1995

The Impact of the Tau Mutation on Reproductive Function in the Golden Hamster

Eliza Leake Thomasson

Virginia Commonwealth University
School of Medicine

This is to certify that the thesis prepared by Eliza Thomasson entitled "The Impact of the Tau Mutation on Reproductive Function in the Golden Hamster" has been approved by her committee as satisfactory completion of the thesis requirement for the degree of Master of Science.

[redacted]

Richard J. Krieg, Jr., Ph.D., Director of Thesis

[redacted]

Dennis W. Matt, Ph.D., School of Medicine

[redacted]

John N. Clore, M.D., School of Medicine

[redacted]

Robert F. Spencer, Ph.D., Interim Department Chair

[redacted]

Hermes A. Kontos, M.D., Ph.D., Dean, School of Medicine

[redacted]

William L. Dewey, Ph.D., Dean, School of Graduate Studies

June 26, 1995

Date

The Impact of the *Tau* Mutation on Reproductive Function in the Golden Hamster

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

By

Eliza Leake Thomasson
B.A. in Anthropology, University of Virginia, 1993

Director: Richard J. Krieg, Jr., Ph.D.
Professor of Anatomy

Virginia Commomwealth University
Richmond, Virginia
August, 1995

DEDICATION

I would like to dedicate this thesis to my aunt and uncle, Tamara and Clyde Toms. You have not only provided me with a place to live, but you also have given me a sense of family and endless support during a very difficult year. My living situation was a little different from that of my friends, but I would not have traded it for the world. I have truly enjoyed being a member of your household. It has been especially great for me to have been with Clyde, Jr. and William as they have grown during the past year. I will never forget your kindness and support.

ACKNOWLEDGMENTS

First and foremost, I would like to thank Dr. George Liechnetz who, in his infinite wisdom, accepted me into this program and allowed me to prove what I always thought to be true. I would also like to thank Dr. Richard J. Krieg, Jr., who always had the time and the patience to help me through this process. He proved to be an excellent advisor as well as a fellow aficionado of fine bagels. Thanks as well to Dr. Dennis Matt and Dr. John Clore, who agreed to sit on my committee and be a part of the wonderful world of hamsters. In addition, I would like to extend my gratitude to the faculty of the Anatomy department for their assistance and guidance in my courses this year.

To my parents, I appreciate your faith and support of me and the path I have chosen. Thank you to Maggie, who serves as my calm head on numerous occasions. To Ann, who always gives me a reason to smile. To George, who constantly reminds me how important it is to stay grounded in reality. And thanks to Matt for all of the great adventures during the past two years.

Finally, a huge thank you to my classmates this year (especially Lorna, Michael, and Erik) for your support and friendship. You have helped me through a very trying year and I will leave here with many great memories. May we all achieve every goal we strive for - we have earned it.

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
C	Centigrade
DD	Constant Darkness
DNA	Deoxyribonucleic Acid
FSH	Follice Stimulating Hormone
g	gram
IP	Intraperitoneally
kg	kilogram
LD	Light:Dark
LH	Luteinizing Hormone
LL	Constant Light
MBH	Medial Basal Hypothalamus
mPOA	Medial Preoptic Area
ug	microgram
mg	milligram
uL	microliter
mm	millimeter
NIDDK	National Institute of Digestive and Kidney Diseases
ng	nanogram
RIA	Radioimmunoassay

rLH	rat Luteininzing Hormone
RNA	Ribonucleic Acid
RP-2	Reference Preparation-2
SCN	Suprachiasmatic Nucleus
wt	weight

ABSTRACT

THE IMPACT OF THE *TAU* MUTATION ON REPRODUCTIVE FUNCTION IN THE GOLDEN HAMSTER

By Eliza Leake Thomasson, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 1995.

Major Director: Richard J. Krieg, Jr., Ph.D., Anatomy

The *tau* mutation affects the circadian system of the golden hamster primarily by decreasing the period of the activity cycle from 24 to 20 hours. To study the effect of this mutation on reproductive function in the golden hamster, 3 experiments have been designed. In the first experiment, wild-type and *tau* female hamsters will be maintained in conditions of 14:10 LD and 11.7:8.3 LD, respectively. Blood samples will be taken and analyzed by radioimmunoassay (RIA) to determine the timing of the proestrous LH surge. In the second experiment, wild-type and *tau* males will be transferred to shortened photoperiods of 10:14 LD and 8.3:11.7 LD, respectively, and testicular length and width will be used as a measure of the onset of testicular regression and subsequent recrudescence. In the final experiment, wild-type and *tau* females will be transferred to conditions of 10:14 LD and 8.3:11.7 LD, respectively, and the time to the onset of anestrus will be recorded. It is expected

that in the *tau* females, the preovulatory LH surge will occur 8.4 hours after lights-on. The onset of gonadal regression, recrudescence, and anestrus will occur 16.7% [(24 hours - 20 hours)/24 hours] sooner in the mutant hamster when measured in absolute time. When the time to the onset of these processes is measured in light cycles, however, it is probable that these events occur within the same number of light cycles in both the wild-types and the *tau* hamsters. The basic hypothesis is that the main impact of the *tau* mutation will be on the timing of these specific reproductive phenomena, but the fundamental physiological characteristics of these events will remain unaffected. These results would suggest that the timing of the preovulatory LH surge and the occurrence of gonadal regression, recrudescence, and anestrus in a shortened photoperiod are driven by the same neural oscillator that regulates the period of the activity cycle in the golden hamster.

INTRODUCTION

The History of the Golden Hamster in the Laboratory

The golden, or Syrian, hamster (*Mesocricetus auratus*) was introduced as a laboratory animal in 1930 by Professor Israel Aharoni, the head of the Department of Zoology at the Hebrew University in Jerusalem, Israel. Professor Aharoni had travelled to Syria to collect specimens of hamsters indigenous to the Middle East for use in the studies of *leishmaniasis* (Black Fever) being conducted by Saul Adler, a parasitologist at the university. Professor Aharoni returned to Israel with a litter of golden hamsters, a species that had never been used in the laboratory. Of the original 8 animals, only one male and 2 females survived the journey from Aleppo, Syria to the laboratory of Mr. Haim Ben-Menachen, the director of the Hebrew University animal facilities on Mount Scopas. Under the care of Mr. Ben-Menachen, these animals reproduced easily in captivity and proved suitable for a number of different experiments. Prior to World War II, offspring from the original 3 hamsters were sent to laboratories throughout Europe and the United States (Adler, 1948; Murphy, 1985). Over the past several years, experiments with golden hamsters have helped to illuminate many of the mysteries of mammalian life, including those of the reproductive system. In addition, the golden hamster has provided scientists a look at the

first known mutation of the vertebrate circadian system.

Gonadal Regression in a Shortened Photoperiod

In their natural environment, many mammalian species are seasonal breeders. For example, goats and rams breed during the fall while ferrets, snowshoe hares, and hamsters are spring breeders. Several experiments have shown that photoperiod is an important factor in the loss, and subsequent recovery, of the reproductive functions of the hamster prior to the breeding season (Gaston and Menaker, 1967; Berndston and Desjardins, 1974; Stetson et al., 1975). Gaston and Menaker (1967) discovered that adult male hamsters receiving less than 12.5 hours of light per 24-hour day experienced testicular regression and loss of spermatogenesis. For those animals maintained in shortened photoperiods, the regression of the testes was consistent regardless of the number of hours of light to which the hamster was exposed. As the body weight of the animals was not affected, it was determined that the effects of photoperiodic treatments were mainly upon the reproductive system. Exposure to less than 12.5 hours of light per day mimics the natural shortening of the days during the autumn. The physiological response of the hamster to shorter days apparently ensures that breeding does not continue under the harsh winter conditions.

It was later discovered that testicular regression was not the sole effect of light deprivation. Histological sections of atrophied testes showed that there was a concurrent degeneration of seminiferous epithelium, reduction in the secretory activity of the epithelium,

disappearance of spermatozoa and spermatids, and a reduction in the diameter of the seminiferous tubules. In addition, there was a decrease in the levels of circulating testosterone, loss of testicular ability to convert tritiated progesterone to tritiated testosterone, decrease in the plasma concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and reduction in the weight of the seminal vesicles and ventral prostates. There was no significant change in the concentrations of protein, RNA, or DNA within the regresses testicles (Desjardins et al., 1971; Berndston and Desjardins, 1974; Chan and Ng, 1994).

The Process of Recrudescence Subsequent to Regression in a Shortened Photoperiod

Exposure of the animals to a long-day photoperiod [LD (light:dark) ratio 14:10] for several weeks was found to be sufficient to reverse the effects of light deprivation. Return to normal concentrations of both hypophyseal and plasma LH and plasma FSH occurred after 10 to 20 days of exposure to a 14:10 LD cycle in hamsters originally kept in conditions of constant darkness (DD) for 60 days. After 20 days of the 14:10 LD cycle, the testes had regained the capacity to convert tritiated progesterone into tritiated testosterone. The circulating testosterone levels were twice normal 50 days after the return to a 14:10 LD cycle. Testicular weight reached control levels within 50 days of consistent exposure to an environment of 14:10 LD (Berndston and Desjardins, 1974). Following regrowth, the testes of animals exposed to a shortened

photoperiod were grossly and microscopically indistinguishable from the testes of animals maintained on a long-day photoperiod (Reiter, 1972). This return to functional capacity was termed "recrudescence".

