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Sex Differences in Circadian Rhythms

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Abstract

Sex as a biological variable is the focus of much literature and has been emphasized by the National Institutes of Health, in part, to remedy a long history of male-dominated studies in preclinical and clinical research. We propose that time-of-day is also a crucial biological variable in biomedical research. In common with sex differences, time-of-day should be considered in analyses and reported to improve reproducibility of studies and to provide the appropriate context to the conclusions. Endogenous circadian rhythms are present in virtually all living organisms, including bacteria, plants, invertebrates, and vertebrates. Virtually all physiological and behavioral processes display daily fluctuations in optimal performance that are driven by these endogenous circadian clocks; importantly, many of those circadian rhythms also show sex differences. In this review, we describe some of the documented sex differences in circadian rhythms.

Circadian rhythms are endogenous biological rhythms with periods of about 24 hours. Circadian rhythms persist in the absence of environmental cues; however, organisms use environmental cues, especially light, to entrain circadian rhythms precisely to the 24-hour solar day (Czeisler and Wright 1999). Synchronizing (or entraining) circadian rhythms to the solar day allows individuals to match physiological and behavioral responses with the appropriate temporal environmental conditions. Endogenous circadian rhythms are present in virtually all living organisms, including bacteria, plants, invertebrates, and vertebrates. Again, light is the most effective entraining agent, or *zeitgeber*. Among individuals of many vertebrate species, light stimulates intrinsically photosensitive retinal ganglion cells, which depolarize and synapse directly onto neurons in the suprachiasmatic nucleus (SCN) of the hypothalamus.

The master biological clock is located within the SCN where, dependent upon species, approximately 20,000–50,000 neurons maintain a transcriptional autoregulatory feedback loop. The molecular mechanism of the mammalian circadian clock has been reviewed in detail elsewhere (Partch et al. 2014). Virtually all cells have the clockwork mechanisms and are organized hierarchically throughout the body with the SCN serving as the master clock organizing all rhythms. The clockwork mechanism comprises an autoregulatory loop as the

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primary mechanism driving circadian rhythms; however, there is increasing evidence of additional processes, including posttranslational modifications (Gallego and Virshup 2007) and cAMP signaling (O'Neill et al. 2008), that are also critical to function. Time-of-day information, based on light intensity, is then relayed from the SCN to other brain regions, as well as to peripheral tissues, via neural and humoral pathways to provoke appropriate responses.

Sex as a biological variable is the focus of much literature and has been emphasized by the National Institutes of Health (NIH), in part, to remedy a long history of male-dominated studies in preclinical and clinical research (Beery and Zucker 2011; Zucker et al. 2021). Given the legions of sex differences in physiology and behavior, the exclusion of females from clinical and nonclinical research has likely had negative consequences for women's health (Beery and Zucker 2011; Zucker et al. 2021). Similarly, there are well-documented temporal differences in physiology and behavior that should be considered across all biological studies (Nelson et al. 2021).

We propose that time-of-day is also a crucial biological variable in biomedical research. In common with sex differences, time-of-day should be considered in analyses and reported to improve reproducibility of studies and to provide the appropriate context to the conclusions. Virtually all physiological and behavioral processes display daily fluctuations in optimal performance that are driven by endogenous circadian clocks; importantly, many of those circadian rhythms also show sex differences. Sex differences exist at multiple levels, from DNA to behavior, throughout the animal kingdom. In this article, we focus on sex differences in biological rhythms and how the neuroanatomical organization and hormonal milieu may transduce these differences or compensate for differences to normalize behavioral or physiological rhythms.

ANATOMICAL DIFFERENCES IN THE CIRCADIAN TIMING SYSTEM

There are three major afferent pathways through which zeitgebers can entrain the SCN; photic information via the retinohypothalamic tract (RHT) from the retinato the SCN, and nonphotic information transduced via the geniculohypothalamic tract (GHT) from the intergeniculate leaflet (IGL) to the SCN, or via direct projections from the dorsal and median raphe to the SCN. All of these afferent structures and the SCN express estrogen receptors (ERs) and androgen receptors (ARs) in various patterns (for reviews, see Bailey and Silver 2014; Yan and Silver 2016; Hatcher et al. 2020; Nicolaides and Chrousos 2020). Indeed, there are sex differences (and species-specific sex differences) in sex steroid receptor expression in the SCN (Iwahana et al. 2008), the retina (Wickham et al. 2000), the IGL (Horvath et al. 1999), and the raphe (Sheng et al. 2004); thus, gonadal and neurosteroids can directly affect the brain's master clock and its afferent pathways to influence the circadian system.

