

## **Influence of sex on sleep regulatory mechanisms**

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## **Abstract**

The majority of species thus far examined exhibit sex differences in sleep and wakefulness. The contribution of biological factors to these sex differences may contribute to gender disparities in sleep disorders. Several lines of evidence suggest that in mammals, reproductive hormones are responsible for the effects of sex on sleep and may have organizational and activational influences on sleep regulatory mechanisms. In humans, exogenously administered estrogens and progestins generally enhance sleep amount and continuity, while androgens appear to have a positive impact on rapid eye-movement sleep, but disrupt sleep consolidation. In rodent studies, however, female reproductive hormones appear to enhance wakefulness while male gonadal hormones reinforce sleep. Rodent studies have also revealed that perinatal masculinization or feminization of the reproductive axis organizes adult sleep-wake architecture. Though the ability of reproductive hormones to interact with sleep regulatory mechanisms has been reported, the influence of sex itself on sleep-wake regulation remains relatively obscure. Recent investigations of the role of sex on sleep and on organizational effects of the sex chromosomes on behavior, may lead to new approaches for treating sleep disorders that exhibit strong gender differences. Treatment of sleep disorders that are more prevalent in women, such as insomnia and restless legs syndrome, has improved in recent years, however a clearer understanding of how sex interacts with sleep may have a positive influence on gender disparities in sleep health.

## **Introduction**

Gender identity is determined by a combination of biological, psychosocial, and cultural influences each of which uniquely impacts health and contributes to the likelihood of developing and/or succumbing to specific illnesses. The complex interactions of these factors with one another and with hazardous agents and behaviors, are responsible for gender disparities in general wellness and

gender predispositions to many diseases<sup>1-2, 3</sup>. In recent years, the increasing attention given to the association of gender with sleep health has been primarily driven by the growing recognition of the health dangers posed by sleep disorders. The key to learning how and why gender is such an important determinant of sleep disorders lies in a strategy that 1) identifies the mechanisms through which biological sex organizes and actuates the sleep-wake cycle, 2) seeks to understand how sex mediates the sleep responses to the environmental stressors and challenges that facilitate disease, 3) determines the biological impact of sex on the comorbidities (e.g. obesity) that are linked to sleep disorders such as sleep apnea. The impact of sex on sleep disorders in women has been reviewed<sup>4</sup>.

### **Gender and sex are not the same thing**

It is important to emphasize that the terms “gender” and “sex” are not interchangeable. Gender refers to sexual identity and its contextual relationship to the prevailing society and culture. The interrelationships of gender determinants such as secondary sex characteristics, psychosocial predispositions, and lifestyle choice change with age and cultural context. Therefore, an individual’s perception of gender roles and gender-specific behavior may evolve with age though most often their allegiance to the male or female gender remains consistent. Sex is much more static and is defined by the 1) complement of sex chromosomes inherited by the parents and 2) the reproductive organs and behaviors produced by sex chromosome complement.

### **Sleep is under the control of circadian and homeostatic processes**

Sleep and its reciprocal daily relationship with wakefulness are governed by two systems: 1) the circadian system which times the rhythm of the sleep-wake cycle and consolidates sleep and wake states into biphasic patterns, and 2) the homeostatic drive for sleep (sleep homeostat) that dictates the amount and intensity of sleep based on the duration of prior wakefulness<sup>5</sup>. Human and

animal studies have revealed that sex and gonadal hormones have influences on both systems but it remains unknown whether sex-induced differences in the sleep-wake cycle are mediated by either or both of these processes.

### **Some effects of sex on sleep may be mediated by the circadian system**

In mammals, sex is determined primarily by sex chromosome complement and propagated by secretion of reproductive hormones (e.g., progesterone, testosterone, and estrogen) from the gonads. Gonadal hormones have two primary effects: organizational and activational<sup>6</sup>. The sex hormone environment around the time of birth plays a major role in organizing the neural circuitry responsible for sex-influenced behaviors later in life<sup>7-12</sup>, including sex-specific mating behavior<sup>13-15</sup>, aggression<sup>16-18</sup>, arousal<sup>19, 20</sup>, and stress responses<sup>21, 22</sup>. During this critical period, between postnatal day (PND) 1 and PND 6<sup>17, 23</sup>, castration in neonatal males causes adult feminization<sup>17, 24</sup>, while androgen administration in neonatal females causes adult masculinization<sup>17, 18, 25</sup>. Several groups have reported organizational influences of gonadal hormones on circadian rhythms. For instance, perinatal androgenization of female rats shortens the free-running circadian rhythm of locomotor activity and alters the ability of 17 $\beta$ -estradiol to alter free-running locomotor rhythms in adults<sup>26-28</sup>. Similarly, perinatal treatment with testosterone propionate in female hamsters eliminates the ability of estradiol benzoate to shorten adult free-running rhythms<sup>27</sup>.

