# THE INABILITY TO PERCEIVE PHOTOPERIOD AFFECTS REPRODUCTION IN MINK

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## ABSTRACT

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The predictable changes in photoperiod throughout the year stimulate changes of reproductive function in most non-primate animals. In mammals, the physiological pathway for photoperiod detection in mammals begins with the eyes, then to the superior cervical ganglia (SCG) which relays the information concerning daylength on to the pineal gland. If the eye-SCG-pineal axis is interrupted, disturbances in reproductive function result.

To separate the roles of the eyes and the SCG in photoperiod- controlled reproduction, 72 prepubertal (6 months old) mink (36 male and 36 female) received one of the following surgical treatments in November or December of their first year: 1) bilateral blinding, 2) bilateral superior cervical ganglionectomy (SCGx), 3) blinding + SCGx, 4) blinding + sham SCGx, 5) sham SCGx, or 6) remained intact. The animals remained under the naturally-occurring photoperiod in East Lansing, Michigan, although one-half of each surgical treatment group was housed indoors or outdoors and both under standard mink ranch conditions. The animals were examined every two weeks from one month post-surgery (January) through two breeding seasons

(March). Body weight was recorded and reproductive development assessed by testicular dimensions or degree of vulvular swelling. Blood samples were collected by jugular venipuncture and the serum from males was analyzed for testosterone (T) and serum from females for estradiol ($E_2$).

The pubertal increase in testes size, vulva edema, T or $E_2$ concentrations did not differ among treatment groups. However, all three blinded mink groups maintained enlarged testes or vulvae significantly longer after the breeding season than sighted mink. Testes involution in blinded males was slower than sighted males with the testes not ascending into the abdomen until July while the sighted males testes ascended in May. The T profile paralleled and slightly preceded that of the testes size in all groups. Testes recrudesence of SCGx males began in November, one month before sham SCGx or intact males. Blinded males showed no recrudesence by the second breeding season. The $E_2$ profiles were too varied within the treatment groups to yield any conclusive results about the effect of the surgeries on $E_2$ levels. The blinded animals of both sexes exhibited no circannual changes in body weight as did the sighted animals. There was no effect of housing on any reproductive parameters measured.

It appears that the eyes are the most crucial component in the eye-SCG-pineal axis in mink since without them the animals become asynchronous with the photoperiod despite

other treatments. There was no spontaneous regeneration of the gonads and the post-breeding season atrophy of the testes was slower in blinded animals indicating that mink require the eyes to detect the photoperiod not only to stimulate reproductive function but to terminate it as well.

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# LITERATURE REVIEW

## 1.0) Introduction

This discussion will focus on photoperiodicity and its role in reproduction of mammals, mainly laboratory species and some carnivores. This phenomenon is poorly understood in carnivores although many are economically valuable especially the family, Mustelidae.

A member of Mustelidae, the mink was the model species for this dissertation, so more time than usual will be spent discussing their reproductive response to photoperiod, and that of a fellow mustelid, the ferret.

The physiological aspects of photoperiodicity will be approached using the pineal gland as the primary neurohormonal structure providing the biological signal concerning length of night and day. The neural circuitry to and from the pineal gland, as well as the hormonal actions and reactions, will be discussed.

This literature review will also concentrate on an aspect of reproduction that this research investigated, the initiation of puberty and the annual onset of the breeding season, and the influence of photoperiod upon it.

## 2.0) Photoperiodicity and Reproduction in Mammals

### 2.1) Investigations of Wildlife Species

Most mammalian species reproduce during the time of year when the probability for survival of both parents and young is maximal (Turek and Campbell, 1979). In temperate regions, the principle cue in triggering the cascade of events which leads to successful parturition and rearing of the young is the annual changes in photoperiod (Menaker, 1971).

The grasshopper mouse inhabits the western United States and breeds during the spring and summer months of long days (March through August). Although the photoperiod is increasing from March until the end of June and then decreasing in July and August, it appears that it is the total amount of light received in a day that is the important cue. Ten hours of light, short days (SD), lead to gonadal regression while 12 to 16 hours, long days (LD), are sufficient to stimulate and maintain enlargement of gonads. However, if the mice are housed under SD for 30 weeks for males or 16 weeks for females, they will undergo spontaneous gonadal regrowth (Frost and Zucker, 1983). Disparity in response between the sexes is not understood although it has been noted in other rodent species as well. This waning of the inhibitory effects of SD permits late-born animals to be reproductively ready for the next

spring breeding season as soon as it arrives although the daylight is not yet 14 hours long.

The white-footed mouse is also a long-day breeder and when adult females are maintained on a schedule of 8 hours of light and 16 hours of darkness (8L:16D) for 6 weeks, their uterine and ovarian weights decrease markedly in weight (Petterborg, 1983). However, a closely related species, the prairie deer mouse, responds differently to short days. Adult females on SD exhibit little disruption of reproductive condition (Whitsett and Miller, 1982).

A relationship between prenatal exposure to photoperiod and subsequent reproductive development has been reported in voles (Horton, 1984, 1985: Nelson, 1985b). Young voles respond to the length of daylight to which their mothers were exposed during gestation, rather than to the photoperiod that the young voles experienced from birth until weaning. A similar response was discovered in the Djungarian hamster by Stetson et al.(1986). This is significant biologically since the young animals can begin to prepare reproductively for the environment they are born into so they will be ready to breed when they are weaned. However, Nelson (1985a) discovered that after a couple of generations of laboratory breeding from wild-trapped ancestors, the descendents have markedly reduced photoperiod sensitivity. Lab raised ground squirrels were also less sensitive to light than wild squirrels (Reiter et

al., 1983). This phenomenon of reduced photoperiod sensitivity after generations of laboratory breeding could be an explanation for apparently conflicting results in photoperiod responses of wild species.

Photoperiodicity has been investigated in species as unusual as pallid bats found in Napa Valley, California. The transition of long days to short days stimulates the change in reproductive function from spermatogenesis and an inability to copulate to sperm storage and the ability to mate but with no sperm production (Beasley and Zucker, 1984). Large wild ungulates such as red deer (Webster and Barrell, 1985) and white- tailed deer (Bubenik et al., 1982), have been shown to respond to photoperiod, specifically artificially produced short days, with early antler growth, early mating and winter coat growth.

Most of the photoperiod work involving wild mammalian species is descriptive with few of the controlled experiments such as those reported using laboratory species.

## 2.2) Investigations of Laboratory Species

The hamster is to laboratory photoperiod studies what the fruit fly has been to genetic research. The Syrian, or golden, hamster has been the most commonly used species although the Turkish and Djungarian hamsters have also been studied.

The golden hamster is a seasonal breeder in which long days are stimulatory to reproduction and short days inhibitory. However, like the grasshopper mouse, maintaining hamsters in short photoperiods (less than 12.5 hours light/24 hours) will not suppress gonadal growth indefinately. After 20 to 25 weeks the animals will become photorefractory and spontaneously regain reproductive competence (Reiter, 1972; Stetson and Tate-Ostroff, 1981; Steger et al., 1982). However, if they are kept in greater than 12.5 hours light/24 hours, they undergo gonadal regression. The fact that the critical photoperiod is 12.5 hours shows that the hamster is capable of fine discrimination in daylength. When an animal has become photorefractory on long days it requires an intervening period of 10 to 11 weeks of short days to make it "photosensitive" to long days again (Reiter, 1973a).

In a biological perspective the refractory phenomenon can be understood. It occurs in nature from December through February when reproductive success would be poor. Spontaneous gonadal recrudescense begins in March and the animals are reproductively competent and photosensitive to the long days in May. This is also the best time to raise offspring and have them survive.

The laboratory rat, not as photosensitive as the hamster, has been selected for maximal reproduction efficiency. It will continue to mate and raise litters

throughout the year if exposed to even a few hours of light. The hamster may be headed toward the same fate since photoperiod induced reproductive changes that occurred in 4 weeks in 1965 now take 6 to 8 weeks (Reiter, 1980a). If a female rat is exposed to constant light, however, she will go into a state of anovulation but with constant vaginal and behavioral estrus (Lawton and Schwartz, 1967). Due to their inability to respond to relatively small changes in daylength, the lab rat is a poor model for photoperiod studies. Their wild cousins, though, are probably as photosensitive as any other wild animals. But in the wild, other factors such as food availibility may override photoperiodic effects on reproduction.

### 2.3) Investigations of Large Domestic Species

Much of the photoperiod manipulation in the environments of large domestic (farm) animals has been done to increase the food or fiber production by those animals (Tucker and Ringer, 1982). This is accomplished either directly by immediate increases in the amount of milk or other product, or indirectly by controlling when reproduction will occur. Although dairy cattle are not a highly photoperiodic species in terms of reproduction, fertility is greater during the long-day months of summer and fall than in the winter (Tucker, 1982). Heifers reared on long days (16L:8D) eat more and gain weight more rapidly than when