There are sex differences in how gonadal hormones affect both organization and modulation of SCN rhythmicity (Zucker et al. 1980; Albers 1981). Gonadal hormones can act directly on the SCN or indirectly via neurosteroid metabolites of gonadal steroids. Indeed, there is evidence that the SCN can synthesize neurosteroids such as progesterone, androsterone,

and allotetrahydroxy-corticosterone (THDOC), which can alter SCN activity (Trachsel et al. 1996; Pinto and Golombek 1999). Although gonadal and neurosteroids and their receptors are in a position to directly modulate the SCN, sex differences in the effects of neurosteroids on the circadian system have yet to be fully investigated.

The SCN projects major efferents to over a dozen brain areas (Kriegsfeld and Silver 2006; Morin 2013), and these targets all express ERs and ARs in various combinations (Bailey and Silver 2014). Additionally, sex differences in function and anatomy of these SCN efferent target sites underlie sex differences in the hypothalamic pituitary adrenal (HPA) and hypothalamic pituitary gonadal (HPG) axes, as well as in sleep architecture and daily activity patterns (Semaan and Kauffman 2010; Morin 2013; Bailey and Silver 2014; Nicolaides and Chrousos 2020), which are discussed below.

PHYSIOLOGICAL DIFFERENCES IN THE CIRCADIAN TIMING SYSTEM

Hypothalamic Pituitary Gonadal (HPG) Axis

One of the most robust sex differences in circadian rhythmicity is found in circadian gating of the HPG axis. In female rodents, the SCN gates circadian timing of the preovulatory luteinizing hormone (LH) surge; however, estradiol concentration must be sufficiently high for the surge to occur (Christian and Moenter 2010; Williams and Kriegsfeld 2012). Similarly, there is a daily LH rhythm in women that also occurs at the onset of activity (Cahill et al. 1998). Males are unable to produce an LH surge, and it appears that this difference lies in sexually dimorphic population of kisspeptin neurons in the anteroventral periventricular nucleus (AVPV) (25 times more neurons in females than males), which project to GnRH neurons (for reviews, see Williams and Kriegsfeld 2012; Bailey and Silver 2014; Yan and Silver 2016). This neuroanatomical sex difference is a result of an organizational effect of gonadal steroids as developmental exposure to testosterone suppresses AVPV kisspeptin neuron numbers (Kauffman et al. 2007; Homma et al. 2009).

Hypothalamic Pituitary Adrenal (HPA) Axis

The SCN also regulates the HPA axis to affect glucocorticoid rhythms (Moore and Eichler 1972). At the level of the pituitary, this occurs presumably through direct and indirect actions of AVP neurons in the SCN and the PVN (Kalsbeek et al. 1992, 2010). The SCN can also regulate sensitivity of the adrenal cortex to ACTH in a circadian manner (Kaneko et al. 1981). There are also sex differences in HPA axis stress responsiveness (Handa et al. 1994, 2021), potentially due to sex differences in corticotropin-releasing factor (Bangasser and Wiersielis 2018) and/or liver X receptor α (Feillet et al. 2016), which may be downstream of sex-specific differences in AVP signaling in the SCN (Rohr et al. 2021). Some of these sex differences in stress responsiveness and the HPA axis can manifest in downstream physiological systems, such as the cardiovascular system.

Cardiovascular System

Various physiological features of the cardiovascular system are regulated by circadian rhythms, including heart rate, heart rate variability (HRV), cardiovascular tone, angiogenesis, and vascular remodeling (Paschos and FitzGerald 2010). The circadian

rhythms of these features are dictated by clock gene loops in the vascular endothelium, hormonal signals, and autonomic nervous signaling ultimately regulated by the SCN. As with many other circadian rhythms, several sex differences in cardiovascular circadian rhythms have been observed.

In general, females have higher resting and active heart rates than males, and this has been observed in humans to persist across the entire circadian day when examining the MESOR of heart rate (Hermida et al. 2002, 2007). For example, in a study in which ambulatory cardiovascular function was observed in referred patients, women had a slightly higher ambulatory heart rate across both the sleep and wake periods of the day (Ben-Dov et al. 2008). This observation persisted in another cohort study examining cardiovascular function in young and elderly populations (Stein et al. 1997). Another study demonstrated that the largest sex differences in heart rate occurred during the inactive phase in humans (Zhao et al. 2015).

