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Estradiol treatment improves biological rhythms in a preclinical rat model of menopause

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ABSTRACT

The perimenopausal transition at middle age is often associated with hot flashes and sleep disruptions, metabolic changes, and other symptoms. Whereas the mechanisms for these processes are incompletely understood, both aging (AG) and a loss of ovarian estrogens play contributing roles. Furthermore, the timing of when estradiol (E) treatment should commence and for how long are key clinical questions in the management of symptoms. Using a rat model of surgical menopause, we determined the effects of regimens of E treatment with differing time at onset and duration of treatment on diurnal rhythms of activity and core temperature and on food intake and body weight. Reproductively mature (MAT, ~4 months) or AG (~11 months) female rats were ovariectomized, implanted intraperitoneally with a telemetry device, and given either a vehicle (V) or E subcutaneous capsule implantation. Rats were remotely recorded for 10 days per month for 3 (MAT) or 6 (AG) months. To ascertain whether delayed onset of treatment affected rhythms, a subset of AG-V rats had their capsules switched to E at the end of 3 months. Another set of AG-E rats had their capsules removed at 3 months to determine whether beneficial effects of E would persist. Overall, activity and temperature mesor, robustness, and amplitude declined with AG. Compared to V treatment, E-treated rats showed (1) better maintenance of body weight and food intake; (2) higher, more consolidated activity and temperature rhythms; and (3) higher activity and temperature robustness and amplitude. In the AG arm of the study, switching treatment from V to E or E to V quickly reversed these patterns. Thus, the presence of E was the dominant factor in determining stability and amplitude of locomotor activity and temperature rhythms. As a whole, the results show benefits of E treatment, even with a delay, on biological rhythms and physiological functions.

1. Introduction

During aging in women, declines in biological rhythms and the loss of ovarian estrogens converge to influence health and disease processes. Functions such as energy balance and metabolism, sleep, and hot flashes—all regulated by the hypothalamus in a circadian manner—are highly sensitive to age, the estrogenic milieu, and their interactions (Landau and Zucker, 1976; Mauvais-Jarvis et al., 2013; Poehlman et al., 1995; Toth et al., 2000). Within the hypothalamus, the suprachiasmatic nucleus, the master circadian clock (Reppert and Weaver, 2002; Schwartz et al., 1983; Yoo et al., 2005), undergoes age-related dampening in its oscillatory rhythms and output (Davidson et al., 2008; Duncan et al., 2013; Gibson et al., 2009; Krajnak et al., 1998; Mattis and Sehgal, 2016; Oster et al., 2003; Sutin et al., 1993; Whealin et al., 1993; Wise et al., 1988; Wyse and Coogan, 2010; Zhang et al., 1996). The suprachiasmatic nucleus projects to, and receives afferent inputs from, other hypothalamic and nonhypothalamic regions, thereby influencing energy balance (e.g., arcuate nucleus [ARC] [Elias et al., 2000; Wang et al., 2014]), thermoregulation (median preoptic area [Mittelman-Smith

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et al., 2015]), and other physiological processes. These regions are highly estrogen responsive due to high expression of estrogen receptors (Chakraborty et al., 2003; Chung et al., 2007; Gundlah et al., 2000; Kruijver and Swaab, 2002; Naugle et al., 2014; Vida et al., 2008).

The perimenopausal period is a point of divergence for the trajectory of quality-of-life during the last third to half of a woman's life. Although there is no question that the most efficacious way to mitigate many of the adverse menopausal symptoms is with estrogen treatment, there are also risk factors. In 2002, the Women's Health Initiative, a large clinical trial of risks and benefits of estradiol (E) treatment in post-menopausal women, was abruptly terminated because of a small but significant increase in adverse cardiovascular and breast cancer outcomes (Kim et al., 2007). This led to a vast decline in the use of E treatment for symptomatic women. However, over the next decade, re-examination of that study revealed health *benefits* of estrogens for women who commenced treatment in the perimenopausal or early postmenopause period (Baber et al., 2016; Stuenkel et al., 2015), suggesting that the timing of replacement is key to health outcomes. Today, the primary clinical questions for symptomatic women without major risk factors (e.g., breast cancer, thromboembolism) center around when and for how long to take E—that is, whether there is a window of opportunity for E's protective effects, and whether effects may be maintained after withdrawal. This question has not been rigorously tested for neurobiological health with only few exceptions (Baxter et al., 2018; Garcia et al., 2016; Garcia et al., 2017a; Garcia et al., 2017b; Gibbs, 2000; Yin et al., 2015a).

Here, we used our laboratory's rat model of surgical menopause to obtain quantitative data about aging, biological rhythms, and the influence of E timing/duration. Previously, we used this model to determine the neuromolecular phenotype of the hypothalamus [arcuate nucleus and medial preoptic area (Garcia et al., 2016; Garcia et al., 2017a; Garcia et al., 2017b; Yin et al., 2015a)] and to measure social behaviors (Garcia et al., 2017a; Garcia et al., 2017b). In general, results showed that it was the absolute absence or presence of E that determined the phenotype, yet there were subtle but significant effects of timing and duration on a subset of outcomes. In the present study, we sought to extend that work through studies of the critical window of E treatment on biological rhythms.

2. Materials and methods

2.1. Experimental animals and husbandry

Female Sprague-Dawley rats were purchased at 3–4 months (young reproductive mature [MAT]; virgin) and 10–11 months (middle-aged [referred to as AG; retired breeder]) from Harlan (Houston, TX). On arrival, rats were randomly pair-housed with a rat of similar age in plastic cages with 140 square inches of floor space. Rats were maintained on a 12:12 LD cycle (lights-on at 0700 hours). Room temperature was ~22° C. Food and water were available *ad libitum*. The composition of the pelleted diet (Prolab RMH, 1800, PMI Nutrition Int'l, LLC) for rats was used throughout the study. The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin, and all work adhered to guidelines from The Guide for the Care and Use of Experimental Animals.