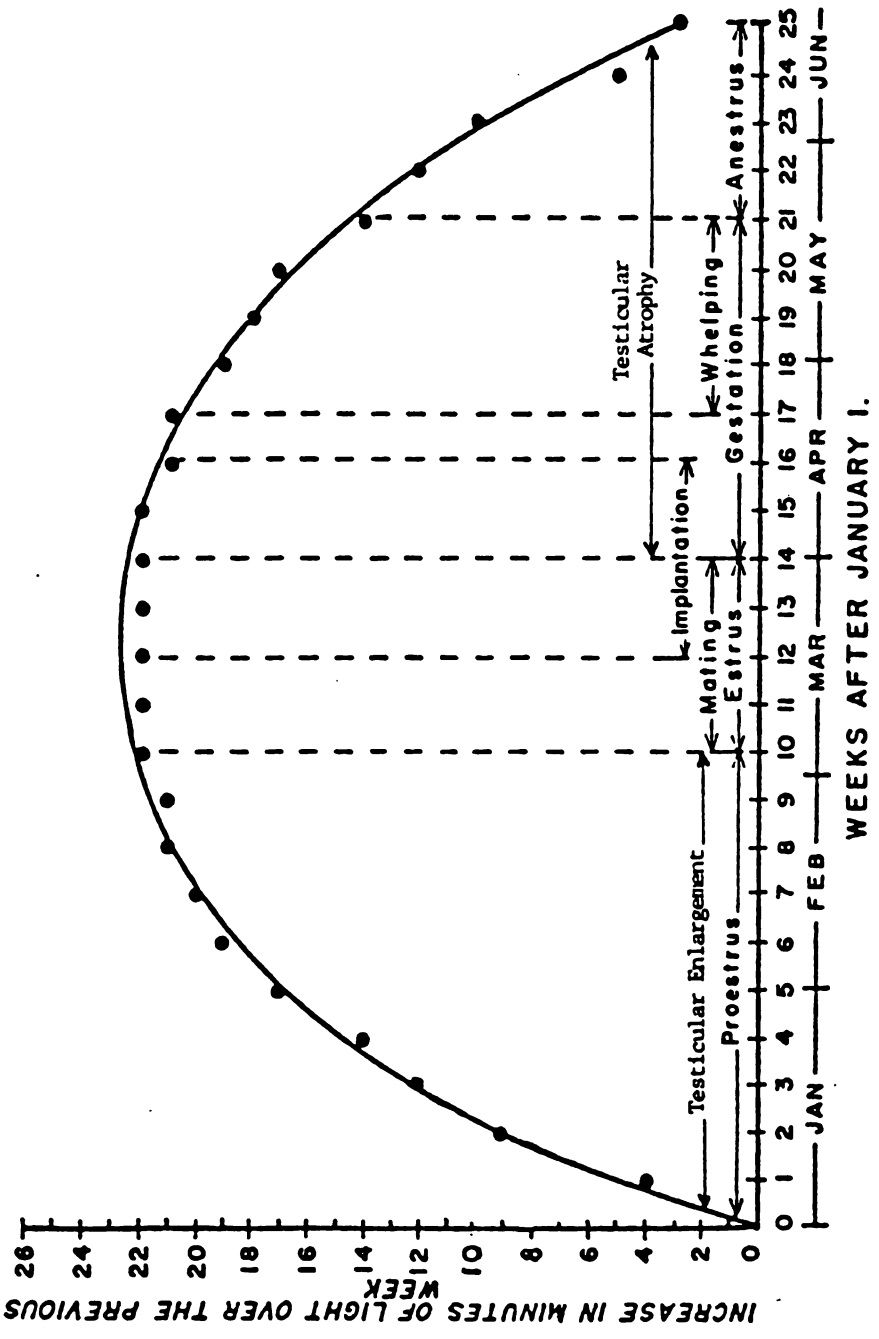
exposed to short days (8L:16D), consequently they reached a larger body size and puberty sooner (Petitclerc et al., 1983a).

The most commonly studied large animal species is the sheep. Most breeds of sheep are photosensitive and the decreasing daylength in the fall stimulates the onset of the breeding season. Rams maintained under short days (8L:16D) showed increases in testes size, and presumably fertility, sooner than rams on long days (Almeida and Lincoln, 1982). Ewes treated similarily followed the same pattern with an earlier onset of estrus (Legan and Winans, 1981). However, if the sheep are kept on short days for more than 16 weeks the sexual competence wanes and their gonads regress. Conversely, if they are maintained under usually inhibitory long days, the gonads spontaneously recrudesce. Sheep appear to have a refractory mechanism as does the hamster (Robinson and Karsch, 1984).

## 2.4) Investigations of Mink and Ferrets

As stated in the introduction, the model species for this research is the mink. Both mink and ferrets mustelids breed during lengthening days although the testes of the male mink begin to enlarge in decreasing daylength (Bostrum et al., 1968; Nieschlag and Bieniek, 1975) prompting some authors to call them "short-day breeders" (Boissin-Agasse et al., 1982) (Figure 1). The mink

Figure 1. Rate of increase of light per week from January 1 through June 24 at the 45th N parallel (calculated from the nautical almanac) and the breeding cycle of mink.


INCREASE IN MINUTES OF LIGHT OVER THE PREVIOUS WEEK
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
JAN
FEB
MAR
APR
MAY
JUN
WEEKS AFTER JANUARY 1.
Testicular Enlargement
Proestrus
Estrus
Mating
Implantation
Testicular Atrophy
Whelping
Gestation
Anestrus

breeding season begins about the first of March and lasts 3 to 4 weeks while that of the ferret starts in May and continues for several months throughout the summer (Pilbeam et al., 1979). Both species are induced-ovulators and the mink displays the embryo diapause/delayed implantation phenomenon (Hansson, 1947). Mink annual cycles of furring and reproduction are closely tied to the photoperiod (Duby and Travis, 1972).

Bissonnett (1932) discovered that ferrets can be induced into early sexual competency by artificially increased photoperiod in the late fall. Hansson published the first detailed report in 1947 on reproductive physiology in the mink. He tried unsuccessfully to hasten the onset of the breeding season by exposing the animals to an earlier dawn and a later dusk in the fall. He caused only uterine and ovarian weights to increase. In 1951, Hammond reported that exposing mink to artificially short days (the actual photoperiod was not reported) in the summer caused the females to exhibit estrus vaginal smears and the males, enlarged testes. Ferrets exposed to 14 hours of light in January experienced estrus earlier than those on natural photoperiod or on constant light. Mink on the same lighting regime did not exhibit estrus (Donovan et al., 1983). Immature female ferrets begin to have ovarian maturation within 22 days after being exposed to long days at 16 weeks to age (Ryan, 1985). In general, ferrets

appear to respond reproductively to light manipulation more quickly than do mink.

Female mink given extra lighting (8.2 minutes light added/day) from February 1 showed estrus and willingness to be mated earlier than females on normal photoperiod (February 15 vs. March 5) (Holcomb et al., 1962). Increased daylength before and after mating also reduced gestation length and increased the number of kits whelped per litter. Both these effects were due to decreased embryonic diapause and earlier implantation. A later attempt was make to produce two litters in the same year. Daylength to the mothers was decreased after weaning (July 1) to that of the winter solstice by mid-July and then gradually increased (Aulerich et al., 1963). The females' vaginal smears were estrual in the fall but there were no matings since the males showed little interest in mating.

Travis and Pilbeam (1980) examined photoperiodic schemes to alter the mink's annual cycle so it so it would be 6 months out of phase with the animals raised in natural light. They abruptly switched mink on the shortest day of the natural photoperiod (December 21) to the longest day. The daylength was then decreased as in nature. The reproductive performance of these mink was followed for the next 3 years. The reproductive performance of both the males and females in the artificial photoperiod was significantly poorer than the controls on natural light.

This could have been due to high ambient temperatures during the "new" breeding season, endogenous reproductive rhythms from the original group of mink, and/or improper light spectra generated by artificial light. These results proved surprising since two mink ranchers had reported earlier in a trade journal that they could get some mink to breed and whelp any time of year (Williams and Turbak, 1970). However, Williams and Turbak's observations were not as carefully controlled as those to Travis and Pilbeam. Some mink will respond to artificial photoperiods but not enough so that it would be feasible for commercial operations.

## 3.0) Physiology of Photoperiodicity

### 3.1) Introduction

In mammals, the pathway from the ambient photoperiod through the brain which eventually effects the gonads and reproductive function, has been partially elucidated. Most of the anatomical components for mediation of photoperiod and reproduction have been identified although interaction has not been fully determined. A simplified model of the cephalic components of the pathway include: 1) the eye as the sole photoreceptor, 2) the suprachiasmatic nucleus as the "time-keeping" portion of the hypothalamus, 3) the superior cervical ganglia as a regulator of input from the sympathetic nervous system to the pineal gland, and 4) the

pineal gland which converts the neural input concerning photoperiod into a chemical signal, melatonin (Figure 2). The pineal will be discussed first and then the other components of the pathway including how they relate to the pineal.

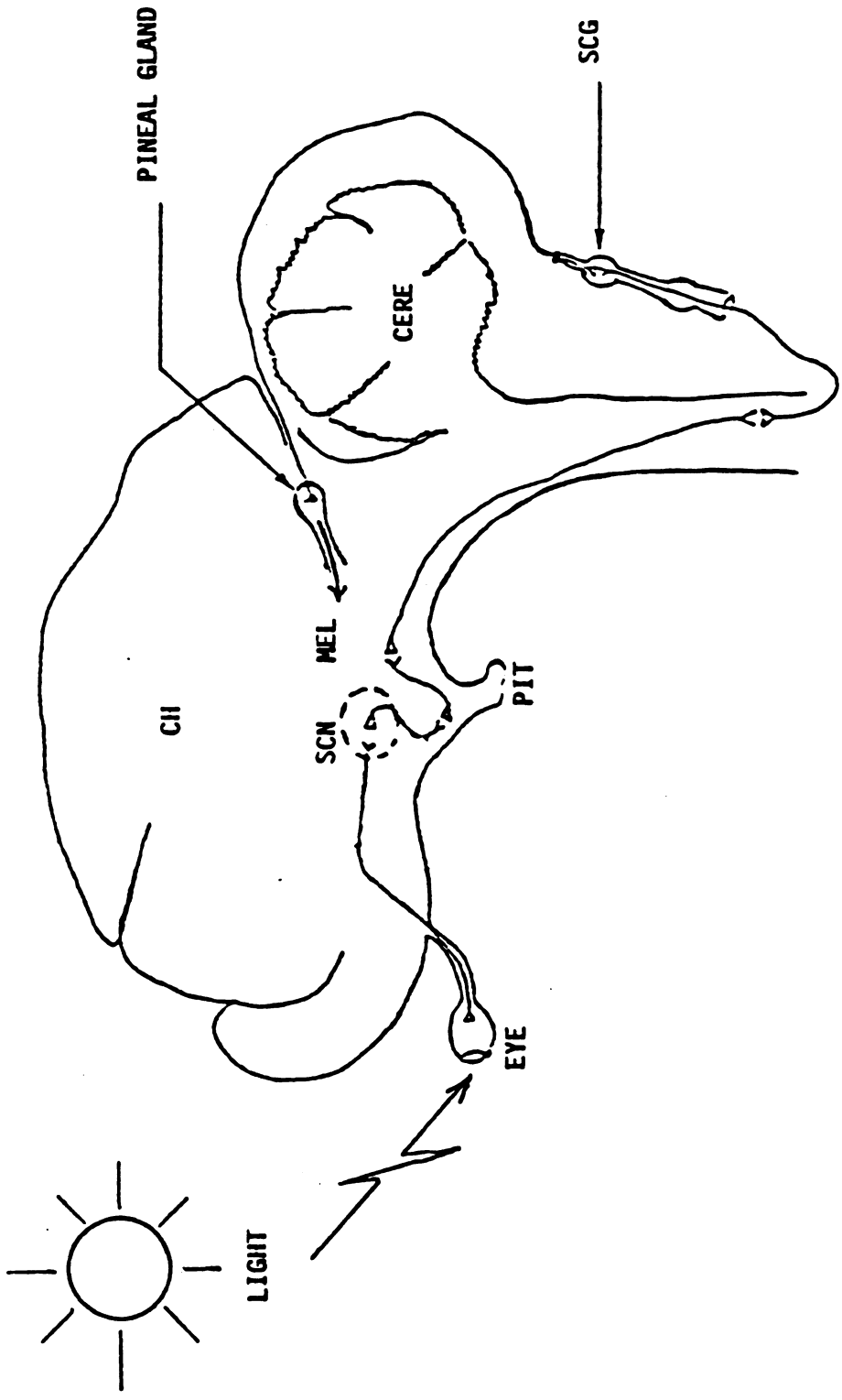
### 3.2) The Pineal Gland

The pineal gland is located between the cerebral hemispheres and anterior to the cerebellum. The pineal varies in size among mammalian species with a general trend of smaller glands in animals living near the equator (small annual changes in photoperiod) and large glands in related temperate and artic- dwelling species (large circannual photoperiod changes) (Reiter, 1980a). The discovery of one of the pineal hormone, melatonin, and its structure revived interest and research on this gland (Lerner et al, 1958; 1959).