Further experiments indicated that daily exposure to a long-day photoperiod was not necessary to induce recrudescence after constant darkness. One 6-hour light pulse every 36 or 60 hours was sufficient to stimulate testicular regrowth. These findings led to the idea that the rhythm of photosensitivity is the cornerstone of the photoperiodic response to light (Stetson et al., 1975). The rhythm of photosensitivity is about 24 hours long and is divided into 2 phases of approximately equal length, a light-sensitive and light-insensitive portion. The hamster is a nocturnal animal. The light-sensitive phase coincides with the active portion of the hamster's circadian activity cycle. This active portion, called subjective night, is about 12 hours in length. The beginning of this portion is marked by the onset of activity (usually wheel-running activity). The insensitive phase occurs during the inactive period of the hamster - subjective day (Elliott, 1976). The effects of light on the reproductive system are wholly dependent on the portion of the animal's circadian rhythm during which the light falls. When the 6-hour light pulse was given after 36 or 60 hours, it fell during the light-sensitive phase. This stimulated testicular regrowth. In contrast, giving the light pulse after 24 or 48 hours had no effect on regrowth because it fell during the insensitive phase (Stetson et al., 1975).

Interestingly, hamsters experience regression followed by

spontaneous recrudescence and regrowth of the testes if they are exposed for a long enough time to shortened photoperiods, or constant darkness. The spontaneous recrudescence occurs 20 to 30 weeks after the introduction of the short photoperiod (Reiter, 1972; Turek et al., 1975; Bittman, 1978; Steger et al., 1982; Nelson and Zucker, 1987). Steger and his colleagues (1982) observed that the testicular weight of male hamsters maintained in a 5:19 LD cycle reached a low after about 7.5 weeks of exposure to the shortened photoperiod and remained at that level until week 19. A sharp rise in weight was visible by week 20. The weight of the seminal vesicles of these animal followed a similar pattern. It has been determined that regression occurs in the first 8 to 10 weeks of exposure, followed by a latency period of 8 to 10 weeks (Elliott, 1976). Once recrudescence has begun, the hamsters are no longer responsive to short photoperiods. This interval is known as the refractory period, during which time gonadal regrowth occurs regardless of the amount of light the hamster receives daily. Nelson and Zucker (1987) attempted to alter the timing of recrudescence by exposing hamsters to occasional long-days during an otherwise constant short-day environment. Neither the time to recrudescence nor the refractory response was changed in those hamsters. Apparently, the timing of testicular regrowth is internal, and appears to be an evolutionary mechanism that ensures the hamsters are prepared to breed by the spring.

Relatively little is known about the processes behind testicular regression and regrowth. The secretory rhythms of the pineal gland (Figure 1), controlled by the circadian pacemaker in the suprachiasmatic

nucleus (SCN), are known to affect the response of the hamster to a shortened photoperiod. Hoffman and Reiter (1965) studied the individual and combined effects of bilateral removal of the eyes (enucleation) and pinealectomy on hamsters exposed to conditions of 1:23 LD. Hamsters that underwent a pinealectomy did not display gonadal regression when exposed to short-day conditions. Hamsters that were blinded experienced testicular atrophy regardless of the light conditions, probably due to the perception of constant darkness. The gonads of the animals that were enucleated and pinealectomized did not regress. It is thought that the pineal gland produces and secretes an antigonadal hormone when the animal is exposed to a shortened photoperiod or constant darkness. Several experiments have led to the theory that melatonin (Figure 2) is the antigonadal hormone in question (Turek et al., 1975; Bittman 1978). Daily melatonin injections or melatonin implants have been found to induce gonadal regression in hamsters exposed to long photoperiods. Yet, just as under extended conditions of constant darkness, spontaneous recrudescence is observed during prolonged melatonin treatments. It is thought that spontaneous recrudescence occurs when the neural target tissues responsible for testicular maintenance become insensitive to the short-day melatonin signal. This refractory period may begin as soon as 15 weeks following the introduction of the shortened photoperiod (Nelson and Zucker, 1987), thus preceding noticeable testicular regrowth by 5 weeks.

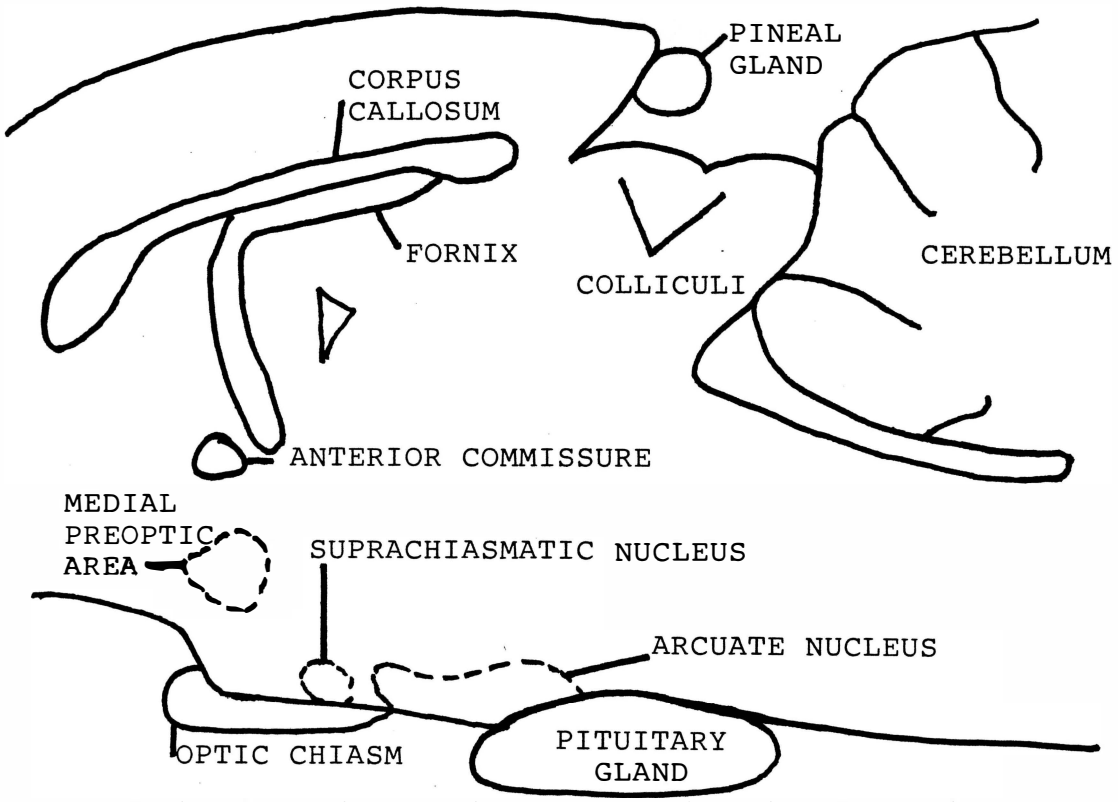

Figure 1. Sagittal section showing the relationship of the pineal gland to the hypothalamic nuclei in the rat brain, which is quite similar to that of the hamster. (Adapted from Paxinos and Watson, 1986).

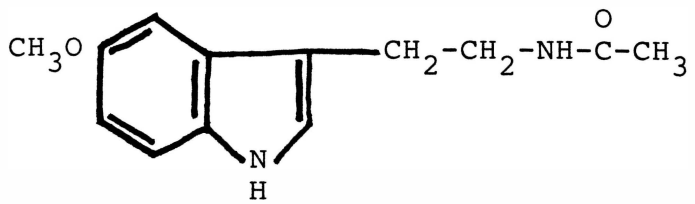

Figure 2. The structure of melatonin

The Preovulatory LH Surge

The reproductive cycle of the female hamster is very well defined in wild-type animals exposed to long photoperiods. The estrous cycle lasts for 4 days and is marked by predictable hormonal shifts. For example, there is an LH surge that occurs within a certain time period on the afternoon of proestrus (Figure 3). As has been documented in female hamsters maintained on a 14:10 LD cycle (lights on at 0500 hours), the LH concentration begins to rise between 1300 and 1430 hours, there is a peak between 1500 and 1600 hours, and by 2100 hours the LH concentration has returned to base-line levels (Goldman and Porter, 1970; Turgeon and Greenwald, 1972; Bast and Greenwald, 1974). Ovulation occurs on the following day, estrus, approximately 9 to 10 hours after the LH peak (Goldman and Porter, 1970).

