DO PHOTOPERIODIC CHANGES IN MELATONIN SECRETION DETRIMENTALLY AFFECT THE FEMALE REPRODUCTIVE CYCLE?

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ABSTRACT

Melatonin, better known as "the hormone of darkness," is secreted by the pineal gland during the night and helps us fall asleep. Because its internal regulation depends on light, melatonin is part of chronobiology, the study of biological mechanisms and their adaptations to lunar and solar related rhythms (Klein et al. 1991). Therefore, photoperiod changes greatly impact melatonin concentration, influencing changes in neuronal and hormonal mechanisms of the photo neuro-endocrine systems, namely reproduction. Research has shown that a disruption in the circadian rhythm of melatonin due to photoperiod changes detrimentally affects the rhythmic function of the female reproductive cycle. Research from journals, articles, and printed books has shown that both exogenous and endogenous features contribute to the reproductive cycle and that the internal mechanisms are entrained by environmental cues. Photoresponsiveness of the reproductive system is mediated by the internal biological clock, transcriptional factors, period genes, photic input, GnRH (gonadotropin-releasing hormone) neurons, and melatonin secretions. Specifically, melatonin secretions directly affect reproductive function either through stimulatory or inhibitory pathways. Seasonally breeding animals interpret photic information according to melatonin secretions. This clearly points to a relationship of melatonin with photic and reproductive qualities. Research is ongoing with various species of animals in hope of uncovering the mystery of the connection between light, melatonin, and reproduction, which may be helpful in understanding the effects of photoneuroendocrinology on the female human.

INTRODUCTION

In the industrial world of the 21st century, work at night seems to be unavoidable. Erhard Haus claims that 17% of the working world is involved in some kind of rotating shift work, permanent night shift, or trans-meridian travel (Haus and Smolensky 2006). Nurses work rotating shifts, wholesale food producers drive through the night, bakers bake in the wee hours of the morning, medical school students intern around the clock, security guards work night duty, and traveling businessmen fly many miles a month. What they all have in common is that they are on arrhythmic sleep schedules. The diabetic frequent flyer knows to keep insulin medication handy because glucose levels can fluctuate in response to confusing messages of night and day the body receives when traveling across several time zones, for traveling eastward shortens the day while traveling westward lengthens the day (Keystone and Kozarsky 2011). The body runs on an internal rhythmic schedule and may not adapt easily to extreme changes in the timing of surrounding environments. The World Health Organization has blamed shift work for the increasing occurrences of cardiac, gastrointestinal, and reproductive disorders and certain cancers (Longo et al. 2012). Melatonin is known to inhibit estrogen dependent disorders, including breast cancer. If sleep schedules are compromised, melatonin may not be able to guard the body from the invasion of cancer. Epidemiologic studies on women show a correlation between shift work employment and the incidence of breast and colon cancers. Melatonin disturbances have shown to affect proper reproductive function as well. Flight attendants and shift work employees, two groups of people who suffer from disrupted day/night schedules and

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increased variance in their melatonin production, have reported changes in their menstrual cycles. These changes may account for fertility issues and the inability to carry a pregnancy through nine months (Dawson et al. 2006). Therefore, women in the working world should be educated about the effects their schedules may have, if any, on their reproductive function if they intend to lead healthy reproductive lives.

This paper will focus on the effects of photoperiodic changes in melatonin concentration on female reproductive function. Although the estrous cycle of female rats is not the same as the menstrual cycle in the female human, experimental results and scientific reviews on rats are analyzed as a means of uncovering trends in animals that may be possible in humans as well.

MATERIALS AND METHODS

The information in this research paper was located using Touro College Library search engines, multiple databases, printed books, science journals, and articles. This paper offers an in-depth analysis of the information collected from these sources. Most of the information is based on experiments and research on the rodent reproductive cycles because not much effort has been made to test human subjects. It is not within the scope of this paper to come to a complete conclusion about the effects of light and the pineal hormone on the menstrual cycle, but this paper will attempt to unveil the mechanisms involved in these processes and offer possible reasons as to why certain effects may or may not be evitable. The role and function of melatonin will be discussed first, followed by a description of the menstrual cycle and the photoperiod. Then, the effects of the photoperiod and melatonin on reproduction will be examined. In addition, the role of the endogenous circadian system, or internal clock, and its impact on the female reproductive cycle will be discussed and compared to the exogenous factors influencing reproduction. Lastly, the far-reaching effects caused by an imbalance of melatonin and possible solutions to eliminate them will be discussed.

DISCUSSION

The Function of Melatonin and its Relation to the Female Reproductive Cycle at the Hypothalamic Level

The suprachiasmatic nucleus (SCN) of the hypothalamus receives photoperiodic information from the environment and uses it to controls biorhythmic and neuroendocrine functions. The suprachiasmatic nucleus is the body's master clock and is the prime controller of the body's circadian rhythm, the rhythm of various functions, processes, and mechanisms that occur in a 24-hour day. Through its retinohypothalamic connection, the suprachiasmatic nucleus interprets photic stimuli and sends this information to the brain and other organs in the form of chemical messages. The main target, another organ in the photo endocrine system, is the pineal gland, also called the diencephalic or deep photoreceptor (Klein et al. 1991). The pineal gland is the body's 'third eye' because it reacts, by secreting melatonin, based on what it sees from the outside environment (Cagnacci et al. 1995). In fact, pinealocytes and photoreceptors of the eye share common origin and components of signal transduction pathways (Lolley et al. 1992). Melatonin, produced exclusively at night, is secreted by the pineal gland after an adrenergic stimulus at the pinealocytes (Cagnacci et al. 1995). Research has shown that ablation of the suprachiasmatic nucleus destroys the circadian rhythm of pineal N-acetyltransferase activity, which can inhibit the proper functioning of the hormone melatonin (Klein et al. 1991). Many studies claim that melatonin inhibits the luteinizing hormone (LH).