Heart Rate Variability

Additional sex differences in cardiac function have been observed when examining time and frequency-domain indices of HRV across the day. Time-domain measures examine the intervals between specific components of polarization events during each cardiac cycle as measured by electrocardiograms. Frequency-domain measures assess the individual frequencies of functions of time-domain plots. For example, R-R intervals are a time-domain measurement, and the frequency-domain of the R-R interval can be determined through a Fourier transform of the R-R plotted over time (Shaffer and Ginsberg 2017).

In the time-domain, one study observed that men had greater R-R intervals across the entire circadian day (Bonnemeier et al. 2003). Men had increased standard deviations of NN intervals (SDNNs), standard deviation of the average NN intervals of each 5 min segment across a 24 h HRV recording (SDANN), and mean of SDANN (SDNNi) across the entire day and greater root mean square of successive R-R interval differences (rMSSD) at night (Bonnemeier et al. 2003). Similar results were reported in a cohort study examining ~33-yr-old adults, where men had higher SDNN, SDANN, SDNNi, and average heart period in ms (AVGNN) than women (Stein et al. 1997). In contrast, a third study observed that women had lower R-R intervals during the inactive phase, but this difference was not statistically significant during the active phase (Extramiana et al. 1999). The same study reported that women had faster cardiac repolarization rates across the entire day, with the exception of the intervals between Q onset and T wave apex (Extramiana et al. 1999). In contrast, one study reported no difference in mean 24 h heart rates between male and female rhesus monkeys (Barger et al. 2010).

Sex differences in the frequency-domain of HRV across the day have also been observed. Women have lower LF/HF ratios than men during the circadian day (O'Connor et al. 2007). Another study reported similar results, where older men had greater LF/HF ratios across the entire day, but younger men only had a greater ratio during the inactive phase (Stein et al. 1997). Men display elevated LF during the active phase (Yamasaki et al. 1996) and across the entire day (Stein et al. 1997). Conversely, women displayed greater HF across the

day (O'Connor et al. 2007), whereas another study reported that women have greater HF between 12:00–06:00 h (Yamasaki et al. 1996).

Last, depression differentially affects HRV across the day between women and men. In women, greater depressive scores were found to reduce the MESOR of circadian variation patterns of vagal activity, whereas the opposite effect was observed in men (Jarczok et al. 2018).

Blood Pressure Rhythms

In general, men tend to have higher systolic and diastolic blood pressure levels than women (Burt et al. 1995), an effect that persists across the day (Hermida et al. 2002, 2007, 2013). Ambulatory blood pressure monitoring in a cardiovascular clinic has also revealed that blood pressure levels are lower in women than men across the day in an ambulatory setting (Ben-Dov et al. 2008). Other circadian-regulated aspects of blood pressure, such as dipper versus non-dipper patterns do not appear to be affected by biological sex (Ragot et al. 1999).

Rodent studies examining circadian differences in blood pressure have generated mixed results. No differences in arterial pressure were reported between male and female rats across the day (Sampson et al. 2008). Conversely, another study demonstrated that male C57Bl/6 and FVB/N mice display greater diastolic and systolic blood pressures across the day (Barsha et al. 2016). Among other potential mechanisms, differences in blood pressure may be driven by circadian rhythms in renal function, as constitutive renal *Bmal1* knockout in AQP2-Cre mice (C57Bl/6 background) led to reduced MESOR blood pressure in males but not females (Zhang et al. 2020).

Last, pharmacological treatment of blood pressure at different times of the day is affected by sex. Aspirin administration in the morning leads to elevated blood pressure in women, and aspirin administration in the evening leads to reduced blood pressure in both sexes, but a greater reduction is observed in women (Ayala and Hermida 2010).

Body Temperature Rhythms

Body temperature fluctuates across the day as a result of circadian regulated behavioral and physiological processes, such as variations in activity or metabolic function (Refinetti 2010). In general, men have lower body temperatures than women across the day. One study examining the effects of oral contraceptives on body temperature reported that men and naturally cycling women had lower MESOR body temperatures, greater temperature amplitudes, and lower nighttime-specific body temperature than women using oral contraceptives (Kattapong et al. 1995). Similar results were observed in another study, in which men had greater amplitudes in body temperature than women (Cain et al. 2010). Several studies have reported sex differences in phase angles of body temperature rhythms, with the nadir of body temperature during the inactive phase occurring 30 min (Baehr et al. 2000) to an hour earlier in women than in men (Cain et al. 2010). In free-running (i.e., not entrained) humans, women have been observed to have shorter intrinsic periods (taus) of body temperature rhythms (Wever 1984; Duffy et al. 2011). However, during internal desynchrony during free-running rhythms, the difference in body temperature taus between men and women is reported to disappear (Wever 1984).