The theory of a critical period for LH release was tested in experiments utilizing phenobarbital (which suppresses the activity of the hypothalamus) and hypophysectomy (removal of the pineal gland) in an attempt to block ovulation (Greenwald, 1971). Animals in this experiment were kept in an environment of 14:10 LD, with the lights on at 0500 hours. Phenobarbital (6.5 mg/100 g body wt) injected at 1300 hours on proestrus successfully blocked ovulation the following day in all of the test animals. Injections given to 16 animals at 1400 hours resulted in partial ovulation in 2 animals, full ovulation in 1 animal, and the other 13 did not ovulate. Injections given at 1500 and 1600 hours were insufficient to block ovulation in any of the hamsters. Removal of the pituitary gland between 1300 and 1500 hours on proestrus

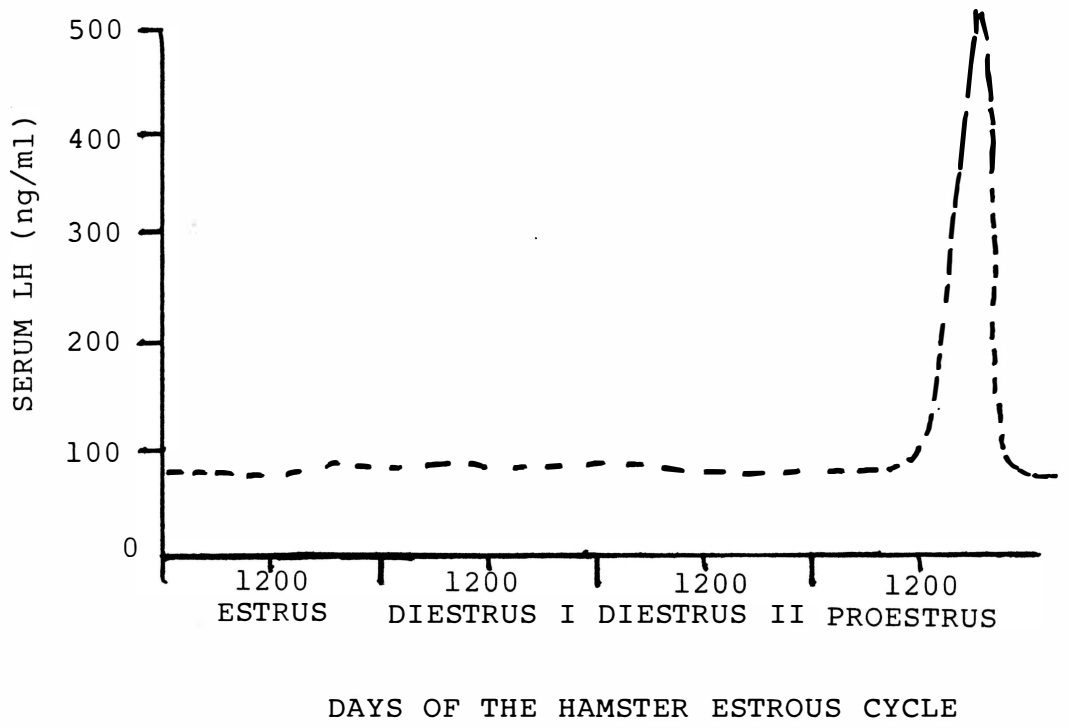

Figure 3. Serum LH levels throughout the estrous cycle of the golden hamster. Estrus is the day on which ovulation occurs. (Adapted from Bast and Greenwald, 1974).

prevented ovulation the next day. Hypophysectomy performed between 1530 and 1630 hours on proestrus blocked ovulation in only 25% of the animals. Based on these results, it appears that the critical period for the release of ovulatory hormones occurs before 1500 hours on the afternoon of proestrus.

The release of LH on the afternoon of proestrus, and subsequent ovulation, are now known to be regulated in the hamster by the presence of estrogen from the maturing follicles. Labhsetwar (1972) found that ovulation could be blocked by the introduction of antiestrogens in the late afternoon of diestrus II. In addition, estradiol benzoate was effective in restoring ovulation in those animals that had been treated concurrently with antiestrogens. Labhsetwar and his colleagues (1973) later attempted to determine whether estrone and progesterone, in addition to estradiol, played a role in the induction of ovulation. This was accomplished by measuring the concentrations of estradiol, estrone, and progesterone in the ovarian venous blood at various points throughout the estrous cycle. In this experiment, estrus was denoted as Day 1, diestrus I as Day 2, diestrus II as Day 3, and proestrus as Day 4. Estradiol was observed to peak in the afternoon of diestrus II, remain high through proestrus and the LH surge, and decline on estrus. Estrone levels remained fairly constant throughout the cycle. The ratio of estradiol to estrone output was high in favor of estradiol on the afternoon of diestrus II, indicating that the observed increased estrogen secretion at this time was related to a concurrent increase in the output of estradiol. The secretion of progesterone was at its

highest on estrus, following the LH surge. From these results, it follows that estradiol has a strong temporal relationship to the preovulatory release of LH.

Once the presence of estrogen was established as important for the ovulatory process, the next step was to elucidate to what extent this hormone was responsible for the cyclic gonadotropin release. Stetson and his cohorts (1978) proposed two models of involvement. The first stated that elevated estrogen levels were mandatory for the release of the gonadotropins. The second speculated that gonadotropins continued to be released in the absence of a high estrogen concentration, but not to the same extent as in the presence of estrogen. They looked at 3 groups of ovariectomized female hamsters maintained on a 14:10 LD cycle. The first group received 5 mm Silastic implants containing crystalline estradiol, the second group received empty implants, and the third group received no implant. Every group demonstrated a daily afternoon peak of LH that temporally resembled the proestrous LH surge, which is typical of the hamster during the short-term following ovariectomy. Within this period, hamsters assume a pattern of daily surges of LH which, as just mentioned above, resemble the timing of the proestrous LH surge. However, this release commonly is not significantly different from the serum LH concentrations at other points during the day. The results of Stetson and his colleagues (1978) showed that the estrogen implant increased the concentration of LH secreted to levels approximating those of proestrus, but only on day 1 of the implant. On subsequent days, observed peak serum LH levels were reduced. The authors suggested the

possibility of an eventual negative feedback response induced by the high levels of estrogen. The data from this experiment support the theory that the cyclic release of gonadotropins is regulated by a neural oscillator, estrogen merely serves to modulate the amounts secreted.

Neural pathways are also important in the reproductive cycle of the hamster. Complete deafferentation of the medial basal hypothalamus (MBH) induces acyclicity in females in addition to inducing a reduction in ovarian, uterine, and pituitary weights. There is also an accumulation of LH in the pituitary gland, which is accompanied by diminished LH levels in the plasma (Norman et al., 1972). Using deafferentation and lesioning techniques, Norman and Spies (1974), examined the integrity of the bilateral pathway from the medial preoptic area (mPOA) (Figure 4) to the MBH. Four animals underwent a bilateral disconnection of the mPOA and MBH, 3 a unilateral severance of the pathway, 5 a bilateral mPOA lesion, and 9 hamsters received a misplaced bilateral lesion (not to the mPOA). These animals were subsequently ovariectomized and treated with injections of estradiol benzoate. Those animals with the bilateral deafferentation or mPOA lesion did not display an estrous cycle or release LH, despite the estrogen supplement. However, hamsters with unilateral pathway disruption or misplaced lesions exhibited both an estrous cycle and a post-ovariectomy LH surge in response to the estrogen injections. These results confirm that the medial preoptic area, the medial basal hypothalamus, and the pathway between these two structures serve as important regulatory components in the effects of estrogen on the reproductive cycle of the hamster.

The reproductive cycle of the hamster is controlled by an endogenous biological clock located within the suprachiasmatic nucleus (SCN) (Stetson and Anderson, 1980). Input concerning light-dark cycles reaches the SCN via the retinohypothalamic tract. The SCN sends connections to the medial basal hypothalamus that probably influence the output of releasing and inhibiting hormones to the pituitary gland (Figures 2, 4). The reproductive cycle can free-run on its own period in the absence of zeitgebers (external entraining agents, i.e. light cues), and is probably dependent on the endogenous rhythms of the SCN. Alleva and his colleagues (1971) noted that female hamsters maintained in conditions of 16:8 LD displayed estrous cycle periods of approximately 96 hours. Among 19 animals, the observed range was 95.85 hours to 96.11 hours. Twenty additional animals were moved from LD 16:8 into conditions of constant light (LL). After an adaptation period of several days, the endogenous estrous cycles of the individual animals were visible. The observed periods ranged from 95.35 hours to 97.54 hours, yet these cycles were normal in all regards despite their varying lengths. Eighteen of these 20 animals displayed endogenous estrous cycle periods that were significantly different from the 96-hour periods observed in animals maintained in a controlled lighting environment. When these animals were returned to LD 16:8, the estrous cycles were restored to the expected period of about 96 hours. Thus, the reproductive cycle of the female hamster has its own endogenous period but might also be responsive to external light cues. This is indicated by the profound response of male hamsters to shortened photoperiods, as