The stability of free-running rhythms in constant conditions and the ability of circadian locomotor rhythms to synchronize to the daily light-dark cycle are also influenced by sex and gonadal hormones<sup>29</sup>. In hamsters, sex differences in the ability to entrain to elongated light:dark cycles are eliminated by neonatal castration in males and females<sup>27, 29-32</sup>. More recent studies in diurnal rodents found that sex differences in circadian free-running rhythms develop after puberty

and coincide with the appearance of gonadal steroid receptors in the suprachiasmatic nucleus (SCN), the location of the primary circadian pacemaker in mammals<sup>31,32</sup>.

While early studies questioned the existence of sex steroid receptors in the SCN<sup>33</sup>, the findings of estrogen receptors in the SCN of humans and rodents (particularly estrogen receptor-beta in mice and rats) adds support to the hypothesis that the effects of gonadal hormones on circadian rhythms, and possibly sleep, could be mediated by an action on SCN neurons, particularly estrogen receptor containing SCN neurons<sup>34-39</sup>. Indeed, estrogen receptor mRNA levels show a diurnal rhythm in the SCN in young and middle aged rats that is blunted in old rats, raising the possibility that the effects of estrogen on the sleep-wake cycle could be influenced by such diurnal variations<sup>35</sup>. Further support linking the reproductive system to the SCN are the findings that the SCN extends projections to estrogen receptor and/or gonadotropin releasing hormone immunoreactive neurons<sup>40</sup> and that estrogen receptor neurons project to the SCN<sup>33</sup>.

### **Sex interacts with the molecular timing system**

The genetic basis of circadian timing has been unmasked at an astonishing rate over the past decade and exhibits a remarkable conservation of molecular components, functionality, and responses to the environment across phyla. The molecular oscillator that generates circadian rhythms in mammals is driven by an auto-regulatory feedback loop of circadian “clock” genes. Many of the core clock genes belong to the PAS (Per-Arnt-Sim) family of transcription factors and their positive and negative feedback interactions are responsible for circadian timing<sup>41</sup>. The discovery and identification of the gene *Clock*, through a mutagenesis screen<sup>42</sup> was the initiating event that has resulted in the characterization of the molecular oscillator in mammals. An innovative study on the impacts of circadian gene expression on sleep-wake architecture demonstrated that a mutation of the *Clock* gene in male mice increased daily wake amount and

decreased total sleep amount<sup>43</sup>. This study was the first demonstration that a clock gene has a direct influence on sleep-wake amount. In similar studies, mice homozygous for a deletion of the *npas2* gene, a homolog of *Clock* expressed primarily in forebrain tissue, exhibited a decrease in total sleep amount and a concomitant increase of wake amount<sup>44</sup>, supporting the hypothesis that components of the circadian timing system directly promote or influence wakefulness.

Mutations and deletions of several clock genes exhibit ancillary effects on sleep-wake patterns and at least two genes, the *npas2* gene in mice and the *cycle* gene in fruitflies, encode sex dimorphisms in sleep processes. *Npas2*, in addition to its role in modulating sleep-wake states, couples cortical circadian gene expression to sleep-wake distribution in a sex sensitive manner<sup>45</sup>. Targeted deletion of the *npas2* gene in mice decreases non-rapid eye movement (NREM) sleep in males but not females in response to sleep deprivation suggesting that some of the effects of sex on sleep may be mediated by circadian clock genes. Further evidence in support of this hypothesis comes from studies in fruit flies bearing a mutation of the *cycle* gene, homolog of the *Bmal1* gene in mammals: the mutation eliminates baseline sex differences in rest amount and amplifies sex differences in recovery from rest deprivation as well as lifespan<sup>46</sup>.

### **Reproductive hormones may organize the sleep-wake cycle**

To date, there has been a notable dearth of animal studies that test whether the organizational hormone environment establishes irreversible sleep-wake patterns in adults. A seminal study reported that neonatal castration in male rats alters sleep responses to injections of estradiol and progesterone<sup>47</sup>. In this study, female gonadal hormones administered to perinatally feminized male mice decreased rapid eye movement (REM) and NREM sleep amount in adulthood. These data not only support previous findings that estradiol and the female gonadal

hormones have an inhibitory effect on sleep amount when exogenously administered <sup>48, 49</sup>, but demonstrate an organizational influence of gonadal hormones on sleep-wake architecture.