#### 3.2.1) Brief Description of Morphology

Long thought to be a vestigial complex, the pineal gland arises embryologically from a tubular evagination of the dorsal diencephalon (Altar, 1982). Pineal parenchyma (endocrine secretory cells) proliferate rapidly reaching the greatest rate of mitosis within the first few days after birth, in the rat. Cell division, mainly of neuroglia, continues until two months of age. In the rat,

Figure 2. Presumed components of the photoperiod sensing and processing system in the mink brain. SCN = Suprachiasmatic nucleus; PIT = Pituitary gland; SCG = Superior cervical ganglia; CH = Cerebral hemisphere; CERE = Cerebellum; MEL = Melatonin; = Nerve pathway.


PINEAL GLAND
SCG
CERE
MEL
CH
SCN
PIT
EYE
LIGHT

neuroglia have a secretory as well as structural role within the pineal but in the mink they seem to function only as support structures (Weman and Nobin, 1979). Since the endocrine cells, or more properly "neuroendocrine cells", arise from neural tissue they are metabolically active with many mitochondria and Golgi apparatuses. These pinealocytes are usually light staining but a few dark glycogen-rich cells can be found (Rouvet, 1982). They are surrounded by a thick network of capillaries. Until recently, it was believed that the only efferent communication from the pineal was by way of these vascular connections. However this year, Korf et al. (1986), reported the discovery of neural projections from the gland.

### 3.2.2) Control of Melatonin Synthesis

The pineal gland serves as a transducer of electrical nerve impulses originating from the retina after photic input into a chemical message, melatonin. The amino acid tryptophan is sequestered by the pineal during the day and is converted in two enzyme-catalyzed steps to the indole serotonin. (The pineal contains the greatest concentration of serotonin in the brain.) At the onset of darkness most of the stored serotonin is converted to melatonin by another two enzyme system, serotonin n- acetyl transferase (SNAT) and hydroxyindole-o-methyl-transferase (HIOMT) (Cardinali, 1981). Since SNAT is the rate-limiting enzyme

step, it has been the most studied.

With the onset of darkness, SNAT actvity increases 50-fold and then it falls near dawn (Deguchi and Axelrod, 1972). The circadian changes are endogenous; they are not driven by the light/dark cycle although they are in phase with it (Binkley, 1983) and each morning the dawn "resets" the internal clock mechanism (Ho et al., 1984). If animals are blinded or in constant darkness, the rhythm persists for a time but then it "free-runs" (Zatz, 1980).

Although SNAT activity is the primary control of changes in melatonin concentrations, melatonin synthesis is also under β- adrenergic control. Synthesis of melatonin from serotonin is stimulated by β-adrenergic activation or darkness, and inhibited by β-adrenergic blockage or light (Oxenkrug et al., 1985). It appears that the adrenergic control point is past the photoperiod control point as determined by the adrenergic antagonist, naloxone (Sugden et al., 1985). Naloxone reduces melatonin concentrations in the blood in the face of darkness-induced increases in SNAT activity (Lowenstein et al., 1984). Neurons with cell bodies in the superior cervical ganglia (SCG), synapse on pinealocytes releasing a β-adrenergic substance, norepinephrine, in response to the phtoperiod (Bowers et al., 1984; Coburn, 1984). If the SCG is excised (SCGx), melatonin is still produced by the pineal but not in phase with the photoperiod (Deguchi and Axelrod, 1972). SCGx

animals lose the ability to measure the length of night and day and have subsequent disturbances in reproductive function (sheep: Lincoln, 1979; ferret: Donovan and VanderWerff ten Bosch, 1956; Herbert, 1967). For example, ganglionectomy in female mink suppressed the stimulatory role of long days and the inhibitory role of short days on post-mating prolactin increases (Martinet et al., 1985). (Prolactin in mink activates the corpora lutea with resulting increases in progesterone causing the termination of embryonic diapause and subsequent implantation). Afternoon injections of melatonin to pineal- and SCG- intact bred mink in long days simulated short photoperiod since secretion of prolactin as reduced and implantation was delayed (Martinet et al., 1983).

Synthesis of melatonin can also be controlled by gonadal hormones. The enzyme, HIOMT, is very sensitive to steriod hormone feedback while SNAT is relatively less so. HIOMT activity in the pineal paralleled the presence of melatonin metabolites in rat urine during proestrus (high estrogen) (Cardinali and Vacas, 1978). However, no such changes in HIOMT were seen during the estrous cycle of the hamster, another nocturnal species implying no estrogen negative feedback mechanism in that species (Rollag et al., 1979). In cultured male rat pineal gland, both testosterone and estradiol increase melatonin synthesis (Daya and Potgieter, 1985). The interrelationship between

hormone-related events and the light/dark cycle of activity of the organ is not yet fully understood but the hormone effects are generally subsidiary to circadian photoperiodic forces.

### 3.2.3) Effects of Melatonin and Pinealectomy on Reproduction

Early research on the function of the pineal gland consisted primarily of removing the gland and noting the subsequent effects. Apparently the investigators expected immediate results like those seen after hypophysectomy. The central tenet guiding researchers was that the pineal and melatonin had negative effects on reproduction, an "antigonadotropic action." The experiments designed to test it were inadvertently nonbiological and led to confusing, inconclusive results (Reiter, 1980b). Reiter (1973a) summed up their conclusions by stating that "pinealectomy is a notoriously unreliable means of illustrating the gonad-inhibiting activity of the pineal gland" and "in some instances has undetectable effects on the reproductive system."

Melatonin sometimes enhanced gonadal development and prompted some scientists to declare it had "counter-antigonadotropic" effects (Hoffman, 1974). Reiter (1980b) offered an explanation for the confusion in a review article illustrating that the method of melatonin administration, the time of day it is administered, and the photoperiodic

history of the experimental animals all effect the hormone's action on the reproductive system. Briefly, it is as follows (the hamster is the model species):

1) Method of Administration: Subcutaneous (SC) implants of melatonin given to males or females completely negates the inhibitory effects of short days producing the counter- antigonadotropic action. It is likely that this response is abnormal since never in nature is an animal exposed to the continous presence of melatonin.

2) Time of Administration: Injections of melatonin given late in the day during stimulatory long photoperiods cause gonadal regression, whereas those given early in the day do not. This discrepancy can be explained by the change in sensitivity in melatonin receptors during the day (Tamarkin et al., 1976; Chen et al., 1980). After a long period of melatonin exposure (all night) the receptors are "down-regulated" and cannot respond to more hormone. However, this phenomenon is short-lived, and by late afternoon the receptors can function again.

3) Photoperiodic History: The length of time the animals have been exposed to stimulatory or inhibitory photoperiods, and the inevitability of photorefractiveness, all determine how they will respond to exogenous melatonin treatment.

In the mid-1960's it was discovered that the gonads of hamsters regressed under short photoperiods but that

pinealectomy or SCGx blocked the effect thus demonstrating an antigonadal role for the pineal (Reiter and Hester, 1966). This response has been demonstrated repeatedly by other researchers (Goldman et al., 1979; Benson and Matthews, 1980; Bartke et al., 1983). However, in the closely related Turkish hamster, pinealectomy induces gonadal regression in long days and does not prevent regression in short days demonstrating a progonadal role for the pineal (Carter et al., 1982). But pinealectomy prevented any further gonadal responses to daylength changes in both species. The pinealectomized golden hamster remains reproductively competent in both long and short days, whereas the pinealectomized Turkish hamster is reproductively inactive in either photoperiod (Goldman and Darrow, 1983). Intact Turkish hamsters must have a certain length of light (15-17 hours/24 hours) to maintain testicular function. Daylengths greater than 17 hours or less than 15 hours induces testes involution (Hong et al., 1986). The golden hamster needs only photoperiods greater than 12.5 hours to maintain reproductive function. It is capable of discriminating between 12 and 13 hours of light per day. This intrinsic difference in daylength measuring ability may account in part for the different responses of the two species to pinealectomy.

Another long-day breeder, the Djungarian (Siberian) hamster, has yet another response to pinealectomy. The

testes will recrudesce within 9 to 12 weeks after pinealectomy whether on short or long days, indicating an antigonadal role for the pineal. However, intact animals exhibit testicular regrowth after 6 weeks on long days, suggesting a progonadal role of the pineal (Goldman et al., 1982).

In the short-tailed weasel, subcutaneous implants of melatonin for 3 or 4 weeks under long days were sufficient to stimulate short-day conditions and cause gonadal regression (Rust and Meyer, 1969).

As discussed above, it is afternoon injections of melatonin that are the most successful in mimicking short days and inducing testicular regression (Tamarkin et al., 1976). Sisk and Turek (1982) demonstrated in hamsters that melatonin, like exposure to short days, increased the sensitivity of the hypothalamus and pituitary to negative feedback by testosterone. They postulated that this was the means by which melatonin had its antigonadal effects. If hamsters were actively immunized against melatonin, it would be expected that they would respond as though they were pinealectomized. However, this immunization failed to negate the antigonadal effects of the pineal in hamsters exposed to a short photoperiod (Brown et al., 1981).

Anestrous ferrets pinealectomized in the fall did come into estrous in the spring although several weeks later

than controls (Herbert, 1969). It was in the second year after surgery that the pinealectomized ferrets became greatly asynchronized from the controls (Herbert, 1972). When the pinealectomy occurred when animals were estrual, the surgery did not cause estrus to cease prematurely but in combination with melatonin injections to stimulate short days, or short days, estrus was shortened (Thorpe and Herbert, 1976). It appears that the pineal gland of the estrus ferret is involved with termination of estrus only when the animal is exposed to what it perceives as a short photoperiod.

Some wildlife species that have responded to melatonin treatments with gonadal regression as they would to short days include the white-footed mouse (Petterborg and Reiter, 1980), the mountain hare (from Scandinavia) (Kuderling et al., 1984), and the pallid bat (Beasley et al., 1984). Melatonin treatments caused wildlife short-day breeders such as the the white-tailed deer (Bubenik, 1983) and red deer (Lincoln et al., 1984; Adam et al., 1986) to become reproductively active. The breeding season of the domestic sheep, a short-day breeder, can be hastened by exogenous melatonin treatments (Nett and Niswender, 1982; Arendt et al., 1983; Lincoln and Ebling, 1985). When pinealectomized ewes were studied, it was discovered that it was the duration of the melatonin infusion, rather than maximum or

overall concentration, which was the critical parameter for inducing reproductive changes (Bittman et al., 1983; Yellon et al., 1985). Carter and Goldman (1983a) reached similar conclusions based on their work with the Djungarian hamster.