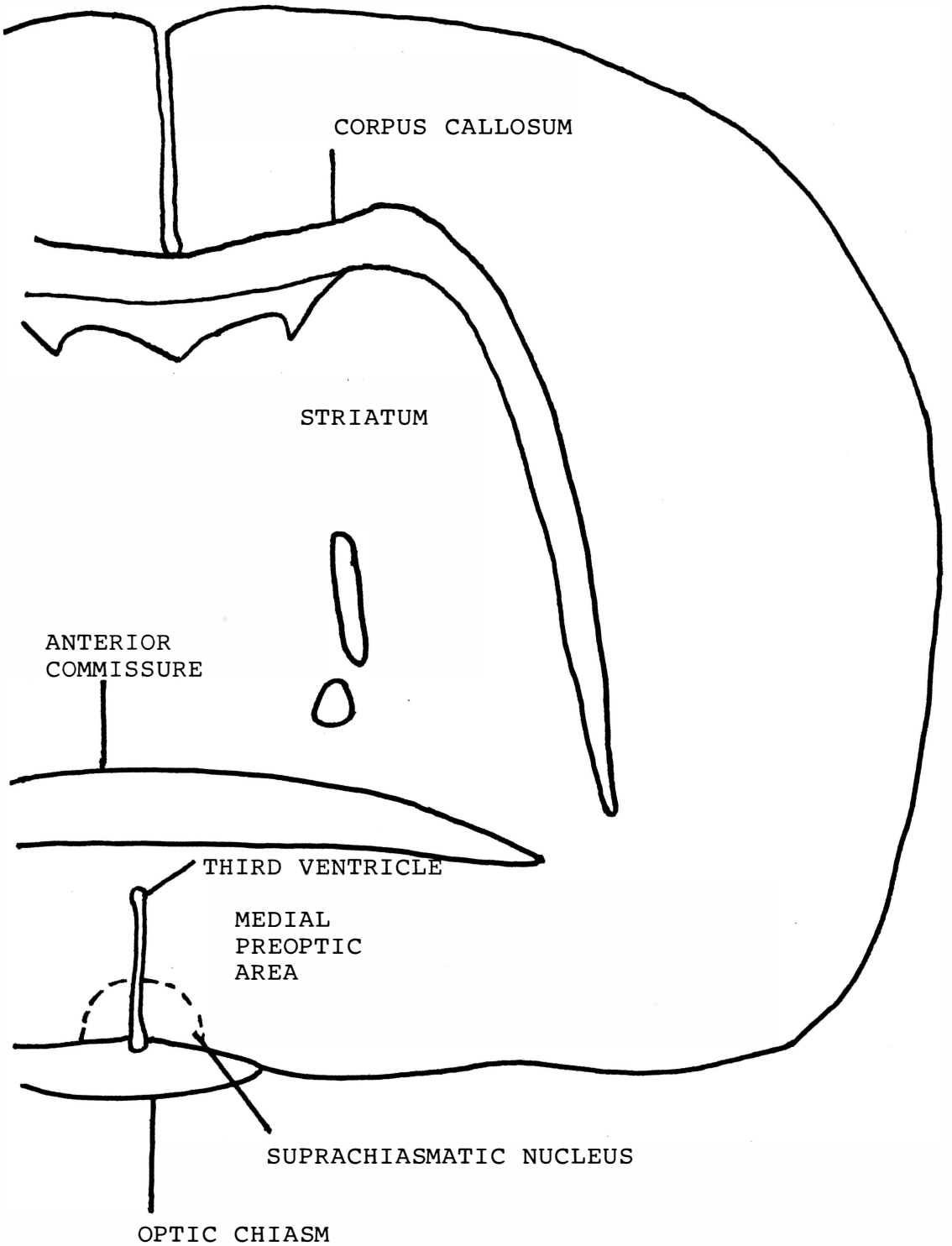

Figure 4. Coronal section detailing the relationship of the medial preoptic area (mPOA) and the suprachiasmatic nuclei (SCN) to the optic chiasm. (Adapted from Knigge and Joseph, 1968).

described above.

The Onset of Anestrus in a Shortened Photoperiod

Female or male hamsters placed in conditions of constant darkness (DD) will display an activity period that is slightly shorter or longer than the 24-hour period observed in a controlled-lighting environment. The circadian oscillator, which entrains to the LD cycle, is allowed to free-run on its endogenous period when there are no external light cues. The reproductive system, which has some components under the control of the circadian oscillator (Stetson and Anderson, 1980; Fitzgerald and Zucker, 1976), is markedly affected by conditions of DD. The ovaries of hamsters that have been blinded involute, show a diminished number of antral follicles, a reduction in the corpora lutea, and a hypertrophy of the interstitium. In addition, the weight of the uterus and the circulating estradiol levels undergo a significant decline (Reiter, 1968; Jorgenson and Schwartz, 1985).

Similar changes are noted when the animals are maintained on a shortened photoperiod. Seegal and Goldman (1975) observed 13 females housed in a 10:14 LD cycle, with lights on at 0500 hours, for several weeks. After 6 weeks in these conditions, 11 of the animals were acyclic. In addition, the timing of the daily peaks of LH, FSH, and progesterone levels were similar to those seen in anestrous females, that is around mid-afternoon (Seegal and Goldman, 1975; Bridges and Goldman, 1975; Jorgenson and Schwartz, 1985). Jorgenson and Schwartz (1985) hypothesize that these hormonal surges inhibit the development of

the follicles and contribute to the degeneration of the ovaries in females exposed to shortened photoperiods.

As shown in the male, melatonin injections can induce effects similar to those found after exposure to a shortened photoperiod in the female hamster (Tamarkin et al., 1976). However, the antigonadal effects of melatonin are dependent on the time of day at which it is introduced. Females were maintained on a 14:10 LD cycle, with lights on at 0600 hours. Injections of 25 ug melatonin were given to 6 animals daily at 0900 hours. Five animals received 25 ug melatonin injections and five received 2.5 ug melatonin injections at 1600 hours each afternoon. At the end of the seven week treatment period, all of the females that received the morning injections had retained cyclicity. By comparison, 100% of the animals injected daily with 25 ug melatonin and 83% of the hamsters treated with 2.5 ug melatonin in the afternoon had become acyclic. The acyclic animals also displayed the expected daily afternoon surges of LH and FSH. Thus, the female hamster demonstrates a clear diurnal rhythm of sensitivity to melatonin as evidenced by the fact that daily injections of this antigonadal hormone produced short-day effects only when administered in the afternoon.

Parallel to the occurrence of recrudescence in the male, the spontaneous restoration of cyclicity is observed after approximately 16 to 20 weeks of exposure to a short-day photoperiod (Seegal and Goldman, 1975; Stetson and Anderson, 1980). After resumption of the estrous cycle, the females become photorefractory and display normal cycles despite the shortened photoperiod. As was previously discussed, this

photorefractory period is most likely due to the desensitization of the neural tissues regulating the reproductive cycle to the short-day melatonin signal (Bittman, 1978).

The Relationship of the Activity Cycle to Hormone Release

It has since been demonstrated that the preovulatory LH surge is temporally related to the activity cycle of the hamster (Stetson and Gibson, 1977; Swann and Turek, 1985). Stetson and Gibson (1977) placed pinealectomized females in conditions of LD 6:18, with lights on at 1000 hours. After a pinealectomy, acyclicity was not induced by a shortened photoperiod nor was there an interruption of normal entrainment to the light cycle. Of 76 hamsters, 63 began their activity period around 1600 hours (lights out) and the other 13 began around 2200 hours. Those animals who began wheel-running around 1600 hours displayed a preovulatory LH surge at approximately 1300 hours. The hamsters that had a later activity onset had a preovulatory LH surge at about 1900 hours. In both groups of animals, the preovulatory LH surge consistently preceded the daily activity period by 3 hours. These results suggest that the activity cycle and the preovulatory gonadotropin cycle are controlled by the same circadian oscillator.

Swann and Turek (1985) monitored 18 ovariectomized hamsters with estradiol benzoate implants maintained under conditions of constant light (LL). After an adaption period, 6 females displayed a single endogenous activity period, 10 exhibited split activity rhythms, and 2 animals were dismissed from the experiment due to unreadable rhythms.

The animals with a single activity record presented with a single LH surge one to 4 hours prior to activity commencement. Those hamsters with split rhythms had 2 daily LH surges corresponding to the 2 activity periods, each occurring up to 4 hours before activity onset. The results of this experiment confirm that the pituitary LH surge and the locomotor rhythm are both regulated by a circadian oscillator. In addition, the appearance of split rhythms suggests that the oscillator is actually composed of at least two separate components. The current theory is that these components are tightly coupled in a light-dark cycle but may become unlinked under constant lighting conditions (Pittendrigh and Daan, 1976).

The *Tau* Mutation

Over the past several years, mutations within nonvertebrate circadian systems have been intensely studied. The mutations of the biological clocks of *Drosophila* (fruit fly), *Neurospora* (ascomycetous fungus), and *Chlamydomonas* (single-celled photosynthetic flagellate) have provided clues about the mechanisms of circadian rhythmicity within those organisms. However, it was not until 1988 that a mutation affecting a vertebrate system was discovered in the golden hamster. The most evident effect of this mutation is the shortening of the circadian activity rhythm, but little is known about its effects on other internal systems.

