 1500 h). When viewed from this alternative perspective (i.e. relative to mid-day rather than dawn), our monkey activity data are in complete agreement with those of Honma et al. (1992), which showed that humans wake-up earlier in summer than in winter.

Also in agreement with previous human studies (Wehr et al. 1993), we did not observe changes in daily average plasma cortisol levels or amplitude, in response to different day lengths. Consequently, if such variations do occur seasonally, they are unlikely to be triggered by changes in the photoperiod per se. A potential effect of other external factors such as temperature, diet or social habits, which show seasonal variations in increased latitudes, cannot be excluded.

Analysis of the DHEAS rhythm did not reveal an effect of day length on either plasma DHEAS mean levels or amplitude, although both values tended to decrease slightly during exposure to a long photoperiod; more conclusive analysis of these parameters would necessitate a larger number of animals, to reduce the impact of high variability between individuals. Nevertheless, the lack of an obvious photoperiodic impact on the magnitude of plasma DHEAS levels contrasts with the marked attenuation of circulating DHEAS concentrations that have been observed during aging (Downs et al. 2008, Perret & Aujard 2005). Similar to cortisol, a significant phase advancement of ~2.5 h was observed for DHEAS under short photoperiods. Considering the reported effects of DHEAS within the central nervous system, this circadian alteration may have important behavioral consequences. Although no receptor has been reported for DHEAS to date, this steroid acts as an excitatory neuromodulator with proconvulsant activity (Carette & Poulin 1984, Demirgoren et al. 1991). It has been reported that DHEAS acts both as a negative allosteric modulator on GABA A receptors and as a positive modulator on glutamate NMDA receptor activity. In support of this view is the finding that DHEAS increases the excitability of CA3 neurons in the hippocampus (Bergeron et al. 1996), suggesting that DHEAS helps to promote a general arousal state. In agreement with the phase-shifts observed for the plasma cortisol and activity rhythms, the phase-advancement in DHEAS rhythm may be interpreted as another component in the adaptation to short winter days, allowing for an earlier increase in the alert/arousal state.

The gene microarray and real-time PCR data demonstrate that photoperiod can influence gene expression in the primate adrenal gland, upregulating the expression of certain genes while downregulating the expression of others. The microarray analysis focused on changes in gene expression that are likely to occur during the annual cycle, especially after the transition from a winter photoperiod to a spring/autumn photoperiod, and from the latter to a summer photoperiod. The three main sets of genes identified (i.e. development, lipid synthesis and metabolism, and immune response) suggest that the adrenal gland undergoes both structural and functional changes as an adaptive response to long-term exposure to both short and long photoperiods. This hypothesis is supported by our observation that homeobox regulators are differentially expressed in both environmental conditions.

The adrenal gland is a dynamic organ that undergoes structural changes as part of its adaptive role in stress.

Figure 5 Expression levels for NCKAP1, FADS1, IL-11, and ACSL1, determined by Taqman qRT-PCR Values are expressed as means ± S.E.M. (N=3). Statistical comparisons were made by one way ANOVA, followed by Bonferroni test *P<0.05, **P<0.01.
response. Ultrastructural changes in this gland have been reported in response to environmental stressors such as noise (Soldani et al. 1999) and heat (Koko et al. 2004). More importantly, from the perspective of the present study, the effect of light on gene expression in the adrenal gland has previously been examined in rodents. For example, a brief light stimulus (400 lux, 30 min) was shown to affect gene expression in the adrenal glands of mice kept under constant darkness, through a pathway that involves the SCN and the autonomic nervous system (Ishida et al. 2005). As demonstrated by Kalsbeek et al. (2007), signals from the SCN are transmitted to peripheral organs, including the adrenal glands, through a pathway that involves vasopressin secretion activating preautonomic neurons of the paraventricular nucleus, and autonomic neurons from the intermediolateral column. Furthermore, exposure of male rats to either constant light or constant darkness was found to upregulate the expression of genes that encode catecholamine biosynthetic enzymes in the adrenal gland (Gallara et al. 2004). In keeping with these previous reports, our results demonstrate that some genes can be activated by exposure to extreme photoperiods (i.e. either short days or long days), and suggest that common pathways may be involved. In the natural environment, photoperiodic extremes are separated by the vernal and autumnal equinoxes (i.e. 12 h light:12 h darkness), which could allow resetting of these genes to a more basal level twice per year.

The gene expression data also suggest that the primate adrenal gland may be capable of responding to changes in photoperiod by undergoing tissue remodeling. These structural changes do not seem to affect the endocrine functions of the adrenal cortex; instead, they likely constitute cellular adaptations that allow the tissue to maintain normal function under environmental conditions associated with long and short photoperiods (e.g., high and low temperatures respectively, and changing food availability). A potential candidate that could underlie the observed changes in gene expression is the pineal hormone melatonin. In mammals, melatonin secretion conveys photoperiodic information to the rest of the body, in part by modulating the secretion of other hormones. In addition, melatonin receptor activation regulates gene expression through a pathway that involves protein kinase C and the extracellular signal-regulated kinases 1 and 2 (Sainz et al. 1999, Roy & Belslham 2002). Expression of MT1 melatonin receptor has been reported in the primate adrenal gland, and it has been suggested that melatonin may play a role in regulating adrenal clock–gene expression (Valenzuela et al. 2008). Therefore, although the autonomic nervous system is known to regulate adrenal gland function, it is plausible that a melatonin-mediated pathway plays a key role in the seasonal modulation of adrenal gland gene expression.

In summary, the present study represents the first multilevel analysis of photoperiod-induced responses in a non-human primate, involving 24-h endocrine profiling, whole animal behavioral observations, as well as gene expression analysis. Although the responses of rhesus macaques to photoperiodic manipulations may be more pronounced than those of humans, the similar organization and endocrine function of the rhesus and human adrenal glands (Conley et al. 2004, Abbott & Bird 2008), emphasize the translational importance of our data. Together, they provide a new perspective on the effects of seasonal variations in photoperiod on primate behavior, physiology, and gene expression, and may have clinical value in the development of therapies for seasonal human pathophysiology, such as seasonal affective disorders (Wirz-Justice et al. 1984).

Declaration of interest

The authors declare that there was no conflict of interest that would prejudice the impartiality of the research reported.

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