Luteinizing hormone is secreted during the follicular phase of the menstrual cycle and is necessary for follicle development and subsequent ovulation. During the follicular phase of the eumenorrheic menstrual cycle, the hypothalamic releasing factor, gonadotropin-releasing hormone (GnRH), stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone from the anterior pituitary. Luteinizing hormone spikes immediately prior and during ovulation, creating a surge of luteinizing hormone mid-cycle, which is followed by the luteal phase and an increase in the ovarian steroid progesterone (Dawson et al. 2006). Consequently, if melatonin inhibits luteinizing hormone, it can inhibit ovulation which is necessary for proper reproductive function and fertilization. In addition, seasonal breeding, favoring long summer days, points to melatonin as a possible inhibitor of reproductive activity. An experiment conducted in Finland, by the Department of Obstetrics and Gynecology of the University of Oulu, sought to discover a connection between seasonal and menstrual secretions of melatonin and gonadotrophins in women. The study found that during a midcycle night, luteinizing hormone was 76% higher in the summer, a 22-hour day, than in the winter, a 5-hour day. Melatonin, on the other hand, was about 50% higher in the winter than in the summer. It is possible that melatonin inhibited luteinizing hormone and, therefore, allowed increased levels during the summer when the day is longer than night (Kivelä et al. 1988). However, these results do not address the fact that luteinizing hormone levels were high at night; if melatonin does inhibit luteinizing hormone, why was there an increase of luteinizing hormone at night, a period during which melatonin is high? It is true that, overall, the summer days have more hours of light and, therefore, luteinizing hormone peaks, but that does not explain why the luteinizing hormone peaked specifically at night when melatonin rises.

Melatonin and Sleep

Sleep disorders and their effects on menstrual disorders is another way to understand the possible effects of melatonin on reproduction. In researching the impact of sleep loss on health, Dr. Michael Thopy, sleep director of Montefiore Medical Center at Albert Einstein College of Medicine in New York, said," if you are supposed to sleep a third of the day, there has got to be a reason for it" (Neergaard 2012). In the results of an experiment done on 26 women by Kari Sveum of Northwestern University in Chicago, Illinois, twice as many subjects with delayed sleep syndrome reported menstrual irregularities than those without delayed sleep syndrome (American Academy of Sleep Medicine 2008). It is not clear from this experiment if sleep itself or a dysrhythmia of the light/ dark perception associated with sleep disorders is connected to menstrual irregularities. This paper will not consider the actual phenomena of sleep to be a key factor in influencing reproductive function; rather the body's interpretation of night and day by the photo endocrine system and subsequent melatonin secretions will be considered a key factor.

Melatonin and Specific Hormones

Melatonin directly impacts the female reproductive cycle by stimulating the release of gonadal hormones according to the information it receives from the suprachiasmatic nucleus. The suprachiasmatic nucleus itself also has a connection to the reproductive pathways via GnRH neurons of the hypothalamus. In contrast to the previous belief about the inhibitory effect of melatonin on luteinizing hormone in the menstrual cycle, an experiment done at the University of California showed an increase of luteinizing hormone following the administration of exogenous melatonin. During two

consecutive days of both the follicular and luteal phases of six normal cycling women, blood assays were checked for melatonin, luteinizing hormone, and other gonadal hormone concentrations. Exogenous melatonin or a placebo was given at 8:00 a.m. on the first days of each phase, and blood was drawn every 20 minutes from 9:00 a.m. to 5:00 p.m. on all four days in order to detect hormonal levels throughout each day. The results found that exogenously administered melatonin increased luteinizing hormone during the follicular, but not luteal, menstrual phase; the stimulatory effect of melatonin was selectively exerted in the follicular phase of the menstrual cycle. Melatonin levels were the same in both the follicular and luteal phases, but only affected luteinizing hormone in the follicular phase. The study acknowledges that this increase may not be felt when looking at the overall luteinizing hormone production during the entire menstrual phase, so it is premature to conclude that this melatonin truly has a stimulatory effect on reproduction (Cagnacci et al. 1995). In addition, the possibility of errors in the experimental procedure and analysis leave room for doubt as to whether melatonin can be categorized as a stimulant. One cannot base a theory on the cycles of only six women and simply categorize the follicular and luteal phases by the first two days of their cycles. In addition follicle-stimulating hormone, which is part of the positive feedback mechanism in increasing luteinizing hormone for mid cycle ovulation (Dawson et al. 2006) (Figure 1), was not affected at all by the exogenous melatonin. Thus, just stimulation to LH in the follicular phase may not be enough to really stimulate menstrual function. In researching the melatonin levels during the menstrual cycle of normal cycling women, Cagnacci reports a delayed melatonin phase in the luteal phase compared to the follicular phase (Dawson et al. 2006). Although melatonin levels were the same in this experiment, it is possible that because melatonin levels are delayed in the luteal phase, they do not affect LH as much as in the follicular phase. Thus, zooming in on LH in the follicular phase does not provide enough of a basis to prove that this melatonin is beneficial to the menstrual cycle as a whole. In addition, when drawing a conclusion about the body's secretion of melatonin and menstrual function, these results are irrelevant because the melatonin used in this experiment was not naturally secreted. Errors in the experiment as well as unnatural conditions make the results of this experiment questionable in reference to melatonin's stimulatory effects on the menstrual cycle.