### 3.3) Interaction of Eyes with Pineal Gland

There are a number of parallels in the embryological development and the biochemistry of the eye and the pineal gland in mammals. Both are evaginations of the diencephalon and have some similar cellular structures. In fact the pineal gland in very young rat pups may be able to detect photoperiod changes without the benefit of eyes (Erlich and Apuzzo, 1985). The retina can synthesize melatonin and has a diurnal rhythm of SNAT (Wiechmann, 1986). However, since pinealectomy is not a natural occurrence, it seems unlikely that the retina serves as a major endocrine organ. Small amounts of Melatonin has been detected in pinealectomized rats and hamsters as well as those species naturally lacking a pineal, alligator and armadillo.

The pineal gland contains rhodopsin kinase, an enzyme previously thought to be restricted to the retina. The enzyme's activity in the pineal is 68% of the level found in the eye (Somers and Klein, 1984). Other biochemical similarities between the eye and the pineal

include hydroxyindole-O-methyltransferase (HIOMT) activity (Cardinali and Rosner, 1971) and the presence of an S-antigen (Kaslow and Wacker, 1977).

The similarities between the eye and the pineal gland in mammals are surprising since the major role of the eyes is very different from that of the pineal. However, both are part of the pathway which mediates the effects of photoperiod on reproductive endocrine responses.

Blinded adult hamsters exhibited gonadal regression 9 weeks after surgery although pinealectomized or blinded + pinealectomized animals did not. However, after 27 weeks only the gonads of the blinded animals recrudesced (Reiter, 1969). The mechanism for the involution is not fully understood but is hypothesized to involve a reduction in prolactin (Prl) and /or luteinizing hormone (LH) release from the pituitary in the blind animals (Reiter and Johnson, 1974). Blind and pinealectomized (or superior cervical ganglionectomized) animals had no reduction in Prl or LH, but blind but pineal-intact white-footed mice gonadal atrophy ia associated with increased melatonin and decreased secretion of LH-releasing hormone (Petterborg and Paull, 1984).

An interesting observation concerning eye-pineal interactions with reproduction was made with the golden hamster mutant, anophthalmic white. These animals are genetically eyeless and are reproductively infertile.

Their gonads are arrested at an immature stage of gametogenesis. However, after pinealectomy fertility was restored (Hagan and Asher, 1983). In this case, the pineal was antigonadal (prevented attainment of full reproductive competence) even though the lack of ocular photoreceptors was genetic and present before birth. This phenomenon is not observed in blind-from-birth humans, but humans are generally insensitive to photoperiod and require very bright light to suppress the circadian rise in melatonin (Lewy and Newsome, 1983).

Within one year after blinding, blinded mink came into estrus at the same time as the intact controls (Thomson, 1954). But later researchers indicated that asynchrony of reproduction in that blinded or pinealectomized animals does not develop for at least 2 years after surgery (Herbert et al., 1978). Some ranch-raised mink experience a progressive retinal degeneration causing blindness (Hadlow, 1984). This has potentially serious negative effects on reproduction causing affected animals to be culled early from the herd.

Pineal-intact, blind ewes continue to follow a circannual reproductive cycle with or without the presence of a sighted ram (Legan and Karsch, 1983). Blinded or pinealectomized cattle exhibit seasonal changes in Prl secretion independent of temperature (Petitclerc et al., 1983b). There seems to be an endogenous control of

seasonality that is partially independent of photic input to the eyes or hormonal signals from the pineal.

### 3.4 Interaction of Hypothalamus with Pineal Gland

In a review article, Kappers, Smith and deVries (1974) discussed the pineal gland's control of hypothalamic activity. They concluded that "the pineal is not a prime mover but a regulator of regulators, or an important center for general homeostasis, very probably exerting its influence primarily on that most important center of integration of vegetative as well as of the cerebrospinal nervous system: the hypothalamus." Since then many of the details as to how the pineal influences the hypothalamus have been elucidated.

Receptors for melatonin have been found in the hypothalamus of rats (Niles et al., 1979). Further studies have localized the receptive areas to the suprachiasmatic nucleus (SCN) and the paraventricular nucleus (PVN) which is on the route from the SCN to the spinal cord and the superior cervical ganglia. Implants of physiological amounts of melatonin into the SCN caused gonadal regression in female white-footed mice within 7 weeks but implants in other areas of the brain had no effect (Glass and Lynch, 1981). Lesions to the SCN prevent male hamsters from responding reproductively to photoperiodic stimuli (Rusak and Morin, 1976). In fact,

pinealectomized rats with SCN lesions did not respond to daily melatonin injections while pinealectomized animals with intact SCN did (Cassone et al., 1986) providing further evidence for the vital interconnection between the pineal and the SCN. The SCN has sometimes been referred to as the "endogenous circadian clock" in the control of reproduction.

Lesions (Pickard and Turek, 1983) and electrical stimulation (Reuss et al., 1985) of the PVN also block short-day induced testicular regression in hamsters and lead to a loss of the nighttime increase in melatonin (Klein et al., 1983).

Mink in short days for 3 months developed greater and more active nuclear volumes and secretory structures in the SCN than mink in long days (Yurisova and Klockov, 1979).

These nucleui, especially the SCN, are critical for the physiological response to photic input to the eyes and mediated by the pineal gland. The mechanism and neural pathways between the SCN, LHRH production, and the gonadotropins has yet to be determined. There is still a great deal to learn before the reproductive response to photoperiod is fully understood.

## 4.0) Photoperiod Control of Puberty and Recurring Cycles

### 4.1) Neural Input and the Pubertal Process

For this discussion the working definition, although general, will be that of Bronson and Rissman (1986) which states ". . . puberty is simply the period of accelerated reproductive development that culminates in functional fertility, as assessed both physiologically and behaviorally."

The prepubertal animal has small, infertile gonads, little sexual interest, and no secondary sexual characteristics. The testes and ovaries produce very small amounts of testosterone and estradiol with no gametes while the pulsatile secretion rate of LHRH and LH from the brain is very slow although the amplitude of the pulses is very large (Root, 1973). Usually a pulse of LH is followed by a pulse of steroids.

As the animal grows older the hypothalamic-pituitary axis matures and becomes less sensitive to the negative feedback effects of the gonadal steriods. As the frequency of gonadotropin pulses increases, the baseline of secretion rises (more hormone is released overall) but the amplitude of the pulses is smaller. This process has been termed "gonadstat resetting" and was first hypothesized by Ramirez and McCann (1963).

The gonadostat theory of puberty has been studied intensively in a few species and thus far has not been

disproven in describing the neural process. The theory describes the changes in the hypothalamic-hypophyseal axis during the onset of the annual breeding season in mature animals. The changes were first described in detail in the female lamb (Legan et al., 1977), and later by Ryan and Foster (1980) then subsequently strongly supported by studies in the male and female rat (Smith et al., 1977; Bhanot and Wilkinson, 1983; Bourguignon and Franchimont, 1984; Matsumoto et al., 1986; Nash et al., 1986; Ojeda et al., 1986), the hamster (Sisk and Turek, 1983), the pig (Elasaesser et al., 1978), the heifer (Day et al., 1984), the male lamb (Olster and Foster, 1986), and the ferret (Ryan, 1984; Ryan and Robinson, 1985; Sisk, 1986).

The increased secretion of gonadotropin near puberty stimulates production of steriod only if the gonads are mature enough to respond (Ojeda et al., 1983a). The brain initiates the sequence of changes leading to puberty, but the gonads complete this maturational process by responding and setting up the hypothalamic-pituitary-gonadal interactions seen in the adult (Ojeda et al., 1980). There does not seem to be a specific physiological "trigger" for puberty but it is more the result of a gradual developmental process.

Aside from photoperiod influence, the time of puberty can be negatively influenced by poor nutrition. Underfed heifers (Petitclerc et al., 1983a), lambs (Foster and

Yellon, 1985), and rats (Holehan and Merry, 1985; Bronson, 1986) will reach puberty later than ad lib fed animals even under a stimulatory photoperiod. However, puberty cannot be delayed indefinately and will eventually occur even in very thin females, but then there may be no further reproductive cyclicity (Bronson and Rissman, 1986). In males, an energy deficit does not appear to be a major factor.

### 4.2) Involvement of the Pineal in the Photoperiodic Initiation of Puberty

As discussed in Part 3.0, the pineal gland is the major neuroendocrine organ that responds to photoperiod, releasing melatonin during darkness but not in light. Therefore, in prepubertal, seasonally breeding mammals, it is the pineal that "codes" for either increasing daylength (stimulatory to "long-day breeders") or for decreasing daylength (stimulatory to "short-day breeders").

Naturally-occurring nocturnal melatonin pulses are infrequent and of small amplitude in the prepubertal lamb. However, as the fall breeding season approaches the frequency and amplitude greatly increase with the largest amount secreted nocturnally the last part of October (Rodway et al., 1985). The importance of this melatonin code in the proper timing of puberty is demonstrated by the fact that pinealectomized lambs experienced puberty 3

months later than intact controls (Kennaway et al., 1985). Puberty did occur but during the second year the pinealectomized ewes were out-of-synchrony with the photoperiod and with the control animals. If lambs are superior cervical ganglionectomized (SCGx) thereby denervating the pineal gland at an early age (6 to 8 weeks), they do not achieve puberty until 65 weeks as compared to 34 weeks for intact controls. Infusing melatonin into SCGx lambs to stimulate long then short days causes puberty to occur with that of controls (Yellon and Foster, 1986).

A practical use of melatonin to advance puberty in the sheep was attempted by Nowak and Rodway (1985). They found that intravaginal implants in 19-20 week old lambs initiated puberty 4 weeks before controls. Implants in younger lambs (7 weeks) did not hasten puberty. This demonstrates that continuously available melatonin mimicked short days in lambs with a brain-gonadal axis mature enough to reproductively respond.

One of the first actions of exogenous melatonin that has been described was the delay of puberty in rats (Chu et al., 1964). Rollag et al. (1982) found that afternoon melatonin injections in golden hamsters did not prevent sexual maturity but once it was attained, melatonin caused rapid gonadal regression. Young Djungarian hamsters raised in short days showed rapid testicular growth when transferred to long days. If they were pinealectomized,

this growth did not occur without melatonin infusions simulating long days (Carter and Goldman, 1983a). The time relative to the onset of light melatonin is administered is of crucial importance due to circadian changes in melatonin sensitivity (Rivest et al., 1985).