Six groups of telemetry monitored rats were used (Fig. 1). Each experimental subject (n = 79) was pair housed with a same age and same treatment partner (n = 79) to study long-term hormone deficiency and treatment on physiological and circadian parameters. Pairs were maintained through the study to enable companionship and minimize stress. Rats were gently handled and their general health checked every 5 days. After adapting to our animal room environment for a week, the estrous cycle of each rat was determined for 10 consecutive days using vaginal cytology as previously described (Yin et al., 2015a). At age 3–4 months, all MAT rats showed regular estrous cycles. At age 10–11 months, the percentage of rats with regular cycles, irregular cycles, and persistent estrus was ~50%, 30%, and 20% respectively, similar to a previous study that did not reveal preovariectomy cycle stage to be a factor in biological outcomes (Yin et al., 2015a). We monitored body weight and food intake for 10 days before ovariectomy (OVX) surgery. Pelleted diet was weighed and delivered to each cage every 5 days. Food consumption was estimated based on the 10-day average of the pair-housed females.

2.2. Surgery procedures

Bilateral OVX was performed under isoflurane anesthesia per our published protocols (Yin et al., 2009; Yin et al., 2015b). For each pair

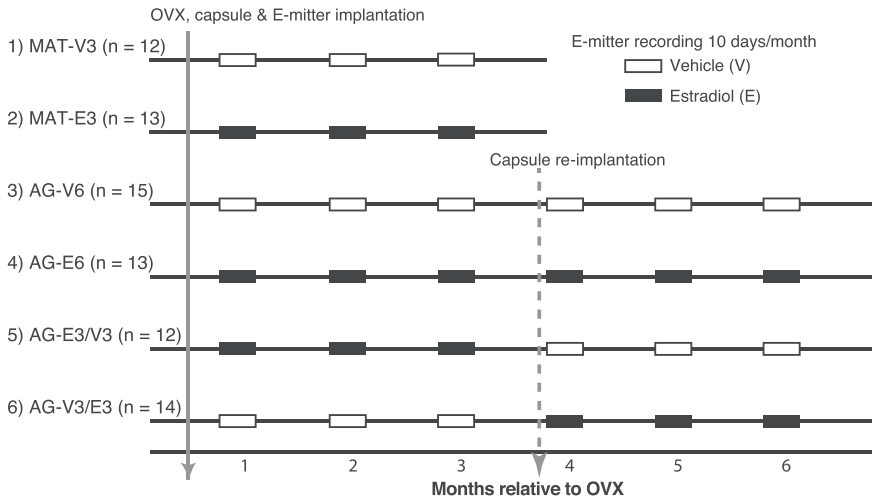

Fig. 1. Experimental design to compare effects of age, and timing and duration of estradiol or vehicle treatment, on circadian parameters. Ovariectomized (OVX) rats with an implanted telemetry device (E-mitter) were recorded on a transmitter/receiver device for 10 days per month beginning at ~4 months of age (reproductively mature, MAT) or beginning at ~12 months of age (reproductive aging, AG). Groups received estradiol (E) or vehicle (V) for 3 or 6 months as indicated, with two of the AG groups switched from V to E or from E to V.

of rats housed together, both were ovariectomized and received the same hormone treatment, but only one rat from the pair was randomly chosen to receive a telemetry device (PDT 4000 E-mitter transponder, Philips Respironics, Bend, OR), implanted in the peritoneal cavity and sutured to the right abdominal muscle during the OVX surgery. At the end of the surgery, a Silastic capsule filled with either 5% 17β-E (Sigma E-8875) in cholesterol (Sigma C-3292) for E treatment or 100% cholesterol for vehicle (V) treatment was subcutaneously implanted intrascapularly, as described (Yin et al., 2009; Yin et al., 2015b). After surgery, each rat was singly housed for 5 days to recover before rehousing with the same partner.

Both MAT and AG rats received a capsule at the time of OVX (described previously), but the AG rats were also subjected to a second surgery 3 months later. At this time, rats were anesthetized (isoflurane), the capsule removed, and a new one implanted for the "switch" groups (E to V, or V to E), or the capsule loosened between the shoulder blades for the "continuous" E or V groups maintained on the same treatment for the entire 6-month period. We have previously confirmed that hormone levels are maintained up to 6 months even without capsule replacement (Yin et al., 2015a). Groups are referred to as MAT-V3, MAT-E3, AG-V6, AG-E6, AG-E3/V3, and AG-V3/E3 (Fig. 1).

2.3. Telemetry and data collection

Recording was performed in a dedicated room, with the temperature and light cycle similar to the general housing room. 5 days before recording started, each home cage containing the pair of rats, one with an E-mitter and one without, was transported to the recording room and placed on a transmitter/receiver device (ER-4000 energy receiver, Phillips Respironics, Bend, OR). Daily activity was recorded continuously and stored at 5-minute intervals, and core temperature data were collected every 5 minutes remotely for ten days per month by a Windows-based automated data acquisition system (VitalView, version 5.0). During the 10-day recording period each month, at the end of days 5 and 10 of recording, we moved rats to clean cages, recorded body weight, and weighed the food tray. Then, rats were transported back to the general housing room. This process was repeated every month for 3 (MAT) or 6 (AG) months.

2.4. Euthanasia and tissue collection

At the end of the experiment, rats were humanely euthanized by rapid decapitation during the lights-on phase. Trunk blood was collected and allowed to fully coagulate at room temperature for serum collection. Body length, waist diameter, pituitary weight, adrenal weight, spleen weight, and uterine horn diameter data were collected to evaluate changes of the endocrine system (Yin et al., 2015b). Brain, pituitary, adrenal, spleen, and uterine tissues were stored for future studies.