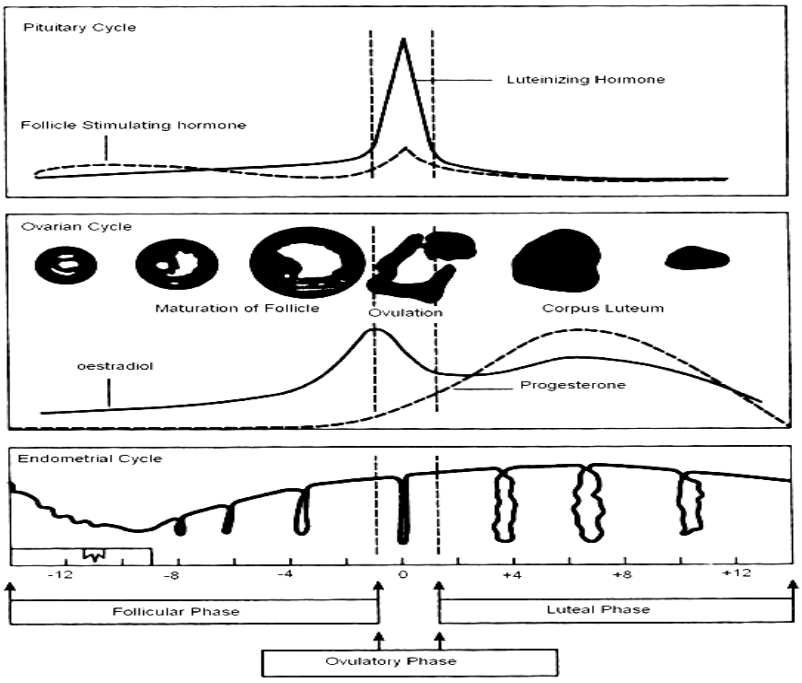

Figure 1: Stages of the menstrual cycle: hormones, in the ovaries, and in the uterus. Luteinizing hormone is needed for the LH surge at ovulation. Progesterone is necessary for proper growth of the uterus. Source: Dawson et al. 2006

High melatonin levels were detected in women with secondary amenorrhea, indicating that the pineal hormone may play a role in inhibiting menstruation. This condition is common in female athletes who strenuously train for sports on a low caloric diet, for exercise training that reduces body fat may lead to a reduced production of estrogen, thereby inhibiting proper function of the menstrual cycle (Dawson et al. 2006). Exercise induced amenorrhea is the body's way of telling itself to conserve energy where necessary and not put too much effort in functions that are not life threatening, like reproductive activity. The exact mechanisms and causes of secondary amenorrhea are not fully understood, but researchers see a relationship between melatonin and the amenorrheic women. Brezinski and Kadva report elevated melatonin levels in women with reduced gonadotropin levels "which may suggest a neuroendocrine pathology underlying this disorder" (Dawson et al. 2006). On the other hand, it is possible that melatonin itself does not reduce gonadotropin levels, but rather it increases in response to reduced gonadotropin levels. Another link between melatonin and the menstrual cycle is their shared ability to inhibit estrogen dependent disorders. Postmenopausal women are at a greater risk for developing breast cancer because of their lost menstrual cycle which is thought to prevent cancer by keeping estrogen levels in check. When circulating blood contains high levels of estrogen, breast cancer, an estrogen dependent disorder, can thrive.

Similarly, melatonin has been found to keep estrogen levels in check by reducing estrogen receptors, thereby decreasing the development of estrogen dependent disorders such as breast cancer and endometriosis. Melatonin's action on estrogen can be helpful, on one hand, in reducing the risk of estrogen dependent disorders but detrimental, on the other hand, if melatonin inhibits healthy levels of estrogen necessary for uterine tissue growth. The ovarian steroid estrogen, made from the precursor estradiol, is needed for preparing the uterus in case of fertilization. It is unclear how much melatonin is helpful and how much is harmful to estrogen in the human body. A study done at Al- Azhar University was designed to investigate the modulatory effect of melatonin on uterine estrogen and progesterone receptors and uterine contractility. Twelve non-pregnant Albino Wistar rats were classified into two groups, one of which was treated with melatonin daily for 15 days. The rats were then scarified to expose their uterine horns, treated with oxytocin, and carefully watched for contractility. In addition, a uterus section of four micrometers thick was mounted on a slide and stained to reveal estrogen and progesterone receptors. The results of melatonin treated rats showed a decreased effect of oxytocin on uterine contractility, decreased estrogen receptors, and increased progesterone receptors (Abu-Allah et al. 2003). These results, however, reflect the responses of the uteri of just six rats and, therefore, cannot be a strong enough basis to conclude the effects of melatonin on uterine receptors. Although it is still possible that melatonin may inhibit breast cancer development through its inhibiting the level of estrogen receptors, at the same time it may be decreasing estrogen receptors necessary for uterine lining development. This experiment does not mention if the decrease in estrogen was beneficial or harmful for healthy reproductive function.