As with hamsters (Reiter and Hester, 1966), pinealectomy abolishes the negative effects short days have on reproduction in the prepubertal white-footed mouse (Johnston et al., 1982) and they also exhibit a circadian sensitivity to light and consequently, to melatonin (Whitsett et al., 1984a). Whitsett and co-workers (1984a,b,c,d) found that exogenous melatonin delayed puberty in mice reared in long days. Exogenous melatonin did not effect age at puberty of mice reared in short days. Interestingly, exposure of short day and/or melatonin-treated males to adult females after weaning hastened sexual development so that puberty was not delayed in the males.

The clinical use of melatonin to treat disturbances in puberty has yet to be attempted in humans but there are reports of the normal, non-pathological changes in melatonin concentrations near puberty. Silman et al. (1979) attempted to determine the changes in serum melatonin concentration during the day in prepubertal children and in those in various stages of pubertal development. They found that with the onset of pubertal development in boys,

there was an abrupt drop in melatonin that remained low during later developmental stages. No such relationship was found in girls. Penny (1982) measured overnight urinary excretion of melatonin in similarily age-grouped children and found that melatonin metablites increased with advancing pubertal development but was very low in adults, both male and female. To further confuse the situation, Waldhauser et al. (1984) reported that although daytime serum melatonin concentrations did not change with sexual maturation, nighttime levels did decrease with development. One explanation could be that serum levels of melatonin decrease with age because clearance from the body and excretion rates are high until adulthood when both serum and urine levels decline.

Melatonin injected in mid-afternoon caused an increase in prolactin in pubertal but not prepubertal children. Decreased growth hormone occurred in the prepubertal group but no change was detected in the pubertal children. There was also no change in LH, FSH, or TSH in either group (Lissoni et al., 1986). There appears to be a change in the pituitary to melatonin with pubertal development but not all of the hormones respond in this particular experimental design.

## 5.0) Concluding Remarks

The future of pineal gland research looks very bright. No longer should it be ignored as a regulator of reproductive physiology, especially when manipulations of the photoperiod are part of the experimental design. The role of the pineal gland may vary among species and thus there would be some initial confusion. However, as more is learned the more we will understand about the control of reproduction by photoperiod.

# INTRODUCTION

Hamsters and sheep have been extensively used in photoperiod and reproduction research. Consequently, a good deal of information has accummulated about their response to changes in photoperiod. However, to fully understand the physiology of photoperiodicity, and the reproductive response to light in particular, more species need to be studied in more depth (Bronson and Rissman, 1986). Only then can an accurate general scheme about mammalian photoperiodism be developed.

Carnivores are an ecologically important order of mammals since they usually reside at the top of the food chain. They are also raised or hunted by humans for skins, or to provide a service (eg. domestic dogs and cats). They are, unfortunately, a neglected lot when it comes to understanding their reproductive responses to photoperiod. The European ferret (Mustela putorius furo) is one of the few carnivores which has been studied to any extent. Limited research on the economically valuable mink (Mustela vison , has been done but it has centered primarily on how photoperiod effects the pelt (Martinet et al., 1984; Rose et al., 1984, 1985) or litter size (Hammond, 1951; Holcomb et al., 1962; Aulerich et al., 1964; Duby and

Travis, 1972; Pilbeam et al., 1979). Most of what is known about mink is in an uncontrolled setting and anecdotal. There has not been much basic research on the mink using it as a model species. Therefore, in this study, an attempt was made to increase what is known about photoperiodicity and reproduction in mink with special attention to how the pineal gland is involved. Perhaps later, practical applications can be made to aid the fur farmer.

## MATERIALS AND METHODS

Objective:

To further understand how information about photoperiod is processed in the central nervous system of a model carnivore, the mink.

Specific Hypothesis:

Alteration of the prepubertal mink's ability to physically receive or encode photoperiodic signals due to removal of the eye and/or disruption of the pineal gland melatonin production pattern results in disturbances in puberty and the annual reproductive cycle.

Statistical Design:

The original statistical design was a complete block with repeated measurement (Gill, 1978) (Table 1). However, due to death losses among the females, their data was analyzed using a different design which deleted litter pairs (Table 2). Thirty-six brown-eyed prepubertal (7-8 months old) pastel mink of each sex from 18 different litters were divided into six surgical treatment groups comprised of three litters per treatment. The surgical treatment groups (twelve animals/group) were either bilaterally blinded, bilaterally superior cervical

Table 1: Main statistical design of a double split plot with sex effect analyzed separately.

| Sources of Variation | df |
|---|---|
| Treatment | 5 |
| Litter pair/treatment | 12 |
| Housing | 1 |
| Treatment x housing | 5 |
| Litter pair/trt. x housing | 12 |
| Bleed | 32 |
| Bleed x treatment | 160 |
| Litter pair/trt. x bleed | 344 |
| Housing x Bleed | 32 |
| Bleed x trt. x housing | 160 |
| Litter pair/bleed x trt. x housing | 424 |
| Total | 1187 |

Table 2: Design for analyzing unbalanced data for females by surgical treatments and housing.

| Sources of Variation | df |
|---|---|
| (within housing) | |
| Treatment | 5 |
| Animal/Treatment | 6 |
| Bleed | 32 |
| Bleed x Treatment | 160 |
| Animal/Bleed x Treatment | 853 |
| Total | 1055 |

| Sources of Variation | df |
|---|---|
| (within treatment) | |
| Housing | 1 |
| Animal/Housing | 21 |
| Bleed | 32 |
| Housing x Bleed | 32 |
| Animal/Bleed x Housing | 853 |
| Total | 1055 |

ganglionectomized (SCGx), blinded and SCGx, sham SCGx, blinded and sham SCGx, or intact controls. Then half of the animals in each surgery group were put in one of two types of housing: inside in lighting-controlled chambers, on naturally-occurring photoperiod, or outside in open sided sheds under commercial mink ranch conditions.

The animals chosen for this study were from litters containing at least two males and two females to form littermate pairs of the same sex. All the animals selected from the same litter were given the same surgical treatment and then placed inside or outside, one male and one female assigned to each type of housing.

The specific contrasts were body weight, size of external genetalia and serum concentrations of steriod hormones within each sex and type of housing for 1) intact vs. blinded, 2) SCGx vs. sham SCGx, and 3) SCGx + blinded vs. sham SCGx + blinded. Specific example: concentrations of testosterone in the blinded males would be contrasted with that of the sighted, intact males. Or, the testes volume of the SCGx males would be contrasted with that of the sham SCGx males. The assumption was made that the intact and sham SCGx groups would show the same response as would the blinded and sham SCGx + blinded groups.

Animal Care:

The mink were housed individually in 60 x 40 x 40 cm wire cages with sheet metal sides at the Michigan State University Experimental Fur Farm. Each cage contained an attached wooden nest box (30 x 30 x 45 cm). The mink were fed a conventional (wet) diet composed of ground whole chicken, commercial mink cereal, ocean fish scrap, beef tripe, liver, trimmings and lungs (Bleavins and Aulerich, 1981). Enough water was added to give the mixture the consistancy of hamburger and it was placed on a wire feed grid on the top of the cages. Unconsumed feed was removed daily before new feed was given. Food and water were provided ad libitum.

The animals housed outside were exposed to ambient temperatures which in central Michigan (42° 42' N latitude, 84° 28' longitude) range from -10° C in January to +30° C in July and to photoperiods of 9 hours/day on December 22 to 15 hours/day on June 21. The animals housed inside were not exposed to such temperature extremes although the photoperiod closely approximated the natural light/dark cycle at an intensity of 160- 220 lux provided by 100 watt incandescence light bulbs.

General Surgical Procedures

All animals received their surgical treatments between November 15, 1984 and December 20, 1984, the year of their

birth. All surgeries were performed under antiseptic conditions using 70% ethanol as a disinfectant. The skin in the surgical area was closely shaved and scrubbed with Betadine surgical scrub. The animals were under ketamine-xylazine induced anesthesia at a dosage of 100 mg ketamine (Vetalar, Parke-Davis) containing 2% by volume xylazine (Rompun, Haver-Lockhart)per kg body weight injected intramuscularily in the thigh. Although this dosage was four times greater than that recommended by Moreland and Glaser (1985), it was found to be necessary in order to maintain a plane of anesthesia deep enough to permit optical enucleation. The average time to initiation of recovery after surgery was 60 minutes. Although longer than necessary, it proved beneficial in that it provided enough time for good clotting and fibrin formation which was then not easily dislodged during the animal's involuntary movements during recovery. The animals were allowed a 1 to 7 day recovery period in a warm room where they were observed 5 to 6 times per day before being returned to their cages.

Specific Surgical Procedures

1. Bilateral Optical Enucleation.

After preparing the area around the eye (shaving and scrubbing) of an anesthetized animal careful cuts with a scapel were made just above the upper and below the lower

eyelid to remove the ribbon of skin containing the eyelashes. Using forceps and then hemostats, the lids were retracted and the eyeball nearly pulled out of the socket by its lower musculature. A loop of 4-0 plain gut suture was slipped over the eyeball and used to ligate the blood vessels and nerves behind the orb. Once ligated the eyeball was cut free just distal to the ligature with a scapel and removed. A 1 cm square piece of sterile Gelfoam (Johnson & Johnson) was then inserted into the eye socket to control any bleeding. The eyelids were sutured together with 4 to 5 stiches of 4-0 PDS suture (monofilament, absorbable, Ethicon) using an interrupted suture pattern. Any bleeding that occured was easily controlled by direct pressure with cotton swabs or gauze pads. The surgical site was wiped gently with a gauze pad dipped in clean saline to remove any clotted blood from the area. The procedure was repeated for the other eye. Two females developed blocked tear ducts from the surgery and their eye sockets repeatedly became swollen and sometimes infected. They were given antibiotics and the fluid behind the eyelid was expressed. During the last half of the study the problem did not recur.

2. Bilateral Superior Cervical Ganglionectomy (SCGx).

This ganglia, located lateral to the esophagus between the manidibles, separates from the cervical sympathetic trunk of the vagus nerve near the posterior ganglia of the

vagus (Field and Taylor, 1969). After the usual surgical site preparation on an anesthetized animal, an incision was made lateral and parallel to the esophagus. When the cutaneous connective tissue and musculature was teased away, the large white vagus trunk was easily seen. It was followed anteriorly to the branch point. The SCG is the more lateral of the two ganglia in that area. It was lifted carefully out of its bed using glass Pasteur pipettes so as not to stretch the nerve, then the nerve on either side of the ganglion was cut and the ganglion removed. Little or no bleeding occurred during the surgery. The skin incision was closed with 4-0 PDS suture using an interrupted pattern. Any disturbed muscles were not sutured since they were usually separated by teasing and had not been cut. The site was gently swabbed with clean saline and the procedure repeated on the other side.