2.5. Hormone assays

Hormone concentrations were measured in terminal serum samples from nonfasting rats, all run in duplicate. Serum E concentrations were measured using an RIA kit (DSL-4800, Beckman Coulter, Webster, TX) in a single assay (coefficient of variability (CV) = 7.68%). Other serum hormones were measured using the Milliplex Rat Magnetic Bead assays (Millipore) with the MAGPIX instrument (Luminex, Millipore). A rat neuropeptide panel (Cat. # RMNPMAG-83K) contained assays for α-melanocyte–stimulating hormone (CV = 0.14%), β-endorphin (CV = 0.32%), and neurotensin (CV = 0.51%). A multispecies steroid/thyroid hormone panel (Cat. # STTHMAG-21K) contained assays for progesterone (CV = 1.16%), triiodothyronine (T3, CV = 0.72%), and thyroxine (T4, CV = 0.81%). Melatonin (CV = 1.27%) was measured on a separate hormone panel (Cat. # RSHMAG-69K). Blood glucose concentrations were measured from a small amount of trunk blood collected at euthanasia using a blood glucose test strip (Contour Next).

2.6. Analysis of diurnal rhythms

Ten-day temperature and activity recording files (5 minutes sampling rate) from each of the recording periods were subjected to cosinor analysis (Borniger et al., 2014) using freely available software (Roberto Refinetti, www.circadian.org/main.html). In determining the presence of 24-hour rhythms in the data set, the statistical significance (alpha) was set to 0.01 and data were corrected for multiple comparisons. The following parameters were determined via cosinor analysis: robustness (or 'prominence', the percentage of the variance accounted for by the best-fit cosine model, corresponding to the coefficient of determination R^2 in regression analysis [Refinetti et al., 2007]); mesor (value around which the rhythm oscillates); amplitude (the difference between the mesor and the peak of the oscillation); and acrophase (time at which the waveform peaks after lights-off). Locomotor activity actograms and temperature profiles were graphed using ClockLab (ClockLab 6 for Windows). Locomotor activity levels during lights-on and -off were

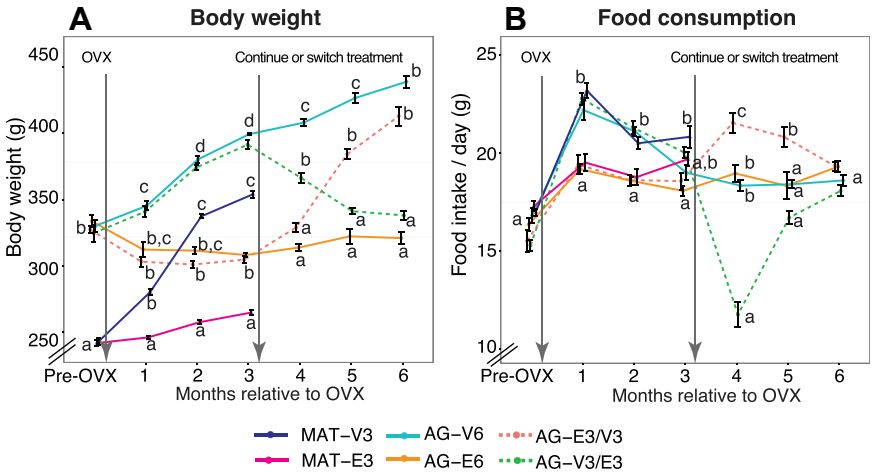

Fig. 2. Effects of age, and timing and duration of estradiol or vehicle treatment, on (A) body weight and (B) food consumption. Body weight significantly increased in rats receiving V compared with E treatment over the 3-month (MAT) or 6-month (AG) post-OVX period. Groups were compared at each time point and those with similar levels are indicated as a, b, c, and so forth. See Supplemental Table 1 for additional statistics. Abbreviations: AG, aging; MAT, mature; OVX, ovariectomy.

analyzed over the 12-hour light and dark phases. To avoid interference by moving and changing cages, data were averaged from 00:00 day 2 to 00:00 day 5 and from 00:00 day 7 to 00:00 day 10.

2.7. Statistics

For data presentation, groups are referred to by age (MAT, AG), treatment E or V, and duration (3 or 6 months), resulting in 6 groups: MAT-V3, MAT-E3, AG-V6, AG-E6, AG-V3/E3, and AG-E3/V3 (Fig. 1). Data collected at euthanasia (serum hormones, physiological endpoints) were analyzed by Student's t-test (MAT-V3 vs. MAT-E3) and one-way ANOVA (the 4 AG groups) to compare group differences. For longitudinal data (body weight, food intake, activity and temperature circadian parameters), we used the LME4 package in R to fit a mixed-effect model with main effects of time, treatment group, and their interaction with a random effect that accounts for repeated measures of the same individuals. We then used the lsmeans package in R to compute the least-squares means and compared Tukey contrasts for the treatment groups at each time point. *p*-values from the mixed models are summarized graphically using a compact letter display (Yin et al., 2015a), with repeated measures comparisons within groups provided in Supplemental Table 1.

3. Results

3.1. Body weight and food intake

Longitudinal monitoring showed that E treatment had profound effects on body weight and food consumption. By 1 month after OVX, body weight was significantly higher in V than E rats in both MAT and AG groups, a pattern that continued over the 3- (MAT) or 6- (AG) month period in the continuous treatment groups (Fig. 2A). Switching treatments 3 months after OVX in the AG females caused a rapid significant loss (V to E) or gain (E to V) in body weight, indicating continued sensitivity to E's presence/absence.