Melatonin was helpful in increasing progesterone receptors, also necessary for uterine development. In this capacity, melatonin acts as a stimulant to reproductive activity. Melatonin's ability to elevate levels of progesterone is yet another connection between the hormone of darkness and menstrual function. Martensson suggests that melatonin is stimulatory to the menstrual cycle, because uterine activity is greater during the dark, the same time that melatonin levels rise (as quoted in Dawson et al. 2006). Progesterone,

meaning “good for gestation," is an ovarian steroid hormone which nourishes the uterine lining before fertilization and continues to nourish the developing embryo. In this sense, melatonin stimulates hormonal secretion at the level of the ovary. Dr. Len Lopez, a nutrition and fitness expert, explains that infertility and spontaneous abortion are common in women with low levels of progesterone because progesterone is needed to support menstrual function and pregnancy. He calls the adrenal gland a thief because it uses progesterone in order to produce the stress hormone cortisol, accounting for the stress-infertility connection (Lopez). However, although cortisol may inhibit progesterone levels, it has not been found to directly affect the menstrual cycle in any other way. A study done on patients diagnosed with fibromyalgia (FM), a rheumatoid like disease with various components on muscular, psychosomatic, and psychoneuroendocrine levels, investigated the effects of fibromyalgia on the H-P-G (Hypothalamic- Pituitary- Gonadal) and H-P-A (Hypothalamic- Pituitary- Adrenal) axes. The experiment consisted of 105 normal cycling women, of whom 63 were diagnosed with fibromyalgia, and 38 women who served as the control. After a night's fast, blood assays were collected and checked for FSH, LH, prolactin, cortisol, progesterone, and estradiol. Cortisol levels were significantly lower in patients compared to controls, while gonadotrophic levels were unaffected, providing no indication that the cortisol levels had an effect on progesterone levels. However, in addition to reduced levels of cortisol, there was an increase in the luteinizing hormone of patients with fibromyalgia also suffering from sleep disturbances. Once again, a relationship can be seen between sleep and melatonin disturbance affecting the synthesis of luteinizing hormone (Gur et al. 2004). It is clear from this experiment that cortisol levels do not affect gonadotrophic levels alone, but can possibly affect LH levels when melatonin levels are altered as well. Alternatively, it is just the melatonin levels that affected the luteinizing hormone and not the cortisol levels at all. Results are questionable because blood assays were taken only once. Because hormones may not suddenly appear in the blood from one minute to the next, extensive monitoring may be required in order to be used as experimental evidence.

PHOTOPERIODIC AND GENETIC CONTROL OF MELATONIN SECRETION AND REPRODUCTIVE FUNCTION

Photoperiodic Responsiveness and Reproduction

Extensive research on the secretion of melatonin and subsequent reproductive function has postulated a balanced relationship between exogenous and endogenous controls. On one hand, light, interpreted by the suprachiasmatic nucleus and hypothalamus, enhances gonadal growth, reproductive hormones, and successful breeding. This indicates that the night hormone melatonin may play a role in inhibiting reproductive function. However, many experiments connect reproductive function to endogenous controls, such as genetics and internal biological clocks, effectively ruling out light and photoperiods as being possible effectors of reproduction. Thomas Dickmeis of the Institute of Toxicology and Genetics in Germany links the two components and suggests that clock gene expression is controlled by light and the suprachiasmatic nucleus (Dickmeis 2009). Strong evidence in both directions demands the answer that both exogenous and endogenous factors control reproduction, and results differ depending on the species in question. For example, when exposed to certain sequences of light and dark, the gametes of some coelenterates mature and are released. This direct reaction to light is unique to this specific

species and not a reflection of the reaction of other animals to light exposure (Scharrer 2006).

In an article, David Kennaway describes the connection between the suprachiasmatic nucleus, melatonin, and reproduction. Studies have found that the suprachiasmatic nucleus vasopressin release stimulates gonadotrophin releasing hormone and, consequently, the surge of LH in the ovary (Kennaway 2005). Suprachiasmatic nucleus projections were found in GnRH immmunoreactive neurons and estrogen receptor cells, suggesting that the circadian system regulates the timing of some menstrual activity (De la Iglesia et al. 1995). Melatonin concentrations change according to SCN activity, and melatonin receptors in the SCN provide an additional link between the circadian timing system and seasonal changes in fertility. Kennaway adds that puberty was delayed in rodents experiencing long hours of darkness, indicating that melatonin probably effects the reproductive maturation of certain animals (Kennaway 2005). Research has shown the direct effects of light on gonadal growth, hormones, and puberty. Because melatonin is the marker for SCN function, and the SCN interprets the light information, responses to light are associated with circulating melatonin levels. The Institute of Zoology in London conducted an experiment on seasonally breeding Syrian male hamsters, involving photoperiodic changes. Both wild type and tau-mutant hamsters were maintained in stimulatory photoperiods for reproduction. The wild type hamsters were allowed 16 hours in the light and 8 hours in the dark (16L: 8D), the tau-mutants were allowed 12 hours in the light and 8 hours in the dark (12L: 8D) (the tau mutation shortens the rhythm by approximately 4 hours resulting in a circadian period of 20 hours instead of 24) (Stirland et al. 1996). The hamsters were then exposed to increasing hours of darkness for 84 cycles, resulting in testicular atrophy and complete regression at 11 hours dark for the wild type and 10 hours dark for the tau-mutant. This inhibition of gonadal growth in increased dark hours seems to show an inhibitory effect of the night hormone melatonin on reproduction (Stirland et al. 1996). Only because Syrian hamsters are long day breeders, prolonged exposure to darkness was detrimental to their reproductive system. These results, however, do not prove that darkness is an inhibitory factor of reproduction in different species of animals. Similar results were found during an experiment with male Djungarian hamsters and photoperiodic changes on gonadal growth. Newborn hamsters from a breeding colony in a long day setting were either kept in a long day (LD) setting or transferred out to a short-day (SD) environment after birth. In order to create four treatment groups that would reflect a variance in photoperiodic changes, at 30 days old, half of long day hamsters moved to the short day environment , and half of the short day hamsters moved to the long day environment (1. LD 2. SD 3. LD-SD 4. SD-LD). Blood samples were taken after 40 days, and at 40, 60, and 90 days of age, 4-6 hamsters in each group were sacrificed and had their testes weighed and coronal brain sections analyzed for GnRH cell bodies. Photoperiod greatly affected testis weights and follicle-stimulating hormone levels while it showed no relation to increased GnRH perikarya. The testis weights of rats transferred from short days to long days was the same as those reared in long days from the start (Figure 2). These results prove that long days are beneficial for the male Djungarian reproductive system. By 90 days, the testes of hamsters reared in long days or transferred to long days were the size of adult testes while SD/ LD-SD hamsters' testes fully regressed. Serum LH levels were unaffected by photoperiods, while FSH increased in LD groups, especially in the SD-LD group (Yellon