3. Sham Superior Cervical Ganglionectomy.

This procedure was the same as SCGx except that the ganglia were only manipulated gently with glass instruments but were not excised.

4. Combination Surgical Treatments.

Blinding + SCGx and Blinding + Sham SCGx were the combination treatments. The procedure for each surgery was as outlined above with the ganglia surgery or manipulation occurring before the blinding.

Collection of Blood

Blood samples were taken by jugular venipuncture every two weeks beginning just before surgery and continuing through the second breeding season in 1986. The animals were lightly anesthetized with an intramuscular injection of the ketamine-xylazine mixture described above at a standard dosage of 0.2 ml for the males and 0.1 ml for females which approximated 10 mg ketamine/kg body weight. This dosage was derived by trial and error and represented a compromise between the level of sedation needed for blood collection and a rapid recovery from anesthesia. The fur in the ventral area of the neck over one jugular vein was shaved to the skin and swabbed with 70% ethanol. Three milliliters of blood were drawn from the jugular vein using a 3 cc syringe and a 20 gauge 1" needle. The blood from each animal was allowed to clot in individually labeled 12 x 75 mm test tubes at room temperature then refrigerated at 4° C overnight. The serum was collected after centrifugation at 1800 x g for 20 minutes the next morning. The serum was frozen at -20° C until assayed for testosterone (males) or estradiol (females).

During the last two-thirds of the study a few animals, independent of treatment, began to exhibit seizures during anesthesia. The seizures were much more violent than the usual resistance to anesthesia, of longer duration, and became worse during subsequent blood collections. Two

animals never recovered and died while under anesthesia. A third animal that failed to recover from anesthesia did not exhibit any unusual seizure activity.

Physical Measurements

After each blood collection several physical measurements were taken. The scrotums of the male mink were palpated, and if testes were located, their length (L), width (W), and thickness (T) were measured with calipers through the scrotal wall. A mean testes volume (TV) was calculated for each animal using a modified equation for a solid elipse (Boisson-Agasse et al.,1982):

$$TV = 4/3 \times L/2 \times W/2 \times T/2$$

The vulvae of the females were examined to determine the amount of estrual swelling or edema. The degree of edema was scored on a scale of 0-3 with zero representing the smallest size and three the largest as described by Travis et al.(1978). The size of testes and the extent of vulvar edema were used as external indicators of the level of sexual competence.

Body weights were measured to the nearest 5 g and an evaluation of the stage of pelage maturation was made. The condition of the pelage/coat was scored on a scale of 1-5, roughly following the molting pattern outlined by Travis and Schaible (1960). A score of 1 represented animals with extremely mottled, patchy coats with many loose hairs. A

score of 5 was assigned to animals with uniform, fully, prime coats with well attached hairs. The scores were not grading scores indicating the quality of the pelt but were used to indicate the condition, or degree of molting, of the coat.

Reproductive Behavior Evaluation

Male and female animals were paired during their first and second breeding seasons to determine if sexual interest paralleled the physical changes in external genitalia. The frequency of pairing followed standard mink farm practice. The animals were placed together the first of March and if the female was not receptive then she was tested again four days later. When a successful mating as determined by "sperm-checking" was obtained, the female was provided an opportunity for a second mating every four days until the end of the breeding season (March 24-30), or until she was willing to accept the male.

The first year the paired males and females were observed for sexual behavior but they were not permitted to fully copulate since it was thought that a pregnancy and lactation might serve as an endogenous "clock-setter", confounding treatment effects in the female. The pairings were random with regard to treatment but were kept within type of housing. The pair was kept together for at least 20-30 minutes while the observer remained out of sight but

checked on the pairs periodically. The second year all the animals that would mate were allowed to do so. The success of the mating was determined by the presence of sperm in post-coital vaginal smears, "sperm-checking" (Shump et al., 1976).

Males were deemed sexually interested if they grasped the female by the back of the neck while emitting a low "chuckling" sound always given during courtship (Linscombe et al., 1982) and attempted mounting and clasping with the front legs. Females were regarded as sexually receptive if they did not vigorously resist the male or attack him and permitted the grasping and mounting.

Radioimmunoassay (RIA) Procedures

1. Testosterone.

Concentrations of testosterone in serum from males were quantified by the method used by Louis et al. (1973) for progesterone but with modifications as described by Kiser et al. (1978) and following: MSU #74 antiserum to testosterone was diluted 1:10,000 and $^{3}H$-testosterone was used at 5000 cpm/200 ul. Only 50 ul of mink serum were extracted and assayed instead of the usual 200 ul.

2. Estradiol.

Concentrations of estradiol ($E_2$) were quantified with modifications of an assay described by England et al. (1974). Modifications included extracting 200 ul serum in

2 ml anhydrous ethyl ether and vortexing vigorously for 2 minutes. After addition of $E_2$-antiserum (1:200,000 in phosphate buffer with EDTA and 1:400 normal rabbit serum), a 2 hour room temperature and 1/2 hour 4° C incubation, $^{125}I$-$E_2$ in buffer was added to all tubes and the tubes vortexed and incubated 1 hour at 4° C. The bound and free $^{125}I$-$E_2$ was separated by the addition of 1 ml cold Dextran-coated charcoal to each tube (2.5 g washed neutral Norit charcoal with 0.25 g Dextran $T_{70}$ in 1000 ml phosphate buffer). This was followed by a 15 minute incubation at 4° C, and centrifugation at 2,500 x g for 15 minutes at 4° C. The supernatant was decanted and counted in a gamma counter.

# RESULTS

In almost all categories, the responses of the animals to the treatments were so distinct that they could be combined into two groups: sighted and blinded, despite other treatment combinations. The sighted group consisted of superior cervical ganglionectomized (SCGs), sham SCGx, and intact animals. The blind group was comprised of blinded, blinded + SCGx, and blinded + sham SCGx animals. Unless otherwise stated, the results will be pooled and discussed in terms of these two categories.

## Body Weight

Sighted males and females showed circannual fluctuations in body weight independent of housing (Figures 3 & 5). The SCGx females were significantly ($p < .05$) heavier after the first breeding season than the other sighted females. Most of the increase was due to the indoor-housed animals in that group. All of the blinded males had a significant ($p < .05$) reduction in body weight after the first breeding season which then did not increase throughout the rest of the study (Figure 4). The blinded females showed more circannual changes in body weight, although not significant, than the blind males but not as much as the sighted females (Figure 6).

Figure 3. Changes in body weight over time in sighted male mink across housing type (n = 6/treatment).

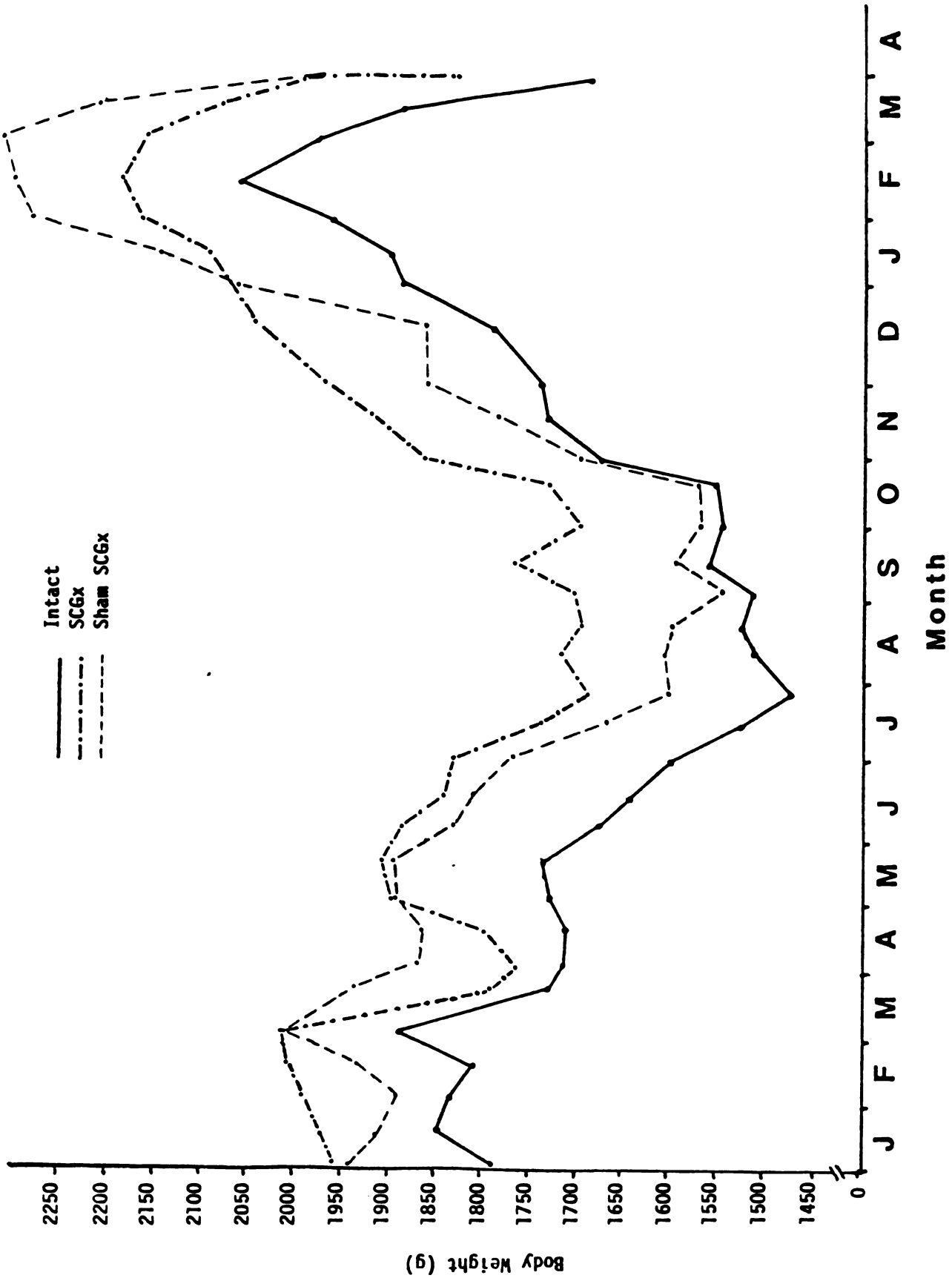

Intact
SCGx
Sham SCGx
Body Weight (g)
2250
2200
2150
2100
2050
2000
1950
1900
1850
1800
1750
1700
1650
1600
1550
1500
1450
0
J F M A M J J A S O N D J F M A
Month

Figure 4. Changes in body weight over time in blinded male mink across housing (n = 6/treatment).