Food intake significantly increased after OVX in all groups, with the most rapid increase within the first month. This was mitigated by E (Fig. 2B). In the continuous V and continuous E AG females, food intake plateaued at 3 months and was similar over the course of the next 3 months. Switching treatments after 3 months in AG

Fig. 3. Activity actograms of representative rats are shown for each treatment group. Each actogram was double-plotted for 10 d/mo for 3 (MAT) or 6 (AG) months. Locomotor activity is represented in 20-minute bins. Both age and hormone treatment affected the consolidation of activity, which decreased in AG rats, and in V compared with E rats. Abbreviations: MAT, mature; AG, aging; V, vehicle; E, estradiol.

rats had dramatic and significant effects. AG rats that were switched from V to E had decreased food intake for the first month after the switch but returned to the levels of the 6-month continuous groups by 2 months after the switch. Switching from E to V also caused a transient increase in food intake, albeit less dramatic, and a return to the continuous group levels by 3 months after OVX.

3.2. Diurnal activity

Representative activity actograms from one rat per treatment group are shown, illustrating effects of age and E treatment on the consolidation of activity rhythms (Fig. 3). Group data were analyzed for mesor, robustness, amplitude, and acrophase. Activity mesor was significantly higher in continuous E compared with continuous V treated rats in both age groups over 3 (MAT) or 6 (AG) months of recording (Fig. 4A). Switching treatment in the AG rats significantly changed locomotor activity mesor by one month after switch, back to the levels of the same treatment continuous (V6, E6) groups. Similar responses to E were seen for activity robustness (Fig. 4B) and amplitude (Fig. 4C). Although the patterns were similar in rats of both ages, mesor, robustness, and amplitude were higher in MAT over AG rats. Activity acrophase relative to dark onset (Fig. 4D) was more modestly affected by age and the E treatment regimens in ovariectomized rats.

3.3. Core body temperature

Representative profiles of core body temperature are shown as time series in Fig. 5. Daily core temperature of rats rose quickly after lights-off, fluctuated through the active (dark) phase, and then quickly declined after lights-on. Analysis of group data was conducted for core temperature mesor, robustness, amplitude, and acrophase. Core temperature mesor (Fig. 6A) was higher in MAT than AG rats; however, effects of E were only observed in the AG groups. The AG-V6 group had the lowest mesor compared with the other three AG groups that had E on board for 3 or 6 months, with the timing/duration having little influence. Temperature robustness (Fig. 6B) was not affected by age but was profoundly and significantly affected by E. Robustness rapidly declined after OVX, reaching a nadir by 2 months in the V groups. When the hormone treatment was switched after 3 months, a rapid transition occurred to higher (V to E) or lower (E to V) robustness. Similar patterns were seen for temperature amplitude (Fig. 6C) and acrophase (Fig. 6D), albeit more modestly.

To further evaluate E's effect on the core body temperature rhythm, we did frequency analysis on a 7-day recording period using a Fourier transform and wavelet analysis. AG rats (12 months) given V and/or E (n = 4 per group) were analyzed during the period 2–3 weeks after OVX. Representative data from one rat per treatment are shown (Fig. 7). Wavelet analysis revealed an ultradian