Sex differences in body temperature have also been observed in other mammals. For example, female rhesus monkeys have lower body temperatures across the day with a greater phase angle than males, but with the acrophases being delayed rather than advanced as observed in humans (Barger et al. 2010). Potential mechanisms for sex differences in body temperature rhythms have been elucidated in mice. One study reported that differences in MESOR body temperatures between male and female mice are abolished after gonadectomy (GDX); GDX led to increased body temperatures in males and eliminated estrous-driven alterations of body temperature rhythms in females (Sanchez-Alavez et al. 2011).

Immune Function Rhythms

Sex differences in the immune system and function are discussed elsewhere in this collection (Moser 2021), and although there are well-studied circadian rhythms in the function of the immune system, few sex differences in the nature of these rhythms have been reported to date. For example, in humans, no differences in circadian rhythms were observed in the expression of Il-6 following lipopolysaccharide (LPS) stimulation; however, women mounted a more robust Il-6 response than men, which was associated with vagal tone and not with gonadal hormones (O'Connor et al. 2007). Female Lewis rats also display more robust responses to an immune challenge (ConA) than males; however, it is driven by biphasic increases in $CD8^+$ and MHC class II lymphocytes from the spleen at the end of both the light and dark phases (Griffin and Whitacre 1991). This sex-specific circadian difference in immune response may underlie the sex differences in development of EAE (experimental allergic encephalomyelitis) in this strain of rats (Keith 1978), and reinforces the necessity of considering both sex and time-of-day as biological variables in future studies of autoimmune disorders.

SEX DIFFERENCES IN BEHAVIORAL RHYTHMS

Sleep–Wake Rhythms

Reports on sex differences in sleep–wake rhythms have been equivocal. Several studies reported that women have greater sleep fractions (Wever 1984) and greater sleep efficiency than men (Goel et al. 2005). These results are consistent with a survey where women stated greater ideal durations of sleep time in comparison to men (Tonetti et al. 2008). However, other self-reporting and survey studies have found no differences in sleep durations between men and women (Van Reen et al. 2013; Randler and Engelke 2019). Opposingly, one activity log study observed that adolescent women sleep less than men (Mathew et al. 2019). Differences between these studies may be a reflection of study design, age, and data-collection methods.

Differences in onset of sleep and activity have also been reported. Several studies reported that women have earlier wake times than men during adolescence (Mathew et al. 2019) and adulthood (Van Reen et al. 2013). One questionnaire study reported that women teachers have earlier bedtimes than men on weekdays, but not weekends (Randler and Engelke 2019), coinciding with a questionnaire study reporting later bedtimes for men than women (Tonetti et al. 2008). Conversely, several groups have reported no sex differences in time of activity onset (Baehr et al. 2000) or sleep onset (Cain et al. 2010). The latter study did report a sex

difference in phase angle of sleep onset in relation to the onset of melatonin secretion (Cain et al. 2010). Last, young adult women (18–30 yr old) are reported to have earlier onsets of stage 1 and stage 2 sleep during the inactive phase than men (Goel et al. 2005).

In terms of chronotype, men generally have later chronotypes than women; this difference begins around age 16 and disappears around age 50 (Roenneberg et al. 2004), consistent with other results (Adan and Natale 2002; Randler and Engelke 2019).

Similar sex differences in sleep and activity exist in rodents. Female rodents have greater variability in the onset of activity that corresponds with varying stages of the estrous cycle (Takahashi and Menaker 1980; Albers et al. 1981; Kuljis et al. 2013; Krizo and Mintz 2015). Female C57Bl/6J mice have longer αs (active phases) during constant darkness (Kuljis et al. 2013). Differences in α may reflect sex-differences in spontaneous firing rates in the dorsal SCN between Zeitgeber time (ZT) 4–6 (Kuljis et al. 2013). C57Bl/6 female mice have greater total percent time awake across the day, primarily during the active phase (Paul et al. 2006). It was also reported that females had reduced non–rapid eye movement (NREM) sleep and increased δ power during the active phase compared to males, and that these effects were driven by gonadal hormones (Paul et al. 2006). Indeed, circulating estrogen and aromatase activity at target sites in the circadian system during development and in adulthood have been implicated in sex differences in activity and circadian coupling in mice (for review, see Hatcher et al. 2020).