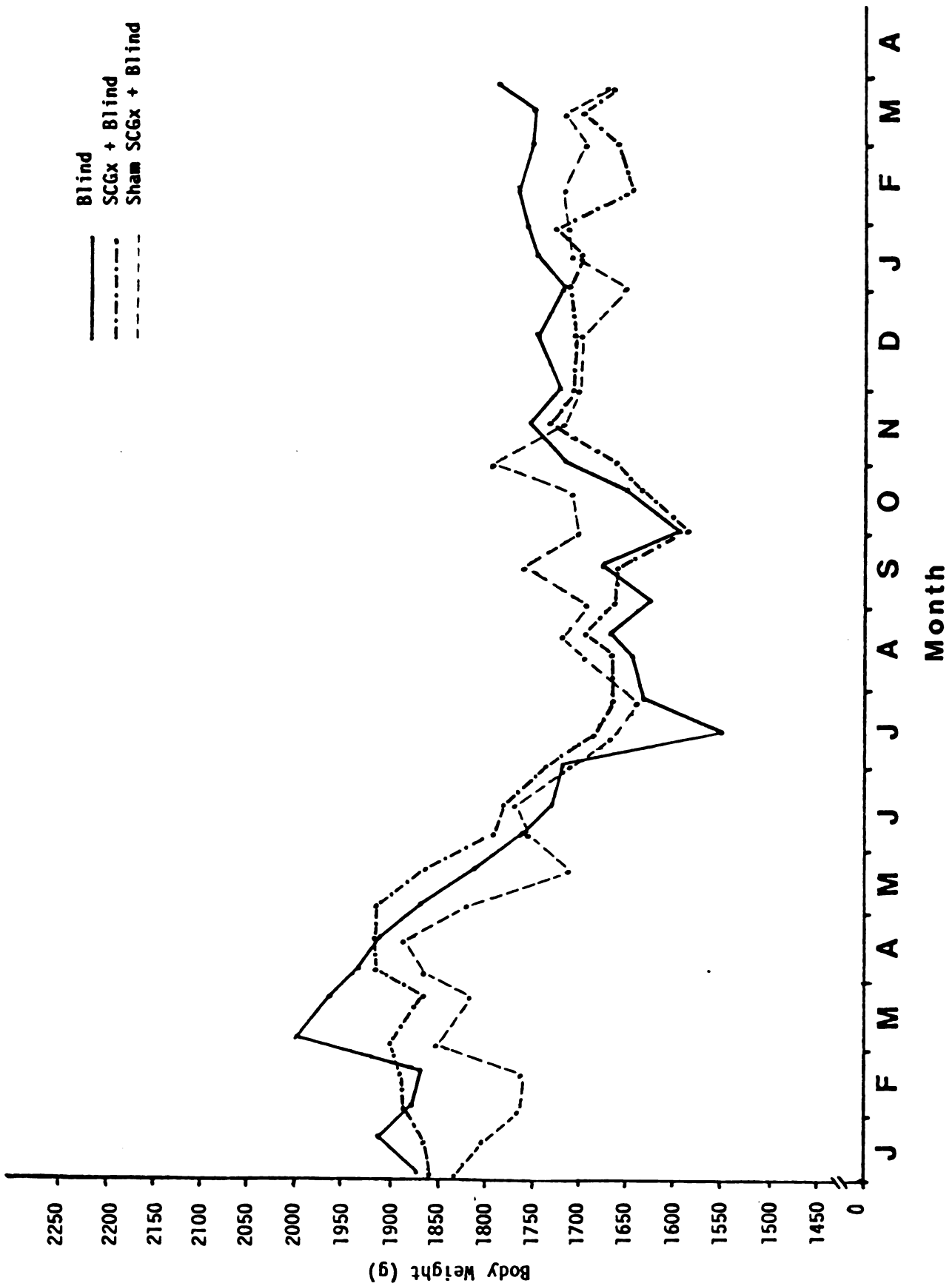

Blind
SCGx + Blind
Sham SCGx + Blind
Body Weight (g)
2250
2200
2150
2100
2050
2000
1950
1900
1850
1800
1750
1700
1650
1600
1550
1500
1450
0
J F M A M J J A S O N D J F M A
Month

Figure 5. Changes in body weight over time in sighted female mink across housing (n = 7 intact, 4 sham SCGx, 6 SCGx).

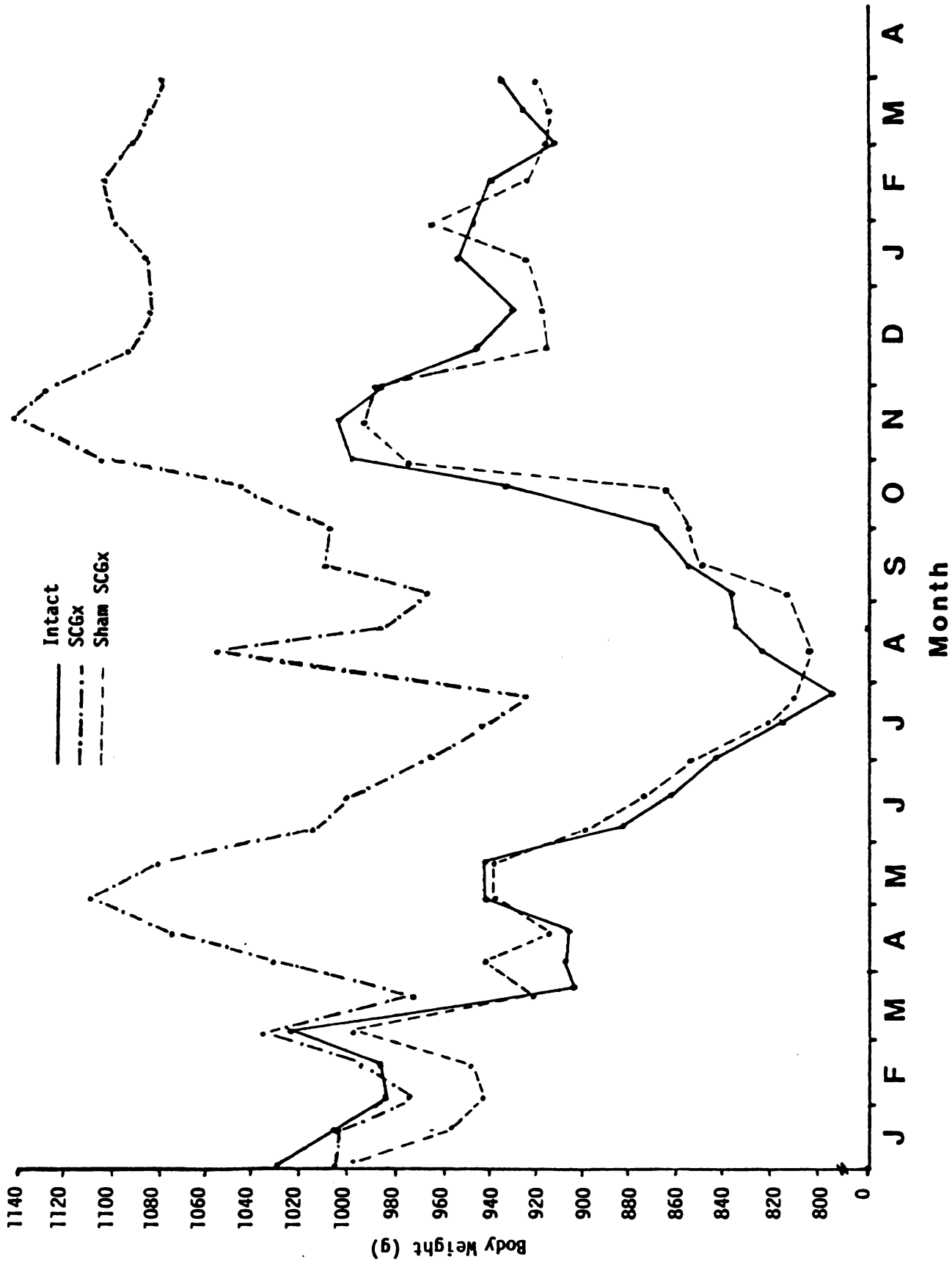
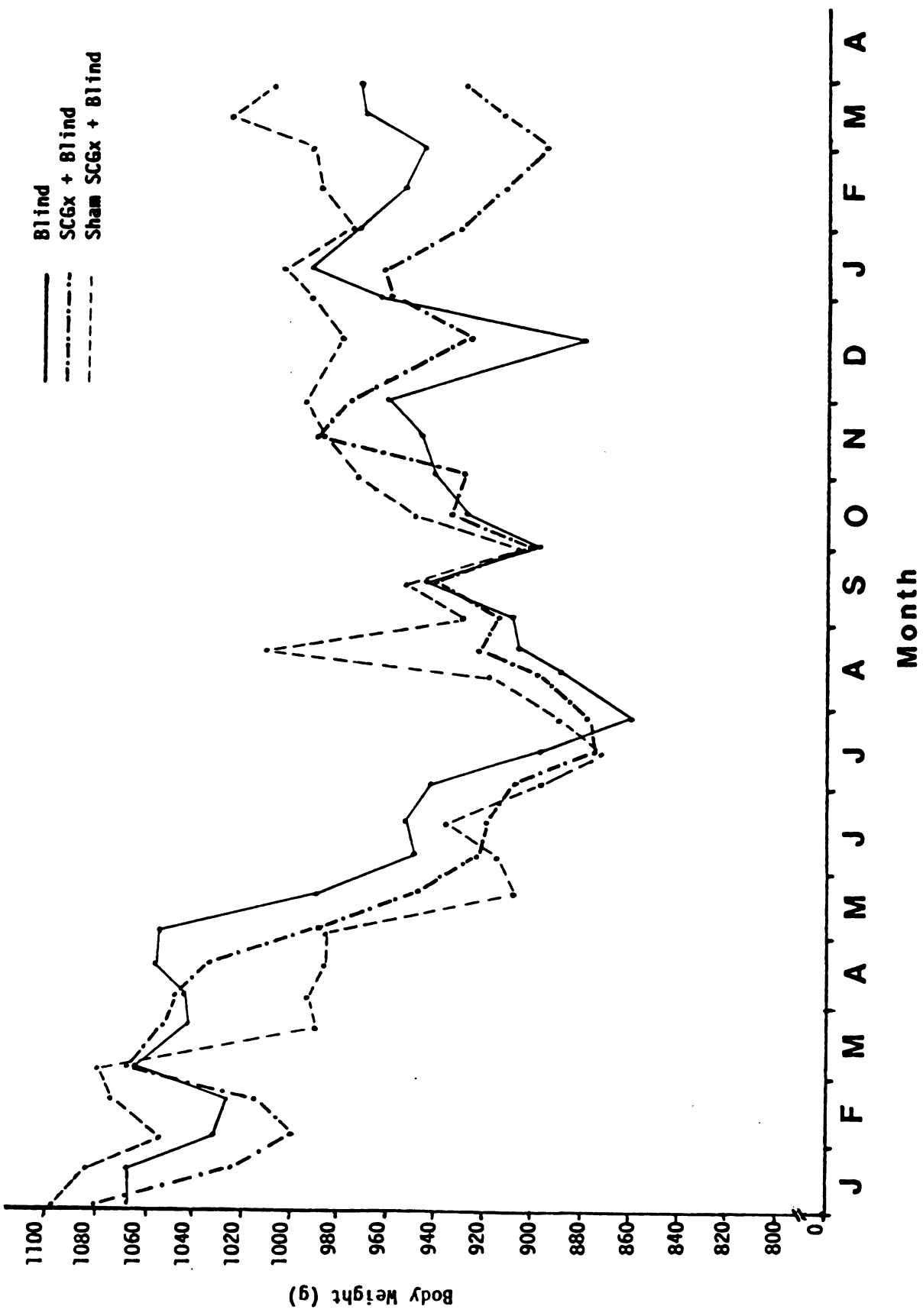

Intact
SCGx
Sham SCGx
Body Weight (g)
1140
1120
1100
1080
1060
1040
1020
1000
980
960
940
920
900
880
860
840
820
800
0
J F M A M J J A S O N D J F M A
Month

Figure 6. Changes in body weight over time in blinded female mink across housing (n = 4 blind, 5 sham SCGx + blind, 5 SCGx + blind).