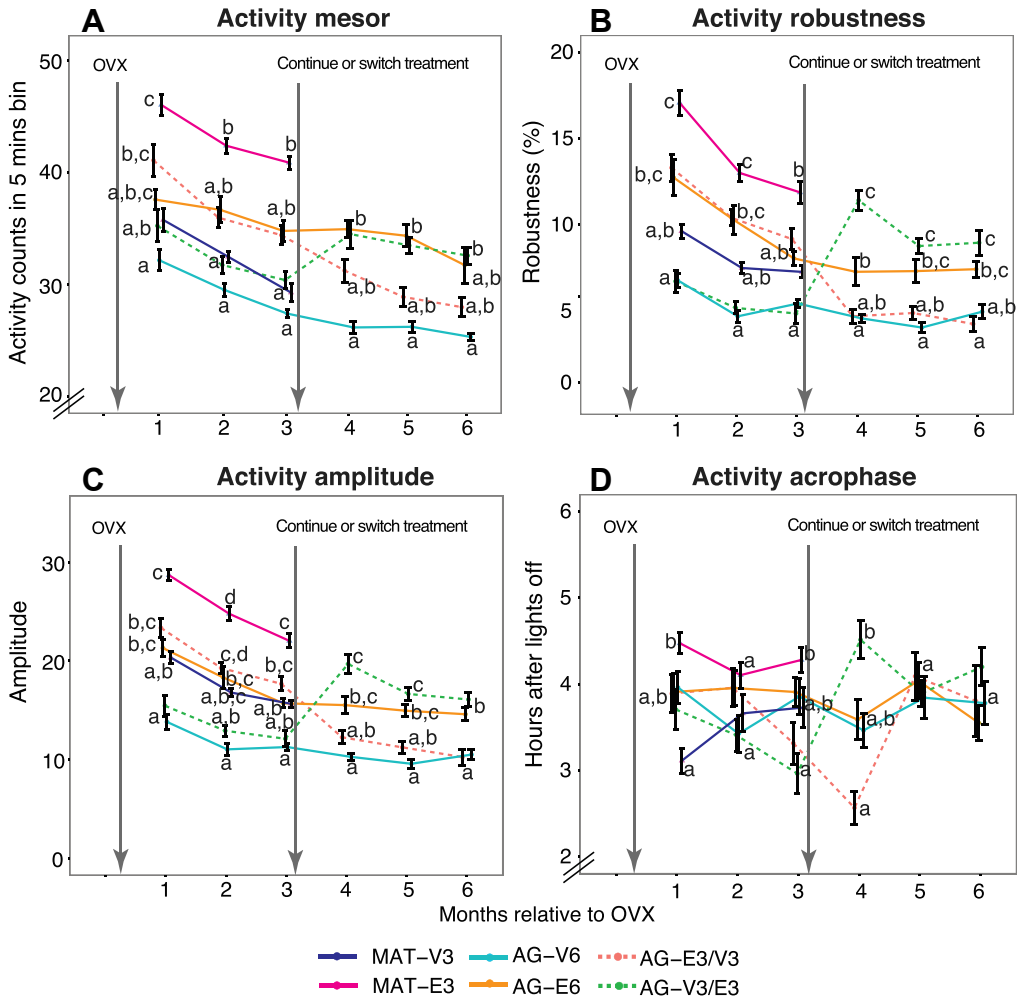

Fig. 4. Effects of age, and timing and duration of estradiol or vehicle treatment, on diurnal parameters of activity. (A) Activity mesor; (B) activity robustness; (C) activity amplitude; and (D) activity acrophase relative to dark onset. Groups were compared at each time point and those with similar levels are indicated as a, b, c, and so forth. See Supplemental Table 2 for additional statistics. Abbreviations: AG, aging; E, estradiol; MAT, mature; OVX, ovariectomy; V, vehicle.

rhythm (3.6–6 hours) in the V rats specifically during the lights-off (active) phase. The E rats did not exhibit this ultradian rhythm.

3.4. Physiological endpoints and serum hormones

In MAT rats, E treatment significantly decreased body length in MAT rats, and decreased waist diameter in all groups (Supplemental Fig. 1). Compared with V counterparts, E-treated rats showed significantly higher pituitary and adrenal indices, and larger uterine horn diameter. In addition, we compared the spleen index as an indication of the immune system. Spleen index was significantly increased by E in AG but not MAT E-treated rats.

Serum hormones and glucose were measured in terminal blood samples (Supplemental Fig. 2). E2 concentrations were measured to confirm efficacy of long-lasting Silastic capsules and were significantly higher in all E-treated groups. Average serum E2 was between 25 and 40 pg/mL in animals receiving E2 capsules, which is in the physiological range of intact proestrus (MAT) or persistent estrus (AG) rats (Gore et al., 2000). Serum T3 was unaffected, and serum T4 was significantly lowered by E in the MAT but not AG groups. Serum P4 was elevated by E2, but this was only significant in MAT rats. E treatment had no significant effects on serum α-MSH, β-endorphin, neurotensin, and melatonin. Nonfasting blood glucose was similar in all groups.

4. Discussion

Perimenopause is a dynamic transitional life stage during which a loss in estrogens is superimposed on an age-related decline in biological rhythms. It is likely that many of the dysfunctions of menopause occur because of the confluence of these processes, including sleep disturbances, anxiety, changes in metabolism, and cognitive and affective impairments (Mauvais-Jarvis et al., 2013; Stuenkel et al., 2015). Although there is strong evidence both from animal models and clinical studies that estrogen replacement mitigates many of these symptoms, whether there is a critical window for intervention to maximize benefits and minimize risks is poorly understood and previously not tested for effects on biological rhythms. Some years ago, our lab developed the rat model of menopause used in the present study to test the critical window hypothesis on the expression of hypothalamic gene networks and on social and affective behaviors controlled by the hypothalamus and other parts of the limbic system (Garcia et al., 2016; Garcia et al., 2017a; Garcia et al., 2017b; Yin et al., 2015a). Results revealed that