1994). Interestingly, the greatest increase in follicle stimulating hormone was found in the SD-LD group, signifying the effects of a variant environment, which ended in a stimulatory setting, as being the most helpful for reproductive function. How exactly light affected reproductive function is unclear because GnRH cell bodies did not increase in response to the light. It was thought that the GnRH immunoreactive cell bodies would increase in response to light, but they did not. Nevertheless, the authors suggest that photo treatment may control post-translation modification of GnRH molecules and effect reproduction (Yellon 1994). A study done on three strains of laboratory male rats, ACI, BUF, and PVG showed photo responsiveness which enhanced gonadal growth. Four groups of rats were raised. Two groups were subjected to the long day (LD) setting, which included 16 hours of exposure to light and 8 hours in darkness (16L: 8D), and the short day (SD) setting, which included 8 hours of exposure to light and 16 hours in darkness (8L: 16D). The other two groups were LD subjected rats with food restrictions (LD+ food restriction) and SD subjected rats with food restrictions (SD+ food restriction). Food restriction groups were fed 70% of the food given to the regular groups. The rats were sacrificed and weighed. In all three strains, short day settings resulted in a slower increase of body and reproductive organ mass. These two studies reflect the stimulatory effects of light on the reproductive function of certain species, unlike other rats that prefer short photoperiods for reproductive growth. The experiment done on the three rat strains, ACI, BUF, and PVG compared the effects of long day photoperiods on reproductive growth to the stimulatory short day photoperiods of Fischer and Brown Norway rats. The results in this experiment showed that long days were preferred in all three strains, indicative of a genetic component involved in these species that controls reproductive responsiveness to photic information (Francisco et al. 2004) (Figure 3). Although the results indicate long photoperiods as stimulatory for these three rat strains, no control of a short photoperiod was actually used in the experimental setup, which would have strengthened the results of this experiment. In all three experiments, light profoundly affected gonadal growth in males, without promise of similar effects on female reproductive organs. Still, the evidence provided in these experiments indicates a direct connection between the exogenous factors, the photic environment, and reproductive function.

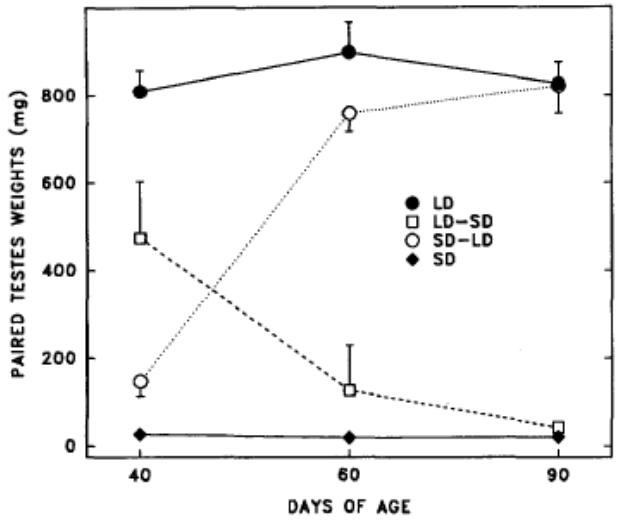

Figure 2: Long day conditions for both LD and SD-LD favored higher testicular weights than SD or LD-SD. Notice the immediate drop in weight of rats switched from LD-SD after only 10 days in their new conditions. Source: Yellon 1994

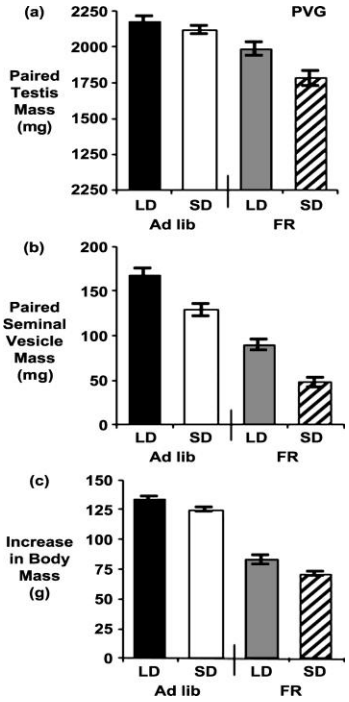

Figure 3: Weight of body and reproductive organs increased under conditions of long days, and normal food. Table for PVG strain; results of both ACI and BUG followed a similar pattern. Source: Francisco et al. 2004

Perspective on Endogenous Controls: Genetics, Peripheral Clocks, and Internal Timing System

How do the endogenous timing system and genetics enter into the scene of light and the SCN controlling melatonin and reproductive function? The answer is that the biological clock reacts according to photoperiodic change. In a sense, the SCN is entrained by the biological clock and genetic components of a specific organism. Animals were created with the ability to adapt to their environment for ultimate survival, and they time anticipated parturition in a season with optimal food availability to increase chances of survival. Therefore, long daylight hours may be beneficial for hamster reproduction but inhibitory to sheep reproduction (Foster and Kreitzman 2004).