In 1988, Michael Menaker and Martin Ralph discovered an unusual animal among those that they had received from the Charles River

Breeding Labs. One male golden hamster exhibited an uncharacteristically short period of circadian rhythmicity under conditions of constant darkness. The normal free-running activity period of the hamster in conditions of constant darkness is approximately 24 hours, but this hamster exhibited a free-running period of about 22 hours. In addition, this animal displayed abnormal entrainment when exposed to a 14:10 LD cycle. Entrainment was observed, but the animal would begin his activity period about 4 hours earlier than the normal animals.

Intrigued by these observations, Menaker and Ralph (1988) began mating this hamster with females who exhibited normal free-running periods. The male was mated with 3 females and a total of 21 offspring were produced. Of the offspring, 10 exhibited normal free-running periods and 10 exhibited the shortened free-running periods under conditions of constant darkness. There was one offspring who presented with unreadable data. Based on the distribution of the mutation within the offspring, it was assumed that the abnormal free-running period was the result of a mutation at a single, autosomal locus. The original abnormal male was thought to be heterozygous for this gene.

Further breeding studies produced animals that exhibited a free-running period of 20 hours under conditions of constant darkness. These hamsters were the result of mating 2 presumptive heterozygotes. Additional crosses were performed among heterozygotes and homozygotes for the mutation. The results of these mating experiments led to the conclusion that the animals with the free-running period of 20 hours

were indeed homozygous for the mutation. These findings enabled Menaker and Ralph (1988) to conclude that they were working with a mutation, which they called *tau*. They assumed that it was partially dominant and occurred at a single, autosomal locus. The primary effect of the *tau* mutation is to shorten the circadian activity period of the carrier.

Although many of the effects of the *tau* mutation are not well known, the mutation has been integral in unlocking one of the mysteries of the mammalian circadian system. For some time it was assumed that the suprachiasmatic nucleus (SCN) in the hypothalamus (Figure 4) contained the cells that might be responsible for the regulation of overt mammalian circadian rhythms (Table 1). A study by Ralph and his colleagues (1990) supported and extended this hypothesis. It involved both normal and *tau* mutant hamsters. The SCN of host animals were lesioned and SCN cells from donors with different genotypes were transplanted. The idea behind this experiment was that if the SCN was indeed the pacemaker of the circadian system, the SCN-lesioned host would display the period of the donor following the transplant. In all cases, the period of the post-transplant rhythm was indeed identical to the rhythm of the donor (Table 2). The period of the restored rhythm was always approximately 24 hours when the donor was wild-type; the period was always about 22 hours when the donor was heterozygous; and a homozygous mutant donor always resulted in post-transplant periods of about 20 hours. There was no apparent effect of the host genotype on the observed post-transplant rhythms (Ralph et al., 1990). The results of this experiment further supported the conclusion that the SCN is the

Table 1. Experimental evidence supporting the theory that the suprachiasmatic nucleus is responsible for the regulation of mammalian circadian rhythms. (From Ralph et al., 1990).

EVIDENCE THAT THE SCN IS THE SITE OF THE CIRCADIAN PACEMAKER:

1. THE SCN IS THE TARGET OF DIRECT AND INDIRECT RETINAL PROJECTIONS REQUIRED FOR ENTRAINMENT TO ENVIRONMENTAL CYCLES

2. THE SCN EXHIBITS STRONG CIRCADIAN RHYTHMS OF GLUCOSE UTILIZATION IN VIVO

3. ABLATION OF THE SCN OR ITS SURGICAL ISOLATION WITHIN THE BRAIN ELIMINATES OVERT BEHAVIORAL RHYTHMICITY AND RHYTHMIC ELECTRICAL ACTIVITY IN THE BRAIN

4. TISSUE EXPLANTS CONTAINING THE SCN CONTINUE TO EXPRESS CIRCADIAN RHYTHMS IN ELECTRICAL ACTIVITY AND VASOPRESSIN RELEASE IN VITRO

5. CIRCADIAN RHYTHMICITY CAN BE RESTORED TO SCN-LESIONED ARRHYTHMIC HOSTS BY IMPLANTATION OF FETAL BRAIN TISSUE CONTAINING SCN CELLS

Table 2. The observed wheel-running rhythm of the SCN-lesioned host following transplantation. (From Ralph et al., 1990).

HOST GENOTYPE	DONOR GENOTYPE	OBSERVED RHYTHM
WILD-TYPE	WILD-TYPE	24 HOURS
WILD-TYPE	HETEROZYGOUS	22 HOURS
WILD-TYPE	HOMOZYGOUS	20 HOURS
WILD-TYPE	CONTROL	ARRHYTHMIC
HETEROZYGOUS	WILD-TYPE	24 HOURS
HETEROZYGOUS	HETEROZYGOUS	22 HOURS
HETEROZYGOUS	HOMOZYGOUS	20 HOURS
HETEROZYGOUS	CONTROL	ARRHYTHMIC
HOMOZYGOUS	WILD-TYPE	24 HOURS
HOMOZYGOUS	HETEROZYGOUS	22 HOURS
HOMOZYGOUS	HOMOZYGOUS	20 HOURS
HOMOZYGOUS	CONTROL	ARRHYTHMIC

site of the circadian pacemaker in mammalian systems.

In addition to shortening the period of the carrier's activity cycle, the *tau* mutation has a distinct effect on the secretory rhythms of cortisol and luteinizing hormone. Loudon and his colleagues (1994) studied the impact of *tau* on rhythmic hormone release in the female hamster. Both normal and *tau* female hamsters were ovariectomized and kept in conditions of constant light. Hormone pulse frequency was measured as was pulse amplitude, hormone half-life, burst amplitude, and mass of hormone per burst. Although a shortened period might suggest a shortened hormone interpulse interval, the opposite effect was found. The *tau* females actually presented a longer interpulse interval than normal females. For LH secretion, this interval was longer by more than 6 minutes (16%). For cortisol secretion, this interval was longer by more than 5 minutes (18%). None of the other secretory characteristics of these particular hormones were apparently affected by the mutation. The conclusion from these experiments was that, in addition to the effect on the circadian system, the *tau* mutation also alters the ultradian pulse frequency of cortisol and LH, each of which are ultimately regulated by different hypothalamic releasing hormones. The mechanisms regulating this effect have yet to be uncovered.

Objectives

From the aforementioned experiments, it can be assumed that the *tau* mutation in the golden hamster shortens the period of the activity cycle but increases the interpulse interval of two important hormones. To

date, these are the only known effects of this mutation. However, it would seem that a homozygote would manifest other characteristics that would differentiate it from a wild-type. If we assume that the day of the mutant is represented by the period of its activity cycle, a homozygote experiences 1.2 days every 24 hours. What, then, are the effects of this variation of the internal clock on the physiology of the hamster, specifically reproductive function? With this question in mind, the experiments set forth in this thesis were designed to examine the impact of the *tau* mutation on four specific aspects of the reproductive system: 1) The preovulatory LH surge 2) The time to testicular atrophy in male hamsters exposed to a shortened photoperiod 3) The rate of recrudescence following gonadal regression 4) The time to the onset of anestrus in female hamsters exposed to a shortened photoperiod.

MATERIALS AND METHODS

General

Adult, sexually mature wild-type hamsters will be purchased from Charles River Laboratories (Wilmington, MA). Adult, sexually mature *tau* hamsters will be obtained from the colony bred in the laboratory of Michael Menaker (Charlottesville, VA). *Tau* males begin puberty about the same time as the wild-type males but *tau* females tend to begin puberty slightly later than their wild-type counterparts (Menaker, personal correspondence). In these experiments, wild-type and *tau* males will be age-matched according to the day of birth. Wild-type and *tau* females will be age-matched according to the day of the onset of puberty.

The wild-type hamsters will be housed in a room with a 14:10 LD (lights on at 0500 hours) cycle. The mutants will be maintained in a 11.7:8.3 (11 hours and 42 minutes: 8 hours and 18 minutes) LD cycle. The latter is a 20-hour cycle that has the same light-dark ratio as the 24-hour 14:10 cycle. Attempts to raise *tau* mutants in a 24-hour light-dark cycle have previously met with questionable results (Ralph and Menaker, 1988; Menaker et al., 1994). *Tau* hamsters raised in a light-dark cycle similar to that of their wild-type counterparts and placed into conditions of constant darkness were found to display an exaggerated phase shift in wheel-running activity after exposure to a

single light pulse. It is thought that the mutation increases the hamsters' sensitivity to residual effects of the previously experienced light cycle. The introduction of the 20-hour regimen has eliminated many of the extraneous reactions of the hamsters to the light-dark cycle (Shimomura and Menaker, 1994). For the sake of clarity in the description of these experiments, "light cycle" will be used in place of "day". In the *tau* animals, the light cycle is 20 hours long, as is their activity period. In the wild-type animals, the light cycle is 24 hours long to match the duration of their activity cycle.