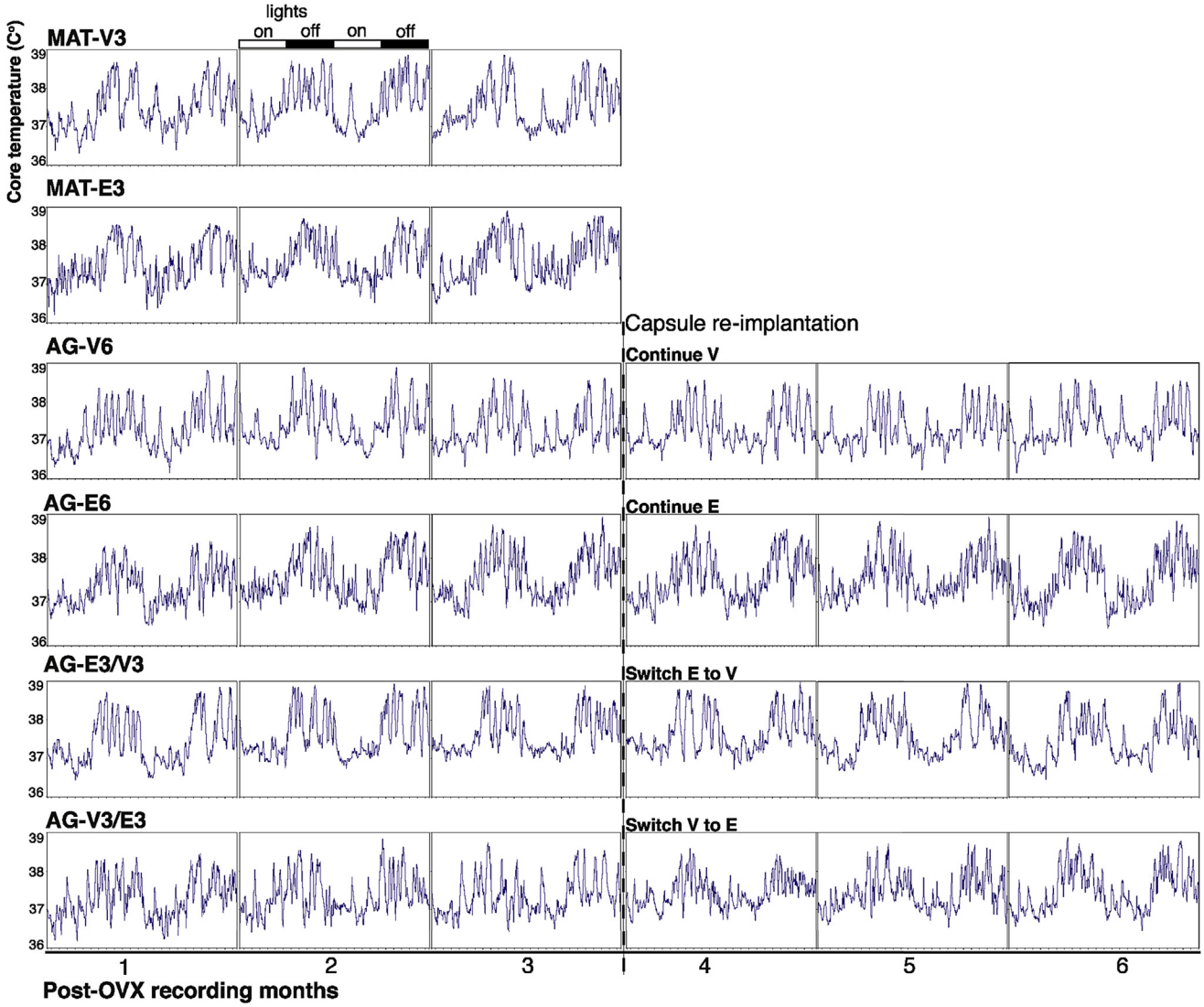

Fig. 5. Core temperature profile of representative rats is shown for each treatment group.

E modulated several of these outcomes, with timing and duration playing a smaller role relative to the absolute presence or absence of hormone. More specifically, in the arcuate nucleus, a cluster of coexpressed circadian genes was identified (*Per1, Per2, Cry1, Cry2, Arntl*) that was decreased by both age and E treatment (Yin et al., 2015a). Another identified cluster was increased by E treatment (but not age) that included genes involved in the hypothalamic control of energy balance (*Igf1, Hcrt, Npy, Ghrh*). Here, we sought to understand the effects of estrogen regimens on the fundamental properties of diurnal rhythms and functional outcomes.

4.1. Physiological effects of age and estrogen treatments

Estrogens play important roles in the regulation of body weight and metabolism in mammals, including humans (Mauvais-Jarvis et al., 2013). The longitudinal experimental design enabled us to study effects of estrogens on basic homeostatic processes involved in body weight maintenance and to determine whether delaying the treatment, or shortening the duration of treatment, would inform whether E timing or duration was an important factor. The protective effects of estrogens against body weight gain were evident immediately at OVX, when both MAT and AG rats underwent rapid and dramatic body weight gain. Animals treated with E at the time of OVX did not undergo any body weight change, and this was maintained through the course of the study in those animals receiving continuous E treatment. When AG rats were switched to the opposite hormonal regimen 3 months after OVX, an immediate body weight loss (V to E) or gain (E to V) occurred by 2 months after switch. This means that neither age nor a delay in E treatment was a limiting factor in the rats' physiological response to ovarian hormone deprivation. When considering the group that switched from E to V, the observation that animals rapidly gained weight indicates that short-term E did not have a protective effect once the hormone was removed.

Several interesting outcomes in the relationships between food intake and body weight were revealed. First, MAT and AG rats consumed about the same amount of food at the start of the study (pre-OVX), yet the AG rats were about 40% heavier than their MAT counterparts. Although we were unable to monitor metabolism in our rats, others have shown that basal metabolism declines with AG in rats, monkeys, and humans (Dominguez and Barbagallo, 2016; Eghlidi and Urbanski, 2015; Purnell et al., 2019), consistent with our results. Our food intake measures were averaged across 10 days, and future work should include time of day of feeding, as prior work has shown that the timing of food consumption during the lights-on (inactive period) is associated with obesity (Fonken et al., 2010). Second, at OVX, both E and V animals