Although many of the effects of sex-specific estrogen described above are thought to drive the differences in activity and sleep described above, there is some indication that there may be differing effects of gonadal steroids and chromosomal sex on these parameters. One study addressed this possibility by using FCG (four core genotype) mice in which genetic sex is uncoupled from gonadal sex (Kuljis et al. 2013). Interestingly, the largest sex differences in this study were found after GDX in FCG mice. After GDX, activity levels rhythm power were reduced in both chromosomal sexes, but the XY mice had the greatest reduction, indicating that gonadal steroids had a greater effect on circadian rhythmicity than chromosomal sex (Kuljis et al. 2013). Because the FCG mice showed no sex differences in these measures prior to GDX, the authors concluded that the role of gonadal steroids was to mask sex differences and normalize the behavior between sexes.

SEX DIFFERENCES IN THE EFFECTS OF DISRUPTION OF CIRCADIAN RHYTHMS

In humans, studies uncoupling endogenous circadian rhythms from the sleep–wake cycle using forced desynchrony and jet lag paradigms have revealed sex differences in cognition, affect, and physiology. In one study, compared to men, women had a higher amplitude of cognition performance and sleepiness after forced desynchrony (Santhi et al. 2016). Circadian misalignment after a 12 h phase shift increased circulating leptin in men, whereas women had decreased leptin coincident with increased ghrelin, resulting in altered food-type cravings between the sexes with no underlying difference in energy use (Qian et al. 2019). Although this study hints at a potential mechanism for sex differences in weight gain resulting from shift work, research in rodents has demonstrated that comparing

circadian metabolic and transcriptional profiles between sexes is not straightforward. In mice, circadian transcriptomic profiling of the liver revealed that in females rhythms in expression were found in genes involved in cell signaling and protein transport, whereas in males the rhythmically expressed genes were involved in drug and steroid metabolism. There were also sex-specific effects of the microbiome on circadian transcription patterns (Wegeret al. 2019). It may actually be a complex interaction among circadian rhythms in the gut microbiota, the circadian clock, and sex that feed back to regulate sex-specific differences in liver function and metabolism (Liang et al. 2015). Thus, it is apparent that future clinical metabolic studies must consider sex and time-of-day as critical biological variables.

Recent work in our laboratory has revealed striking sex differences in the effects of circadian disruption by exposure to dim light at night (dLAN) on many aspects of rodent physiology, behavior, and immune function. Although chronic mild circadian disruption by exposure to LAN (8 wk) in adulthood has similar effects on food intake resulting in obesity (Fonken et al. 2013; Aubrecht et al. 2015), LAN exposure during adolescence alters timing of food intake in male but not female mice, resulting in differential weight gain (Cissé et al. 2017). Brief disruption of circadian rhythms by as few as three nights of exposure to dLAN also alters brain physiology and behavior in a sex-specific manner in adult mice. Adult female mice displayed decreased anxiety-like behavior and had concurrent increases in *VEGFR1* and *IL-1B* expression in the brain, whereas males had reduced *BDNF* expression after three nights of dLAN exposure (Walker et al. 2020). These sex-specific effects of circadian disruption are not limited to adolescent and adult rodents however, because there appear to be transgenerational effects of circadian disruption that are sex-specific for both the sex of the parent and the sex of the offspring. In an experiment where adult male and female hamsters were exposed either to dark nights or to dLAN for 8 wk prior to conception, the male offspring of either sires or dams with preconception disrupted circadian rhythms had blunted immune responses and altered febrile response to an immune challenge compared to their female littermates (Cissé et al. 2020). However, only the female offspring of dams exposed to dLAN had enhanced bactericidal capacity of serum collected after an immune challenge, and none of these immunological effects were a result of altered maternal care (Cissé et al. 2020). This series of experiments makes it increasingly clear that even mild disruption of circadian rhythms has immediate and enduring sex-specific effects in animals at all stages of life, and these effects can be transgenerational in a sex-specific manner.

CONCLUDING REMARKS

Sex differences exist in circadian rhythms at all levels of analysis. In common with most areas of basic, clinical, and translational research, females have been understudied in circadian rhythm research. Furthermore, consideration of time-of-day as a biological variable is nearly nonexistent in most areas of research (Nelson et al. 2021). In this review, we have presented compelling evidence that there are critical sex differences in circadian rhythmicity in all aspects of biology across the life span. Furthermore, disruption of circadian rhythmicity by inappropriate exposure to LAN, shift work, or jet lag has sex-specific detrimental effects on physiology, behavior, and immune function, not only for those exposed, but potentially for future generations of their offspring as well. From

a research standpoint, to improve reproducibility of studies and to provide the appropriate context to the conclusions, time-of-day must be reported and considered in experimental design and analyses of data. In common with eliminating male bias in research, unmasking time as a critical biological variable is long overdue.

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