Light itself is not necessarily the marker of growth and reproductive function; rather it tells the organism what time it is so that it can perform its various seasonal functions. An experiment was done on pinealectomized hamsters under conditions of constant dim light. Two groups received melatonin concentrations which corresponded to either the winter or summer months: more melatonin corresponded to the long winter night and less melatonin corresponded to the short summer night. Hamsters, long day breeders, treated with summer-like melatonin infusions were reproductively active while the winter group was not. A similar experiment done on sheep, which are short day breeders, showed a higher reproductive rate in the winter rather than the summer (Foster and Kreitzman 2004). These findings prove that reproductive response to external cues is at the genetic level and differs across various species. Notice how the hamster and sheep reacted in response to the melatonin concentration; these animals used the blood melatonin level as a marker of the season and became reproductively active depending on their endogenous timing system. This evidence strongly suggests that melatonin mediates the effect of photoperiod changes on reproductive function because the animals take cues from their melatonin levels. Melatonin levels change according to input from the environment, thus additionally proving how the suprachiasmatic nucleus controls reproductive function. Colin Pittendrigh

advocated for the consideration of the biological endogenous clock, its mechanism and entrainment by light as factors working together to control various functions. He remarked, "there are common mechanisms—built of different concrete parts—in circadian systems and photoperiodic effects everywhere" (as quoted in Foster and Kreitzman 2004).

A study conducted by researchers at the University of Illinois was designed to investigate the effects of SCN lesions on melatonin secretion and subsequent reproductive activity. The evidence of the study strongly suggests that the effects of photoperiod on reproductive function are controlled by melatonin secretions. Ovary intact ewes were subjected to unilateral or bilateral lesions of the suprachiasmatic nuclei, or sham lesions, placebo lesions which served as the control (sham lesion is when the identical brain surgery is performed, but does not target the brain tissue that the experimental group is testing). After recovery from the surgery, the ewes were exposed to alternating short day (9 hours of exposure to light and 15 hours in darkness) and long day (16 hours of exposure to light and 8 hours in darkness) photo regimens. Melatonin secretions in the unilateral and sham lesioned ewes remained normal, high at night and low during the day, while the bilateral lesioned ewes experienced disrupted and abnormal melatonin secretions. Almost all reproductive activity, with few exceptions, followed the pattern of melatonin—normal in unilateral and sham lesioned ewes and abnormal in bilateral lesioned ewes— indicating that melatonin is a direct effector of reproductive function (Scott et al. 1995). Like the previous experiment, this study strongly highlights the direct link between melatonin and reproduction, which are both controlled by photic messages. However, the experimental procedure in the study of the ewes is questionable, rendering the results questionable as well. An experiment must have only one variable in the experimental group which is not in the control group in order to accurately test the effect of the variable. In this experiment, the SCN lesion was the variable between the experimental and control group of sham lesions, but the alternating short and long days may have interfered with the experiment at hand. It is possible that the bilateral SCN lesions were responsible for the altered melatonin secretions, but because there was also `a constantly changing photoperiod, it is also possible that the disrupted light patterns caused altered melatonin levels and subsequent disruption in cyclicity. The authors mention that this experiment does not prove that the SCN generates reproductive function, because even ewes with abnormal cyclicity ovulated, but they do not address the fact that the SCN lesion may not have impacted melatonin at all. It is unclear if the SCN lesion was the sole cause of disrupted melatonin secretion because additional environmental factors added to the variability.

The rhythmicity of the SCN is traced back to transcriptional factors, *Clock* and *Bmall*, which code for period genes *per1*, *per2*, and *per3*, which are believed to play a role in fertility (Boden and Kennaway 2006). An experiment recorded in the Journal of Reproduction reports how cellular rhythmicity and reproductive function were affected in various mice knockouts. Mice with the *per1* and *per2* gene knockout maintained normal reaction to a light/dark cycle and did not show changes in reproductive activity, while the period of the *per3* knockout was shortened by .5 hours. When transferred to constant darkness, all rhythmicity was lost in the *per1* and *per2* knockout mice. Mice with the *Clock 19* knockout were also entrained to the normal light dark cycle and lost all rhythmicity when placed in total darkness. LH levels were normal in the *Clock* knockout mice, but prolonged and irregular estrous cycles did not allow for appropriate embryonic development and pup survival. The *Bmall* knockout mice had disrupted rhythmicity of the

SCN in a normal light/dark cycle, and they also showed reduced activity because of an inability to adapt to dark periods. Their reproductive functions were also harmed by delayed puberty, small ovaries and uteri, and the inability to establish viable pregnancies (Boden and Kennaway 2006). After analyzing the results stated in Boden's article, it can perhaps be concluded that the *Bmall* gene is a necessary endogenous feature that controls reproduction in response to environmental cues. All gene knockouts lost their rhythmicity in darkness, but only *Bmall* knockouts showed arrhythmic patterns even in a normal light/dark cycle. The *Bmall* knockout was the most affected by reproductive issues, while the other knockouts showed no or little effect on reproductive functions. It is possible that certain genes, more than others, are involved in regulating reproductive functions in response to environmental light. In regard to the effects of disrupted circadian rhythms in shift workers, an article in "Cancer Causes Control" attributes the desynchronization of the SCN to a delayed adaptation of certain genes to new environmental stimuli. The anterior portion of the suprachiasmatic nucleus adapts faster than the posterior portion, and it is possible that this delayed adaptation is responsible for circadian desynchronization at the genetic level (Haus and Smolensky 2006). These genetic factors contribute to an organism's responsiveness to light and reproductive activity.