Prior to the onset of the experiment, the animals will be housed 4 to a cage (each cage containing age-matched animals of the same sex and genotype), as described in previous experiments (Bast and Greenwald, 1974; Seegal and Goldman, 1975; Stetson et al., 1975). Food and water will be provided *ad libitum*, and the room temperature will be maintained at 21 -23 °C. During the time in constant darkness, the males will be housed 2 to a cage while the females will be housed individually to prevent the influence of one animal's estrous cycle on the period of another.

Determination of the Estrous Cycle

There has been some debate as to what technique is the most accurate in monitoring the phases of the hamster estrous cycle. Several investigators have relied on the presence of a daily vaginal discharge (Orsini, 1961; Labhsetwar, 1972; Norman et al., 1972; Bast and Greenwald, 1974), while others prefer to use a vaginal lavage (Alleva et

al., 1971; Bridges and Goldman, 1975).

The vaginal discharge method was proposed by Margaret Orsini (1961) as a simplified means of checking the progression of the hamster estrous cycle. Her observations led her to propose that there was a characteristic post-estrous discharge that was present on the day following estrus in normally cycling hamsters. This discharge could be present external to the vaginal opening or it could be extruded through slight pressure at the sides of the orifice. According to Orsini, the discharge seen on diestrus I is thick, white, opaque, and stringy. Subsequently, a waxy plug is observed on diestrus II, there is no discharge on proestrus, and the discharge on the day of estrus is a translucent and slightly stringy mucus. Acyclic females consistently present with small waxy blebs or a white, non-mucous substance. This method of testing the regularity of the estrous cycle is good when working with large numbers of animals.

The vaginal lavage method has been used with great success in many rodents. A small amount of saline is loaded into an eye dropper which has had the end cut off and flamed until smooth. The filled dropper is placed over the surface of the vagina and the area is washed. The saline solution and suspended cells are retrieved and placed onto a slide to be viewed under the microscope. In rats, each day of the estrous cycle has a characteristic cellular composition. Nucleated epithelial cells are seen on proestrus; flat, squamous, cornified cells are indicative of estrus; small cells (leukocytes) are observed on diestrus I and II. It must be noted that although these particular cell

types are conspicuous on these days, one actually expects to see a mixture of cells. The relative predominance of cells will be recorded and the actual days of the cycle will be deciphered after accumulation of adequate data. There are differences, however, in the estrous smear cell composition of the hamster as opposed to that of the rat. Alleva and his colleagues (1971) used the vaginal lavage method to determine cyclicity in their female hamsters but observed nucleated epithelial cells on diestrus I instead of on proestrus as in the rat. Lisk (1985) claims that the amount of cellular material and mucin discharged is too copious for the smear to be adequately analyzed. There is also the added risk of inducing pseudopregnancy by accidentally inserting the dropper into the vagina. Some investigators have used a combination of the discharge and lavage methods to confirm cyclicity (Stetson and Gibson, 1977; Stetson and Anderson, 1980).

The initial phase of these experiments will be designed to determine which of these two methods (vaginal discharge and vaginal lavage) is most appropriate for reliably predicting the day of proestrus. Ten wild-type females, maintained in a 14:10 LD cycle, and 10 *tau* females, kept in conditions of 11.7:8.3 LD, will be monitored for 20 light cycles. Once every light cycle, each animal will be checked for vaginal discharge, a vaginal lavage will then be performed. The testing will be done between 0800 and 1200 hours with the wild-type animals, and 3 to 6 hours after lights-on with the *tau* mutants. Observations will be charted daily and used to assess which of these two methods is the most accurate for the determination of the days of the

estrous cycle in both wild-type and *tau* females.

Preovulatory LH Surge

Ten wild-type and 10 *tau* mutant female hamsters will be maintained in LD cycles of 14:10 and 11.7:8.3, respectively, and observed for signs of a regular estrous cycle. Only those animals that display a regular estrous cycle will be used in this experiment. After the animals display 3 consecutive estrous cycles, they will be catheterized with an intra-atrial bleeding catheter on diestrus II. The wild-type hamsters will have blood samples drawn every hour from 1200 to 2000 hours on the day of proestrus. The mutant hamsters will have blood drawn every 50 minutes from 5.85 hours (5 hours and 51 minutes) after lights on to one hour after lights off. For both groups of animals, the sampling will begin half-way through the illuminated portion of the cycle. The *tau* hamsters will be sampled every 50 minutes because this period represents the same relative interval in a 20-hour day that 1 hour represents in a 24-hour day.

The blood samples will be drawn using a heparinized syringe and each sample will have a volume of approximately 250 uL. The collected samples will be centrifuged, the plasma will be collected and frozen. The red blood cells will be suspended in saline and returned to the animal after the subsequent sample. The first sample will be replaced with an equal volume of saline.

Each sample will be assayed individually through double antibody radioimmunoassay (RIA), using a rabbit antirat LH antiserum from NIDDK

(Bethesda, MD; NIDDK, LH antiserum S9). Iodination of LH (NIDDK rLH Iodination Material) will be done using the chloramine-T method. The antibody-hormone complexes will be precipitated using goat antirabbit gammaglobulin, and the bound fraction will be counted. The detectable minimum and maximum LH concentrations correspond to 0.1 and 1.9 ng NIDDK rat reference preparation (LH RP-2). We anticipate intra-and interassay coefficients of variation to be between 5% and 10%. We expect the rat LH RIA to be appropriate for our hamster samples since Loudon et al. (1994) found that, "Homogenates of hamster anterior pituitaries and plasma pools containing high LH concentrations diluted in parallel to the standard curve across the detectable range".

Testicular Atrophy and Subsequent Recrudescence in a Shortened Photoperiod

A total of 10 wild-type, raised in conditions of 14:10 LD, and 10 *tau* mutant males, raised in conditions of 11.7:8.3 LD, will used in this study. Prior to the onset of the experiment, all of the animals will be anesthetized with sodium pentobarbital (50 mg/kg; IP) and the length and width of the right testis will be measured through the shaved scrotal skin (Nelson and Zucker, 1987). As described below, a laparotomy will be performed on 2 of the wild-type and 2 of the *tau* males in order to obtain a more precise measure of the length and width of the testes prior to exposure to a short-day photoperiod. The skin over the abdomen will be shaved, cleaned, and incised, and an incision will be made along the linea alba. The right testicle will be *brought*

into the abdomen and measured (Rusak and Morin, 1976). All testicular measurements will be performed using a vernier caliper. The length and the width of the testes will be used to calculate the volume of the testes throughout the duration of the experiment. The volume will be determined with the formula: volume= $4/3\pi ab^2$, where a=length and b=width of the testes (Krieg et al., 1987). Following this procedure, all of the animals will be placed into shortened photoperiods consisting of 10:14 and 8.3:11.7 LD, respectively. Beginning 10 light cycles after the transfer to the shortened photoperiod, the lengths and widths of the right testes will be measured every 7 light cycles to check for regression and subsequent recrudescence. An external testes width of less than 8 mm is a definitive sign of regression, whereas a width of more than 12 mm is considered to be normal (Nelson and Zucker, 1987). In addition, laparotomies will be performed on 2 randomly chosen wild-type males and 2 randomly chosen *tau* males every 7 light cycles to obtain more accurate measurements of the testicular length and width. Those measurements obtained during the laparotomies will be used to determine the volume of the testes throughout the experiment. These observations will continue for 31 weeks which should be an adequate amount of time for recrudescence to occur in both the wild-type and *tau* mutant males.

Time to Anestrus in a Shortened Photoperiod

Ten wild-type and 10 *tau* female hamsters will be monitored for cyclicity. Only those animals that display regular estrous cycles will

be used in this experiment. The wild-type animals will be placed in conditions of 10:14 LD, the *tau* females will be maintained on an 8.3:11.7 LD cycle. The animals will be checked once every light cycle for signs of a regular estrous cycle. An animal is thought to be acyclic once she has passed 8 light cycles without an estrous smear (Bridges and Goldman, 1975) or a proestrous discharge. This experiment will continue for 8 weeks, which should be a sufficient length of time for anestrus to occur in both the wild-type and *tau* mutant females.

Analysis of Results

The data from these experiments will be analyzed by analysis of variance (ANOVA) and the appropriate post-hoc tests. A "p" value of less than 0.05 will be accepted as significant. For the study of the preovulatory LH surge in both genotypes, the relative time of the surge after lights-on and the amplitude of the surge will be compared. The absolute number of days and the number of light cycles to the onset of gonadal regression, recrudescence, and anestrus will be compared to determine differences in these parameters.

EXPECTED RESULTS

Preovulatory LH Surge

The pre-ovulatory LH surge is known to occur around 1500 hours on proestrus in those wild-type females that are maintained on a 14:10 LD cycle, with the lights on at 0500 hours (Goldman and Porter, 1970; Turgeon and Greenwald, 1972; Bast and Greenwald, 1974). This is approximately 10 hours after lights-on, or 5/7 of the way through the light portion of the LD cycle. In the *tau* females, we expect an LH surge on the afternoon of proestrus within the same relative time period, 5/7 of the way through the light portion of the LD cycle. As the light portion of the *tau* lighting regimen is 11.7 hours (11 hours and 42 minutes), the LH surge is expected to occur 8.4 hours (8 hours and 24 minutes) after lights-on on the day of proestrus. The observed plasma levels of LH in the *tau* females should be similar to those recorded in the wild-type females.