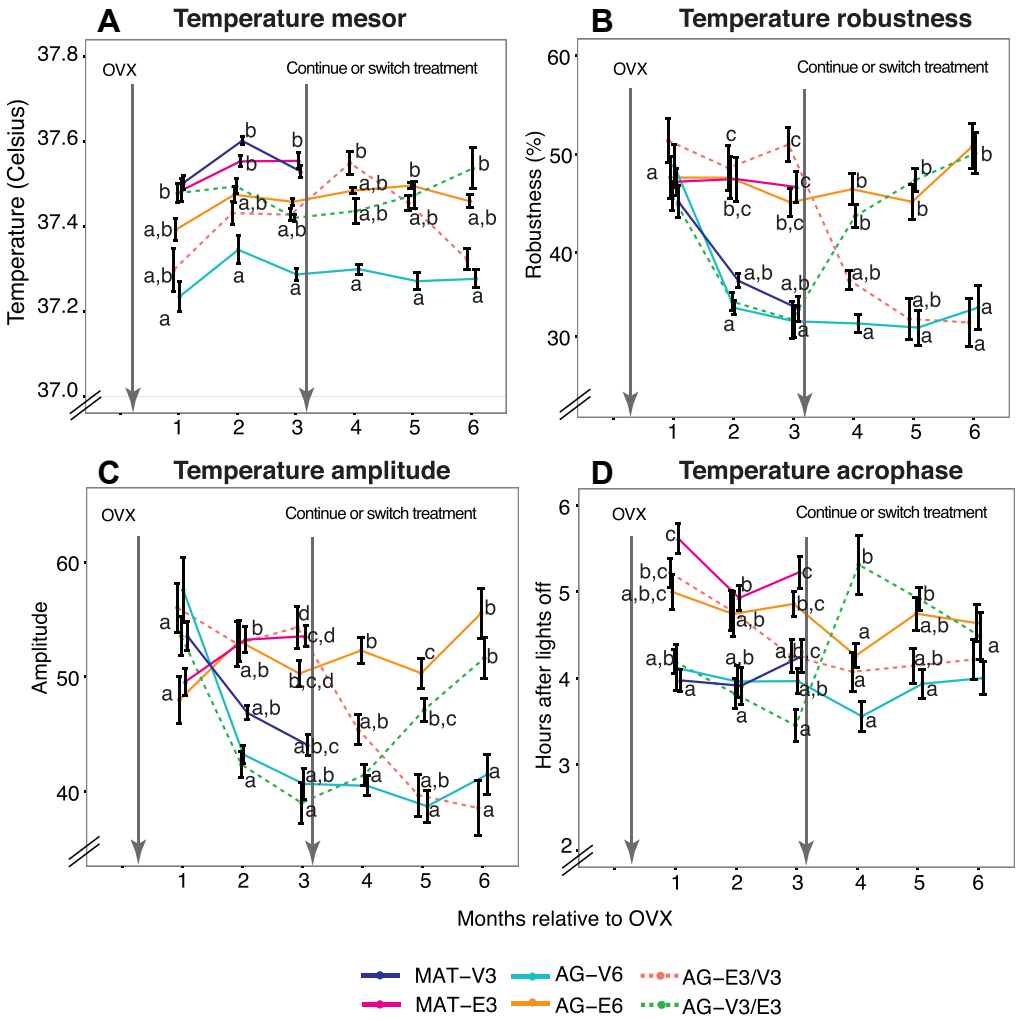

Fig. 6. Effects of age, and timing and duration of estradiol or vehicle treatment, on diurnal parameters of temperature. (A) Temperature mesor; (B) temperature robustness; (C) temperature amplitude; and (D) temperature acrophase relative to dark onset. Groups were compared at each time point and those with similar levels are indicated as a, b, c, and so forth. See Supplemental Table 3 for additional statistics. Abbreviations: AG, aging; MAT, mature; OVX, ovariectomy.

increased food consumption, albeit significantly more so in the V groups, and irrespective of age. This pattern of food consumption was transitory in the continuous V groups, as it peaked 1 month after OVX and subsequently declined to a plateau. By contrast, the continuous E groups gained weight more slowly after OVX and maintained food intake at a flat level. This suggests a dysregulation of the body's ability to maintain homeostasis of energy balance in the absence of estrogens. Third, the switch groups were revelatory in showing a significant decrease in food intake in the AG-V3/E3 group, and an increase in the AG-E3/V3 group, both peaking 1 month after switch. As a whole, these data show a somewhat tenuous relationship between food intake and absolute body weight, and results are consistent with an age-related decrease in basal metabolism.

4.2. Diurnal rhythms of locomotor activity

It is well known that estrogens affect circadian activity. In intact rats, when serum estrogens are elevated on estrus, daily locomotor activity is also elevated (Albers et al., 1981; Wollnik and Turek, 1988). Ovariectomized rodents given E capsules show higher daily locomotor activity levels compared with V (Morin et al., 1977). Similarly, circadian locomotor activity declines with AG (Gibson et al., 2009; Zhang et al., 1996). Our present study confirms and extends prior work by inclusion of the different estrogenic treatment regimens. Overall, estrogen treatment resulted in much stronger and more consolidated rhythms of activity in rats of both ages. All of the parameters examined (mesor, robustness, amplitude, and acrophase) were higher in E compared with V rats, and the first three were also affected by age, being higher in MAT than AG rats. The beneficial effects of E treatment on the maintenance of rhythmicity was also apparent, particularly for activity robustness and mesor, consistent with the literature (Blattner and Mahoney, 2014; Ogawa et al., 2003). In the AG switch groups, rats maintained the ability to respond to delayed replacement (V to E) of E, indicating a maintenance of sensitivity of the brain even 3 months after OVX. In the group given E for 3 months, and then switched to V (E to V), animals quickly transitioned to a similar level of diurnal function as the continuous V group, indicating that E must be on board for the maintenance of diurnal activity.