### **Exogenous hormone treatments alter sleep-wake states**

Though the interaction of sex with the sleep-wake cycle is complex and fluid with respect to age and existing environmental pressures, healthy adult women tend to exhibit more slow wave sleep than men, more sleep spindles <sup>50</sup>, and also a higher sleep drive <sup>51</sup>. Exogenously administered estrogens have been shown to enhance REM sleep and shorten onset to sleep latency <sup>52, 53</sup> as well as decrease wake amount <sup>54, 55</sup>. Progesterone and several of its metabolites exhibit sleep promoting abilities that are generated by progesterone's actions as a GABA<sub>A</sub> receptor agonist. When given exogenously, progesterone and its GABA<sub>A</sub> active metabolite, pregnanolone, behave similarly to the benzodiazepine class of sleep-promoting agents and induce acute increases of NREM sleep amount in females <sup>56, 57</sup> and males <sup>58, 59</sup>. In animal studies the progesterone metabolites pregnanolone and allopregnanolone enhance NREM sleep amount and latency to NREM sleep through the agonistic actions on GABA<sub>A</sub> receptor subtypes <sup>60, 61</sup>.

Androgen administration appears to have a mild positive influence on REM sleep amount <sup>62</sup> and has been reported to induce sleep apnea onset in men and women <sup>63-65</sup>. Androgen administration in hypogonadal males also has negative impacts on breathing during sleep <sup>62, 66</sup>. Sleep disordered breathing tends to have a disruptive influence on sleep continuity resulting in increased fragmentation of sleep states and shorter amounts of time spent in slow-wave sleep. In this regard, androgen administration or replacement may compromise sleep hygiene through negative influences on respiratory function.

### **Animal studies reveal disparate influences of gonadal hormones on sleep**

Animal studies examining the impact of gonadal hormones on sleep-wake architecture have been more conclusive than human studies. Female guinea pigs exhibit reductions in REM and total sleep amount during the stage of estrus, which is characterized by peak estrogen levels and rising levels of progesterone <sup>67</sup>. In rats, ovariectomy increases SWS and decrease wake amount <sup>68</sup>, while estrogen treatment in ovariectomized mice reduces nighttime REM sleep amount <sup>49</sup> and combined injections of estrogen and progesterone reduce daily NREM and REM sleep amounts <sup>48</sup>. Ovariectomy results in a selective increase in the amount of night-time paradoxical sleep in female rats, abolishing the sexual dimorphism at night, but not during the day <sup>69</sup>. Taken together, these studies support the hypothesis that the female gonadal hormones promote wakefulness at the expense of REM and/or NREM sleep.

The surprising lack of information on the effects of sex on sleep in mice, the primary mammalian genetic model for the study of the sleep-wake cycle, led us to test the hypothesis that sex affects sleep under baseline and sleep deprivation conditions in this species. We have recently reported sex differences in sleep-wake amount, distribution, intensity, and recovery from sleep deprivation, several of which are dependent upon the presence of the gonads <sup>70</sup>. The first major finding of these experiments was that female mice were awake 1.5 hours a day more than male mice. The increased wakefulness occurred primarily during the dark phase, the active time in nocturnal rodents. Interestingly, a similar wake profile has been described in female fruitflies <sup>71</sup>. A second major finding was that sleep-wake patterns are more consolidated in female mice, as indicated by a lower number of arousals from sleep and more sustained bouts of wakefulness during the normal active period. The third important result was that following sleep deprivation, female mice gained back more NREM sleep during the recovery opportunity compared to males, both in terms of absolute sleep time and as a percent increase over baseline. The majority of these

differences were eliminated by gonadectomy in males and females, suggesting that the primary influences of gonadal hormones on the sleep-wake cycle of mice are activational.

### **Age interacts with the ability of reproductive hormones to influence sleep**

The synthesis and secretion of androgens, estrogens, and progestins in mammals has a variable longitudinal profile throughout the lifespan. Though the profile of sex steroid secretion exhibits several prolonged periods of stability, abrupt and dramatic adjustments in the magnitude and timing of steroid secretions, as well as the regulation of the gonadotropins that govern steroid production, are hallmarks of ontogeny and aging.

Puberty is most concisely defined as the time when reproduction is first possible <sup>72</sup>. Although sexual maturity (i.e. the time when reproductive capacity is maximal) may occur gradually over a prolonged period of time <sup>73</sup>, puberty signals a synchronous reorganization of the neurohormones of the hypothalamic-pituitary-gonadal (HPG) axis. The specific hormonal events that occur during puberty are well conserved across mammalian species, however, the timing of these events is highly variable across and even within species <sup>9, 74-76</sup>. A number of studies have been carried out, particularly by Carskadon and her colleagues <sup>77</sup>, to indicate that both the homeostatic and circadian regulation of sleep are influenced by pubertal events. A recent study showed that sleep intensity, as measured by NREM delta activity, was stable between 9 and 12 years of age in male subjects, while NREM delta activity decreased in female subjects during this time period <sup>78</sup>. The authors interpreted these results to indicate that the maturation of the change in NREM delta activity to the adult-like condition occurs at an earlier age in females than in males. Another study in 4-14 month-old children found that sex differences in sleep-wake architecture develop around 10 months with girls beginning to display more sleep time <sup>79</sup>.