An experiment done on female golden hamsters questions the genetic component of estrous cyclicity. Two groups of hamsters, one with normal hamsters and the other with tau mutant hamsters, which were kept under constant lighting conditions, exhibited identical estrous periods of 96 hours. The authors of this experiment suggest separate mechanisms controlling circadian periodicity and estrous periodicity because of the identical estrous period found in groups with differing circadian genes (Refinetti and Menaker 1992). From these results we conclude that light may play a major role in controlling reproduction, because two genetically different strains reacted identically in their reproductive cycle. Nevertheless, it can be suggested that genetics still does play a role in photo responsiveness but was not seen here because the mutated Casein kinase 1e (from the tau mutant) may not have an effect on the hamster's SCN interpretation and reproductive activity. The fact that estrous periods were prolonged in constant light reflects the hamster's identity as a long day breeder that is reproductively active during long daylight hours and does not discredit the endogenous timing system that controls reproductive function.

Research on the endogenous biological clock reveals mechanisms other than the suprachiasmatic nuclei, or specific genes that control various functions. A study done on glucocorticoid production and the circadian clock shows an adrenal circadian clock involved in adrenocorticotropic hormone release, besides for stimulation from the primary circadian oscillator (Dickmeis 2009). Control at the level of the adrenal gland proves how circadian rhythmicity exists in some peripheral organs and may function with or without input from the retinohypothalamic tract (Figure 4).

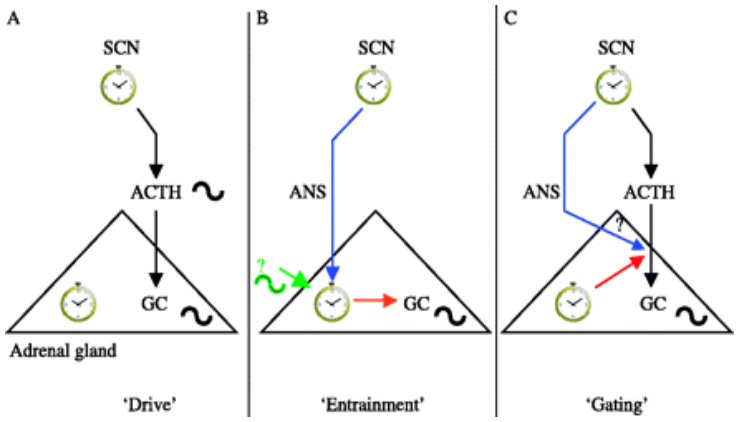

Figure 4: Glucocorticoid synthesis is stimulated by the 1. Master clock=SCN. 2. The adrenal gland's internal clock via input from the autonomic nervous system. 3. A combination of a & b. Source: Dickmeis 2009

Additionally, there may be other endogenous features controlling circadian rhythmicity even though sources of these mechanisms are unknown. In an article titled, "Stability, Precision, and near 24-hour period of the human circadian pacemaker," Czeisler reports that there is endogenous circadian rhythmicity even in the absence of periodic cues from the environment. An experiment was designed to lengthen the daylight hours of human subjects by delaying bedtime by four hours. After about a month on this schedule, the estimated intrinsic periods of melatonin, cortisol, and core body temperature were measured and proved to be similar to standard levels. The intrinsic circadian periods for all 24 subjects estimated between 24 and 24.35 hours, even though subjects experienced a 28-hour "day" (Czeisler et al. 1999).The results presented here reflect the workings of an internal biological clock that was not tricked by outside factors and continued functioning according to its internal circadian rhythm. Similarly, it is possible that reproductive function is also controlled by endogenous rhythmicity like the melatonin levels in this experiment, but this specific experiment did not test gonadal hormones. The authors also mention a study done on submariners who experienced 18 hours of artificial light while undersea on naval duty for six weeks; melatonin levels were only about .01 hours longer than the subjects in the present study and, once again, provide a basis to assume that an internal rhythm regulates hormonal function aside from the SCN.

Lee and McClintock from the Committee on Biopsychology at the University of Chicago, present a study on the seasonal variation in fecundity in female rats under constant lighting conditions. In the absence of environmental cues, which were always thought to be a trigger for reproductive function, these rats displayed increased reproductive activity in long summer days. The results of this experiment performed in two laboratories once again point to an endogenous feature controlling the rhythmicity of life functions aside from the level of the photoreceptor. Those conducting the experiment were under the impression that the female rats would respond like male rats had in a previous experiment in which rats exhibited seasonal variation in physiological measures of pineal and gonadal activity in a controlled lab. Five cohorts of reproductively mature Sprague-Dawley female rats of different ages chosen from a colony of 200 entered the experiment at different points of the year. Stimulatory light conditions of 14 hours exposure to light and 10 hours in darkness were constant throughout the two years of the experiment, as well as temperature, barometric pressure, and circulating fresh air (modified by a heating/cooling unit in the lab). Fecundity was measured by short, regular, and variant cycles which increase the chances of fertilization. Even though the rats had no indication of the time of year, fecundity peaked in the summer months with the appearance of shorter and more regular estrous cycles (Figure 5) and higher rates of mating. The second laboratory housed 3000 Wistar rats under 12 hours exposure to light and 12 hours in darkness for 19 months. Again, reproductive success and fecundity peaked between May and August, proving that these laboratory rats display the same seasonal fecundity as wild rats, probably because of an endogenous mechanism controlling reproductive function. It is well established that these rats prefer long days for reproductive success, but the fact that fecundity peaked during the summer months (even though stimulatory lighting conditions were available all year round) proves that an endogenous feature controls the reproductive function in these female rats. However, like the authors suggested, it is possible that changes in humidity levels throughout the experiment were indicative of the time of year and may have been the trigger of increased fecundity. In addition, male reproductive activity and its response to