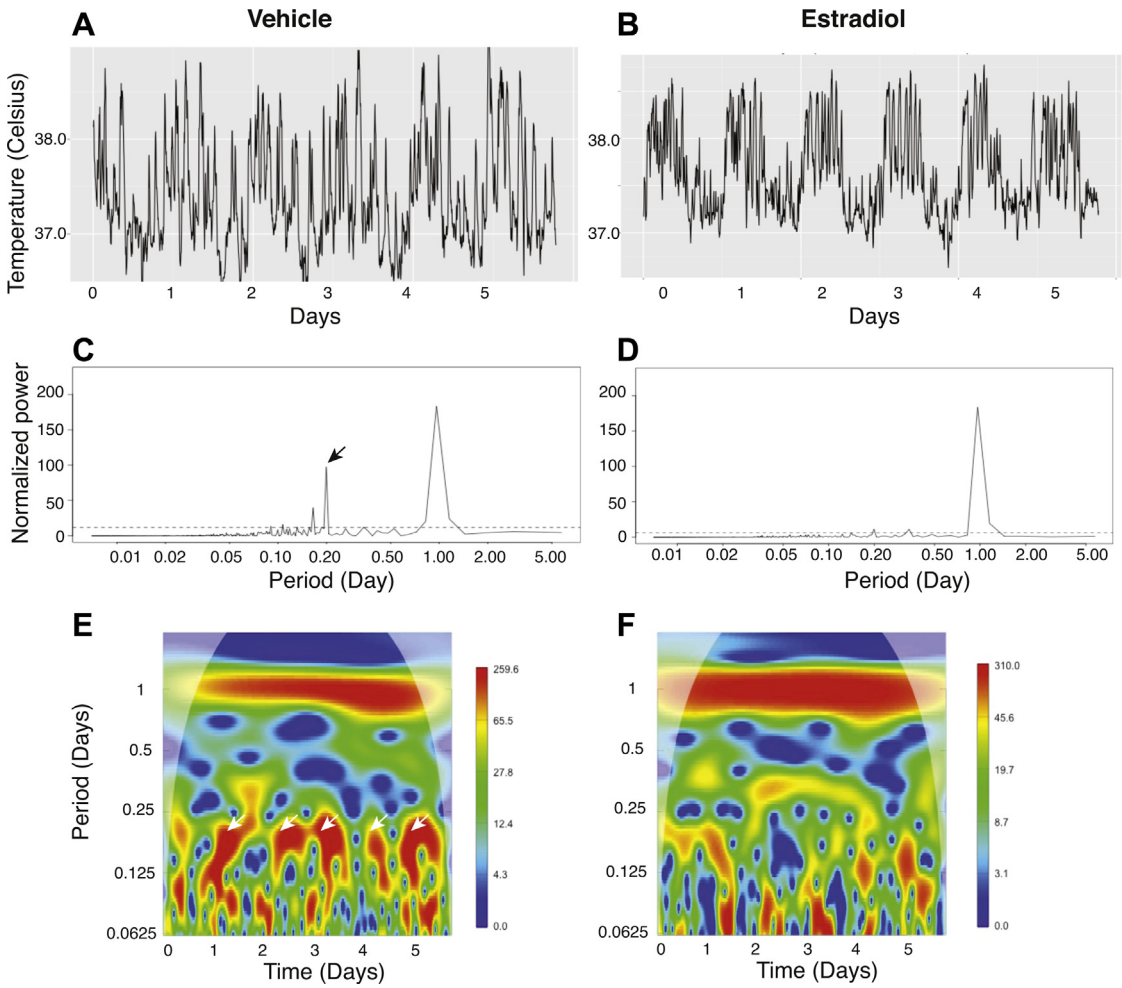

Fig. 7. Representative examples of core body temperature in two 11-month-old ovariectomized females that received a vehicle (left) or an estradiol (right) capsule are shown. (A, B) 6 days of continuous recording of core temperature is shown with the light:dark cycle indicated above the recording. The vehicle rat (A) had large-amplitude excursions from the curve; the E rat (B) had much smoother temperature fluctuations with clear peaks at lights off. To analyze these time series, a Fourier transformation was conducted to generate periodograms. In the vehicle (C) and E (D) rats, there was a defined peak at 24 hours (indicated as 1 day on the x-axis); the vehicle rat also had several smaller peaks with a period of 0.15–0.25 (3.6–6 hours, the latter indicated with the arrow). This ultradian rhythm is also visualized by wavelet analysis, which shows the period plotted on the y-axis and time (days) on the x-axis for the vehicle (E) and E (F) rat. Both rats had a clear 24-hour circadian peak (large red horizontal bar at y=1.0, albeit larger for the estradiol animal), but the vehicle rat also had an ultradian rhythm during light-off across the time series between 0.125 and 0.25. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.3. Diurnal rhythms of core body temperature

The regulation of body temperature is of particular interest to perimenopausal women, as dysfunctions in thermoregulation are the basis for hot flashes that can profoundly impair sleep and quality of life (Freedman, 2001; Freedman et al., 1995; Frishman, 1995). Our core temperature data collected every 5 minutes made it possible to conduct high-resolution circadian and ultradian analyses. Results revealed that while both E and V rats showed 24-hour rhythmicity of body temperature, the V rats exhibited frequent high-amplitude excursions and more irregular cycles. E treatment stabilized these greater amplitude fluctuations of core temperature, especially during the active (dark) phase. Of the parameters examined, temperature robustness and amplitude were particularly sensitive: while MAT and AG rats were similar to one another after OVX and this was maintained in E-treated rats, robustness and amplitude decreased rapidly in rats given V. This effect of E was seen even after a 3-month delay in onset of treatment, and the effect was lost in rats in which E was switched to V. These results may relate to, or even be a basis for, hot flashes at menopause, which have been proposed to be triggered by a rapid resetting of the hypothalamic thermoneutral zone (Freedman, 2001).

Our results indicate that in the presence of E, rats are able to make rapid adjustments to body temperature, whereas in the absence of E, frequent excursions from the baseline result in a less rhythmic pattern. Wavelet analysis conducted in a subset of rats revealed an ultradian rhythm occurred in V-treated but not in E-treated rats. This result contradicts an earlier wheel-running study using castrated male and female rats, with or without E treatment, and found an ultradian rhythm in male but not female rats (Wollnik and Dohler, 1986). That same group conducted such work across the estrous cycle and found that females exhibited ultradian rhythms only on metestrus and diestrus, when E level is low (Wollnik and Turek, 1988). This latter finding is interesting because in the present study, only the V rats (low E) exhibited ultradian rhythms. The mechanism and function of this ultradian rhythm is an intriguing topic requiring future investigation.

5. Conclusions

Our data indicate that E status has profound effects on circadian activity the core body temperature rhythms in reproductively mature and aging female rats, but that this effect was not hindered by a delay in E treatment nor was it maintained when E was removed. When translated to humans, a 3-month period in a rat's life is estimated to be ~5 years (Quinn, 2005; Sengupta, 2013). If these results extrapolate, they mean that several years' delay in E treatment in women may still prove beneficial to biological rhythms. However, the results also imply that once E is withdrawn, symptoms may recur. As a whole, the robust effects of E treatment on body weight and biological rhythms of activity and temperature support the importance of this hormone as a viable pharmaceutical option for symptomatic women.

Disclosure

The authors certify that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.08.029.

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