The reproductive hormone environment in adults is characterized by stable levels of the gonadotropins and testosterone in the male, and by the presence of regular cyclicality in the HPG axis of the female. In a study of sleep across the menstrual cycle in women, REM latency was significantly shorter during the postovulatory (luteal) phase when progesterone levels are high as compared to the preovulatory (follicular) phase<sup>80</sup>. This disruption of sleep on the night of proestrus, when progesterone levels are high, is consistent with reports of disrupted sleep in menopausal women following the addition of progestins to hormone replacement therapy<sup>81</sup>. The finding that *c-fos* expression in many hypothalamic nuclei involved in sleep regulation is highly estrogen dependent, indicates a possible direct action of steroid hormones on sleep-wake centers<sup>82</sup>.

Middle to old age females have consistently been shown to have greater amounts of NREM (stages 3 & 4) sleep and NREM delta activity compared to age-matched males<sup>83-89</sup>. In women, menopause begins between 45 and 55 years of age and is induced by the failure of the ovary to respond to gonadotropins<sup>90-92</sup>. The hallmark of menopause is the arrest of the menstrual cycle resulting in the decrease and eventual cessation of estradiol secretion by the ovaries. Estrogen levels are very low in postmenopausal women who report disturbed sleep associated with hot flashes<sup>93</sup>. The incidences of hot flashes and sleep disturbances is reduced with estrogen replacement therapy (ERT)<sup>93-95</sup>. Estrogen replacement therapy also results in improved sleep in menopausal women who do not report symptoms such as hot flashes<sup>96</sup>. Other studies in humans indicate that ERT increases slow wave sleep in post menopausal women<sup>97</sup>. This is consistent with the observation that women who receive long term ERT show an increase in growth hormone, (which is associated with slow wave sleep) release at night<sup>98</sup>. In men, aging brings about a gradual decline in testosterone production, often referred to as the andropause, which begins between 45 and 60 years of age with there being about a 1-2% annual decline in blood testosterone levels<sup>99</sup>.

The reduction of testosterone secretion is more moderate and gradual than that of estrogen during menopause<sup>100-102</sup>. Sleep disturbances are a common complaint in men receiving anti-testosterone treatment for prostate cancer and in women receiving anti-estrogen treatment in response to breast cancer<sup>103</sup>.

### **Genetic sex has hormone-independent influences on behavior**

To date there have been no studies that directly examine the impact of genetic sex (XY or XX) on the sleep-wake cycle, however an impact of sex chromosome complement on sleep-related processes and behaviors has been demonstrated in mice. For instance, both aggressive behaviors<sup>104</sup> and immune responses<sup>105</sup> can alter the sleep-wake cycle. Separate studies have demonstrated that chromosomal sex acts independently of the gonads as a determinant of aggressive behavior<sup>106</sup> and autoantigen-specific immune responses<sup>107</sup>. These studies have taken advantage of an exciting mouse model of genetic sex in which the sex chromosome complement is the opposite of gonadal sex<sup>108</sup>. This mouse line was initiated by a spontaneous deletion of the testis determining gene “*Sry*” from the Y chromosome of MF-1 outbred mice and the subsequent insertion of the *Sry* transgene onto an autosome<sup>109</sup>. The potential ability to examine sleep-wake states in this and similar mouse models may unmask interactions between genetic sex and gonadal sex on sleep-wake regulation. Such an analysis could become valuable to distinguish potential organizational influences of the sex chromosomes from activational responsibilities of reproductive hormones on the sleep-wake cycle.

### **Significance**

The majority of studies that examine sleep-wake regulation have focused on males, despite the clear evidence that the reproductive hormone environment influences sleep and wakefulness. The few studies that have compared sleep-wake regulatory processes in male and females have

reported pronounced gender differences, although the underlying physiological processes linking the sleep-wake cycle to gender remain poorly understood. While accumulating evidence from clinical and experimental studies indicates that the maintenance of adequate sleep time and sleep quality are important for many aspects of overall health and neurocognitive performance, very little is known about how gender may affect the health and performance consequences associated with inadequate sleep. Furthermore, while it is known that gender influences stress responses and that stress hormones can influence sleep-wake patterns, little is known about how gender influences the effects of stress on sleep or how sleep loss affects the response to stress. These gaps in the knowledge base underscore the need for more effective genetic and behavioral models to clarify how the influences of sex on the sleep-wake cycle may cause gender disparities in the incidence and severity of sleep-wake disorders.

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