constant environmental factors must be taken into consideration when investigating the fecundity and mating success of the female rats (Lee and McClintock 1986).

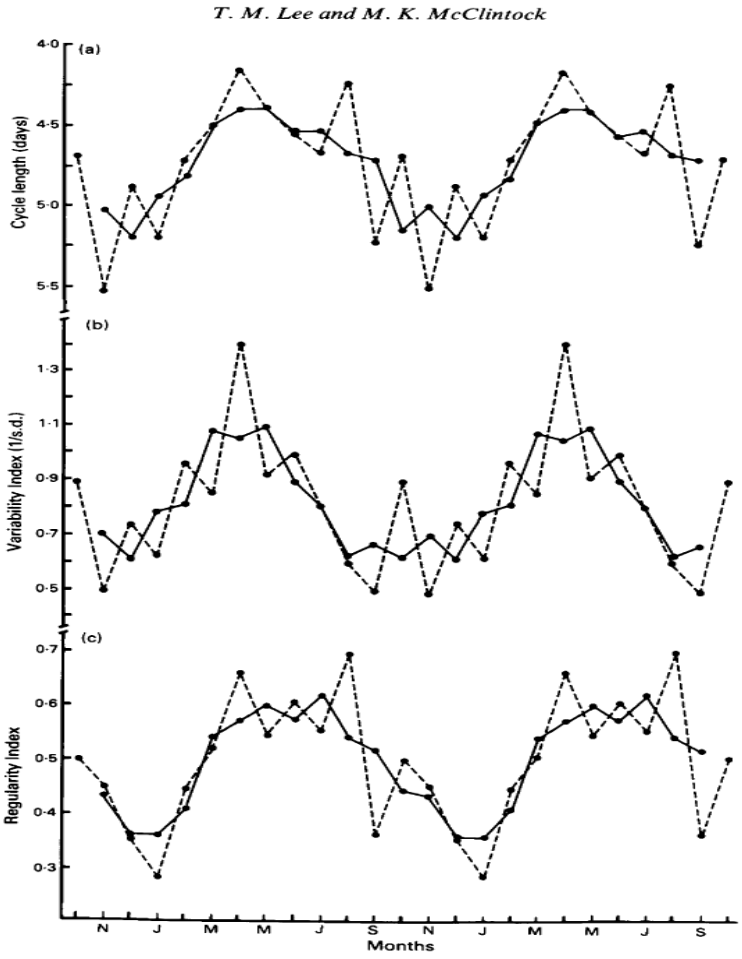

Figure 5: Fecundity peaked between May and August in both years of the experiment. In lab 1, Fecundity was measured according to regularity, variability and length of estrous cycle. Bold lines are the average, and dotted lines are the surrounding range. Source: Lee and McClintock 1986

FURTHER RESEARCH:

Both endogenous and exogenous components are inherent in the photo responsiveness of melatonin and subsequent reproductive function. Disrupted light/dark cycles, melatonin secretions, and genetic factors can contribute to reproductive malfunction and disordered cyclicity. Besides for light telling the body what time it is and keeping rhythmic functions running in a healthy manner, sunlight can also help fertility because of the vitamin D it gives off to the environment. An experiment done on female Holtzman rats compared the reproductive capacity and fetal development of healthy and vitamin D deficient rats. Although the vitamin D deficient rats were capable of reproduction, overall fertility was reduced by 75% and litter sizes by 30% (Halloran and Deluca 1980). We can hypothesize that sunlight, as the main source of natural vitamin D, may be helpful for reproductive function, although no direct experiment relates actual sunlight to fertility. Workers who use the daytime for sleeping to compensate for a night lost sleep may be harming their chances of sunlight exposure which directly and indirectly helps fertility. There have been very few experiments done on humans testing the effects of the light/dark cycle on melatonin secretion and reproductive function, because it is difficult to keep

human subjects under constant light or dark conditions for long periods of time. Therefore, scientists use epidemiological studies to investigate the effects of disrupted light exposure on melatonin and gonadal activity (Kennaway 2005), although results of these studies are not as direct as human experimental results would be.

CONCLUSION

Photoperiodic changes in melatonin concentrations affect female reproductive function because of photic and genetic factors. Limited research has been done on human subjects to test the effects of constant photoperiodic changes on the female menstrual cycle, but evidence from experiments on animals may hint to trends that can be evident in the female human as well. However, as mentioned before, each species runs on its unique biological clock that interprets environmental information based on endogenous features, so experimentation on animals does not really provide conclusive evidence on human subjects. Nevertheless, the mechanisms involved in melatonin secretion and reproductive function can be similar in animals and humans and can be used as a springboard for further research on the photoresponsiveness of human reproduction. Researchers suggest that workers minimize their exposure to constantly changing schedules in order to avoid possible disorders that may arise from the desynchronization of circadian function.

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