Testicular Atrophy and Subsequent Recrudescence in a Shortened Photoperiod

The time to testicular regression and recrudescence in male hamsters is expected to take less absolute time in the *tau* males but should occur in approximately the same number of light cycles as in the wild-type males. When male hamsters are placed in a shortened

photoperiod, testicular regression will be noticeable between 7 and 8 weeks, or 49 to 56 light cycles. Recrudescence will begin between 18 and 20 weeks (Elliott, 1976; Steger et al., 1982), or 126 to 140 light cycles, after the introduction of this lighting regimen. When *tau* hamsters are placed into conditions of 8.3:11.7 LD, gonadal regression should be apparent within 49 to 56 light cycles. This would be 40.8 to 46.7 absolute days, between 6 and 7 weeks, after the introduction of the shortened photoperiod. Recrudescence in the *tau* hamster is predicted to occur within 126 to 140 light cycles in the short-day photoperiod. This is the equivalent of 105 to 116.7 absolute days, or 15 to 17 weeks in wild-type animals. As with the wild-type animals, the gonads following recrudescence should return to pre-regression size and function.

Time to Anestrus in a Shortened Photoperiod

As with gonadal regression and subsequent recrudescence, the time to anestrus in the *tau* females exposed to a shortened photoperiod should take less absolute time, but approximately the same number light cycles, as the time to the onset of acyclicity in wild-type females maintained in a short-day environment. Females hamsters that are maintained in a 10:14 LD cycle should become acyclic within 7 weeks (Seegal and Goldman, 1975; Tamarkin et al., 1976), or 49 light cycles. The *tau* females that are housed in the shortened photoperiod should also become acyclic within 49 light cycles. This is the equivalent of 40.8 absolute days, or almost 6 weeks in wild-type animals.

DISCUSSION

The *tau* mutation in the golden hamster is not responsible for any gross changes in the appearance of the affected animal. Instead, a *tau* mutant hamster presents with an activity period that is 16.7% shorter in duration than that of a wild-type hamster. Assuming that the period of the wheel-running activity is perceived as a day by the animal, the *tau* hamster experiences a day that is 20 hours in length. Therefore, a *tau* mutant experiences 1.2 days every 24 hours. With this in mind, we expect that the reproductive phenomena of the *tau* hamsters will be functionally similar to those of wild-type animals, but will occur in 83.3% of the time that it takes for these events to transpire in wild-type hamsters.

The pre-ovulatory LH surge has been demonstrated to be temporally related to the activity cycle of the hamster (Stetson and Gibson, 1977; Swann and Turek, 1985). In cases where a split activity cycle is observed, there are also 2 LH surges (Swann and Turek, 1985). It follows that if the frequency of the wheel-running activity is increased, the frequency of the proestrous LH surge will also be increased. The pre-ovulatory surge in the *tau* females is expected to occur every four activity cycles, which will be once every 80 hours instead of once every 96 hours, as in the wild-type female. The LH surge in the wild-type females occurs approximately 10 hours into a 14

hour light phase, or 5/7 of the way through the light portion of the cycle. Using 5/7 of the light phase to determine the timing of the surge, we estimate that the proestrous LH surge in the *tau* female will occur about 8.4 hours after lights-on. It is reasonable to assume that the plasma LH concentration is sufficient to induce ovulation as there are no known problems breeding the *tau* hamsters in the laboratory. *Tau* litter sizes range from 8 to 18, with an average size of 11 or 12, which is comparable to the litter sizes of wild-type hamsters. In addition, the gestation period of the *tau* female is the same is in the wild-type, 16 days (Menaker, personal correspondence).

It should be kept in mind that Loudon and his colleagues (1994) found that the *tau* mutation increased, not decreased, the secretory interpulse LH interval in ovariectomized hamsters. There is a possibility that the mutation will affect the LH surge in a similar manner in our experiments. It may be observed that even though the activity cycle is occurring more frequently in the *tau* females, the proestrous LH surge may be delayed. The episodic LH secretion in ovariectomized hamsters observed by Loudon and his cohorts (1994) is a circahoral rhythm, whereas the preovulatory LH surge represents a circadian rhythm. In this regard, the potential for significant changes in the timing of the preovulatory LH surge due to the *tau* mutation might be diminished.

It is interesting to consider the effect that light has on the various activities of the hamster. In the prolonged absence of light, the animal free-runs on its endogenous activity period and the

reproductive organs begin to involute. Gonadal regression is an evolutionary mechanism that ensures that the hamster will not reproduce when the environmental conditions are poor. Although the *tau* mutant is not known to exist outside of the laboratory, it is not unreasonable to expect that both the male and female mutants experience a loss of reproductive function in the absence of light.

It was already noted that these animals experience 1.2 days every 24 hours. To avoid confusion, the term "light cycle" has been used instead of "day" in this thesis. It is predicted that regression, the onset of acyclicity, and recrudescence will occur in the same number of light cycles in the *tau* mutants as in the wild-type hamsters. These processes appear to transpire more quickly in the mutants because their light cycle is only 20 hours in length. Regression, which takes about 7 to 8 weeks in the wild-type hamster (Elliott, 1976; Steger et al., 1982), is proposed to occur within 6 to 7 weeks in the *tau* males. Although it takes less absolute days, the number of light cycles to the onset of regression is proposed to be equal for the wild-type and *tau* males. Recrudescence, which takes 18 to 20 weeks in the wild-type males (Elliott, 1976; Steger et al., 1982), is expected to occur within 15 to 17 weeks in the *tau* males. Anestrus usually takes place 6 to 7 weeks after the introduction of the shortened photoperiod (Seegal and Goldman, 1975; Tamarkin et al., 1976). Yet, in the *tau* females, acyclicity will be expected within 6 weeks. Again, although it is anticipated to take less absolute time for these events to occur in the *tau* mutants, the number of light cycles to the onset of recrudescence

and anestrus is proposed to be equal for both strains of hamster.

Melatonin is thought to be the hormone responsible for the loss of reproductive function in both the male and female hamsters (Turek et al., 1975; Tamarkin et al., 1976; Bittmann, 1978). Again, referring to the findings of Loudon and his colleagues (1994) concerning the impact of the mutation on the secretory rhythms of cortisol and LH, it is interesting to ponder what effect, if any, the *tau* mutation has on the production and output of melatonin. The secretions of the pineal gland (including melatonin) are controlled by the suprachiasmatic nuclei (SCN), which are now known to be the circadian pacemaker (Ralph et al., 1990). Perhaps the mutation will somehow delay the onset of gonadal regression and acyclicity by decreasing or inhibiting the production of melatonin. Regardless, we predict that the *tau* mutation will affect only the timing of the aforementioned reproductive functions, the inherent characteristics will remain intact. Just as transplantation of the SCN from the *tau* mutant reproduced the shortened activity cycle in the wild-type host, similar transplantation experiments would be expected to alter the timing of the preovulatory LH surge and the photoperiod-induced onset of gonadal regression, recrudescence, and anestrus in an SCN-lesioned host.

The investigator who chooses to work with the *tau* mutant hamster is sure to be challenged in a number of ways. Firstly, there is a lack of knowledge as to the effect of the mutation on the internal systems of the hamster. Secondly, the *tau* hamsters must be kept in a special light cycle in order to minimize the side effects of the lighting regimen on

the experiment (Ralph and Menaker, 1988; Menaker et al., 1994). The 11.7:8.3 LD cycle provides the same ratio of light to dark per 20 hours as the 14:10 LD cycle provides per 24 hours (Shimomura and Menaker, 1994). However, this cycle consists of 11 hours and 42 minutes of light and 8 hours and 18 minutes of dark. This means that the investigator may be taking samples or recording observations at odd hours. A special animal room with an adjustable light timer must be available when working with these animals. In addition, the *tau* mutation is now thought to exert its influence by destabilizing the components of the circadian system (Menaker et al., 1994). In light of this view, it may be wise for the investigator to expect unforeseen results in any work with the *tau* mutant hamsters.

The *tau* mutant hamster is easily able to thrive and reproduce in the laboratory, where the lighting schedule can be adapted to its unusual circadian period. It is interesting to ponder how this strain of golden hamster would fare in the natural world, which is a 24-hour lighting environment. Given what is known about the *tau* mutation, it would appear that a mutant would be a severe disadvantage in the wilderness. The hamster is a nocturnal animal. Even in the laboratory, the onset of wheel-running activity is recorded around the time of lights-off. Ralph and Menaker (1988) noted that the heterozygote for the mutation began wheel-running activity about 4 hours prior to lights-off in a 14:10 LD cycle. If the *tau* hamster begins his activity before nightfall, he places himself at a greater risk of attack by predators. Successful breeding would also present a challenge to the *tau* mutant.

The time of reproductive latency in the wild-type hamster approximates the length of the winter hibernation period. If this interval in the *tau* hamster is shorter by 16.7%, as predicted in this thesis, the *tau* mutants will begin their breeding season when the environmental conditions are still unfavorable. It is possible that *tau* hamsters exist outside of the laboratory. However, their chances of survival in the natural world are greatly decreased by the impact of this mutation on the activity and reproductive cycles.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adler S. Origin of the Golden Hamster *Cricetus auratus* as a Laboratory Animal. Nature 162: 256, (1948).

Alleva J.J., Waleski, M.V., Alleva F.R. A Biological Clock Controlling the Estrous Cycle of the Hamster. Endocrinology 88: 1368, (1971).

Bast J.D. and Greenwald G.S. Serum Profiles of Follicle-Stimulating Hormone, Luteinizing Hormone, and Prolactin During the Estrous Cycle of the Hamster. Endocrinology 94: 1295, (1974).

Berndston W.E. and Desjardins C. Circulating LH and FSH Levels and Testicular Function in Hamsters During Light Deprivation and Subsequent Photoperiodic Stimulation. Endocrinology 95: 195, (1974).

Bittman E.L. Hamster Refractoriness: The Role of Insensitivity of Pineal Target Tissues. Science 202: 648, (1978).

Bridges R.S. and Goldman B.D. Diurnal Rhythms in Gonadotropins and Progesterone in Lactating and Photoperiod Induced Acyclic Hamsters. Biology of Reproduction 13: 617, (1975).

Chan W.Y. and Ng T.B. Effect of Photoperiod on Testicular Histology in Golden Hamsters and C57 and Balb/c Mice. Archives of Andrology 32: 101, (1994).

Desjardins C., Ewing L.L., Johnson B.H. Effects of Light Deprivation upon the Spermatogenic and Steroidogenic Elements of Hamster Testes. Endocrinology 89: 791, (1971).

Elliott J.A. Circadian Rhythms and Photoperiodic Time Measurement in Mammals. Federation Proceedings 35: 2339, (1976).

Fitzgerald K.M. and Zucker I. Circadian Organization of the Estrous Cycle of the Golden Hamster. Proceedings of the National Academy of Science USA 73: 2923, (1976).

Gaston S. and Menaker M. Photoperiodic Control of Hamster Testis. Science 158: 925, (1967).

Goldman B.D. and Porter J.C. Serum LH Levels in Intact and Castrated Golden Hamsters. Endocrinology 87: 676, (1970).

Greenwald G.S. Preovulatory Changes in Ovulating Hormone in the Cyclic Hamster. Endocrinology 88: 671, (1971).

Hoffman R.A. and Reiter R.J. Pineal Gland: Influence on Gonads of Male Hamsters. Science 148: 1609, (1965).

Jorgenson K.L. and Schwartz N.B. Shifts in Gonadotropin and Steroid Levels That Precede Anestrus in Female Golden Hamsters Exposed to a Short Photoperiod. Biology of Reproduction 32: 611, (1985).

Knigge K.M. and Joseph S.A. A Stereotaxic Atlas of the Brain of the Golden Hamster. In: Hoffman R.A., Robinson P.F., Magalhaes H. (eds.). The Golden Hamster: Its Biology and Use in Medical Research. Iowa: The Iowa State University Press, pp.283-319, (1968).

Krieg R.J., Jr., Rogers J.P., Jr., Seibel H.R. Influence of a Prolactin- and ACTH-Secreting Tumour on Oestrous Cyclicity, the Pro-Oestrous Surges of LH and Prolactin and Ovarian Hypertrophy in the Rat. Journal of Endocrinology 114: 41, (1987).

Labhsetwar A.P. Role of Estrogen in Spontaneous Ovulation: Evidence for Positive Feedback in Hamsters. Endocrinology 90: 941, (1972).

Labhsetwar, A.P., Joshi H.S., Watson D. Temporal Relationship Between Estradiol, Estrone and Progesterone Secretion in the Ovarian Venous Blood and LH in the Peripheral Plasma of Cyclic Hamsters. Biology of Reproduction 8: 321, (1973).

Lisk R.D. The Estrous Cycle. In: H.I. Siegel (ed.). The Hamster: Reproduction and Behavior. New York: Plenium Press, pp. 23-51, (1985).

Loudon A.S.I., Wayne N.L., Krieg R., Iranmanesh A., Veldhuis J.D., Menaker M. Ultradian Endocrine Rhythms Are Altered by a Circadian Mutation in the Syrian Hamster. Endocrinology 135: 712, (1994).

Menaker M., Shimomura K., Ihara N.L. The *Tau* Mutation Destabilizes the Circadian System of Golden Hamsters. In: Fifth Symposium on Biological Rhythms, Hokkaido University Press, Sapporo. In press (1994).

Murphy M.R. History of the Capture and Domestication of the Syrian Golden Hamster (*Mesocricetus auratus Waterhouse*). In: H.I. Siegel (ed.). The Hamster: Reproduction and Behavior. New York: Plenium Press, pp. 3-20, (1985).

Nelson R.J. and Zucker I. Spontaneous Testicular Recrudescence of Syrian Hamsters: Role of Stimulatory Photoperiods. Physiology of Behavior 39: 615, (1987).

Norman R.L. and Spies H.G. Neural Control of the Estrogen-Dependent

Twenty-Four-Hour Periodicity of LH Release in the Golden Hamster. Endocrinology 95: 1367, (1974).

Norman R.L., Blake C.A., Sawyer C.H. Effects of Hypothalamic Deafferentation on LH Secretion and the Estrous Cycle in the Hamster. Endocrinology 91: 95, (1972).

Orsini M.W. The External Vaginal Phenomena Characterizing the Stages of the Estrous Cycle, Pregnancy, Pseudopregnancy, Lactation, and the Anestrous Hamster, Mesocricetus Auratus Waterhouse. Proceedings of Animal Care Panel 11: 193, (1961).

Paxinos G. and Watson C. The Rat Brain in Stereotaxic Coordinates. Australia: Academic Press, plate 78, (1986).

Pittendrigh C.S. and Daan S. A Functional Analysis of Circadian Pacemakers in Nocturnal Rodents. V. Pacemaker Structure: A Clock for All Seasons. Journal of Comparative Physiology 106: 333, (1976).

Ralph M.R. and Menaker M. A Mutation of the Circadian System in Golden Hamsters. Science 241: 1225, (1988).

Ralph M.R., Foster R.G., Davis F.C., Menaker M. Transplanted Suprachiasmatic Nucleus Determines Circadian Period. Science 247: 975, (1990).

Reiter R.J. Changes in the Reproductive Organs of Cold-Exposed and Light-Deprived Female Hamsters (*Mesocricetus Auratus*). Journal of Reproductive Fertility 16: 217, (1968).

Reiter R.J. Evidence for Refractoriness of the Pituitary-Gonadal Axis to the Pineal Gland in Golden Hamsters and Its Possible Implication in Annual Reproductive Rhythms. Anatomical Record 173: 365, (1972).

Rusak B. and Morin L.P. Testicular Responses to Photoperiod Are Blocked by Lesions of the Suprachiasmatic Nuclei in Golden Hamsters. Biology of Reproduction 15: 366, (1976).

Seegal R.F. and Goldman B.D. Effects of Photoperiod on Cyclicity and Serum Gonadotropins in the Syrian Hamster. Biology of Reproduction 12: 223, (1975).

Shimomura K. and Menaker M. Light-Induced Phase Shifts in *tau* Mutant Hamsters. Journal of Biological Rhythms 9: 97, (1994).

Steger R.W., Bartke A., Goldman B.D. Alterations in Neuroendocrine Function During Photoperiod Induced Testicular Atrophy and Recrudescence in the Golden Hamster. Biology of Reproduction 26: 437, (1982).

Stetson M.H. and Anderson P.J. Circadian Pacemaker Times Gonadotropin Release in Free-Running Female Hamsters. American Journal of Physiology 238: R23, (1980).

Stetson M.H. and Gibson J.T. The Estrous Cycle in Golden Hamsters: A Circadian Pacemaker Times Preovulatory Gonadotropin Release. Journal of Experimental Zoology 201: 289, (1977).

Stetson M.H., Elliott J.A., Menaker M. Photoperiodic Regulation of Hamster Testis: Circadian Sensitivity to the Effects of Light. Biology of Reproduction 13: 329, (1975).

Stetson M.H., Watson-Whitmyre M., Matt K.S. Cyclic Gonadotropin Release in the Presence and Absence of Estrogenic Feedback in Ovariectomized Golden Hamsters. Biology of Reproduction 19: 40, (1978).

Swann J.M. and Turek F.W. Multiple Circadian Oscillators Regulate the Timing of Behavioral and Endocrine Rhythms in Female Golden Hamsters. Science 228: 898, (1985).

Tamarkin L., Westron W.K., Hamill A.I., Goldman B.D. Effect of Melatonin on the Reproductive Systems of Male and Female Syrian Hamsters: A Diurnal Rhythm in Sensitivity to Melatonin. Endocrinology 99: 1534, (1976).

Turgeon J. and Greenwald G.S. Preovulatory Levels of Plasma LH in the Cyclic Hamster. Endocrinology 90: 657, (1972).

VITA