Blind
SCGx + Blind
Sham SCGx + Blind
Body Weight (g)
1100
1080
1060
1040
1020
1000
980
960
940
920
900
880
860
840
820
800
0
J F M A M J J A S O N D J F M A
Month

External Genitalia

There was an interaction ($p < .001$) between time of bleed and surgery as well as among surgery groups for testes volume. Testes size in sighted males was smallest in June while the blinded males did not achieve minimal size until September (Figures 7 & 8). The SCGx males began testes recrudesence in November, significantly earlier ($p < .05$) than other sighted males that did not start to show regrowth until the end of December. Recrudesence did not occur in blinded males.

There was an interaction ($p < .001$) between time of bleed and surgery as well as among surgical groups for vulvar edema. The blind females maintained enlarged vulvae longer than sighted animals and did not show an estrual swelling the second year (Figures 9 & 10). The vulvar enlargement of the sighted animals in the second year was less than the first.

Steroid Hormones

Testosterone (T) concentrations paralleled and preceded the changes in testes size in sighted and blinded males. There was no effect of housing on T levels but there was an interaction ($p < .001$) between time of bleed and surgery as well as among surgery groups. The blind males had higher T levels for a longer period of time than the

Figure 7. Changes in testicular volume over time in sighted male mink across housing (n = 6/treatment).

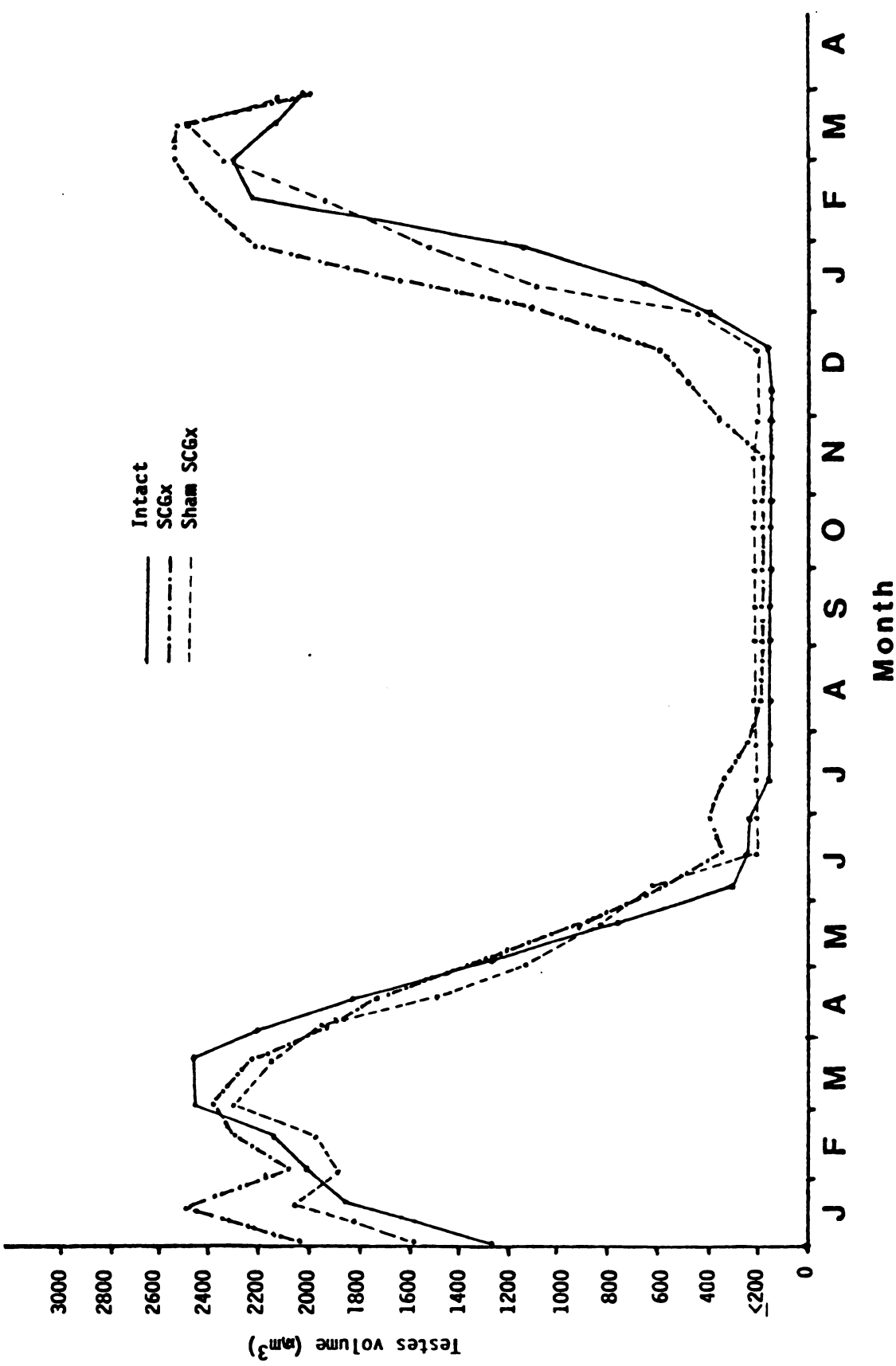

Intact
SCGx
Sham SCGx
Testes volume ($mm^3$)
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
≤200
0
J F M A M J J A S O N D J F M A
Month

Figure 8. Changes in testicular volume over time in blinded male mink across housing (n = 6/treatment).

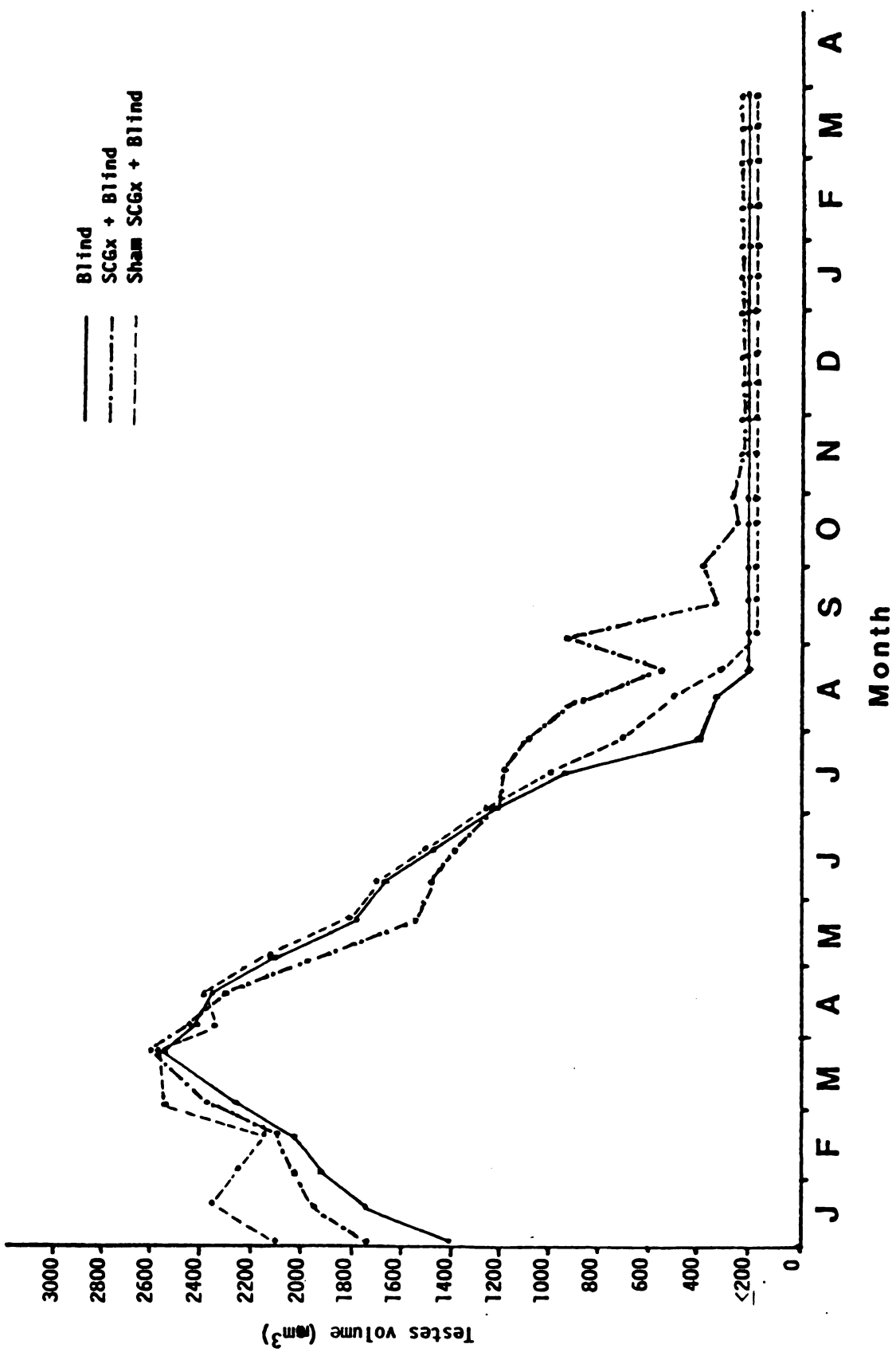

Blind
SCGx + Blind
Sham SCGx + Blind
Testes volume (mm3)
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
<200
0
J F M A M J J A S O N D J F M A
Month

Figure 9. Changes in vulvar edema over time in sighted female mink across housing (n = 7 intact, 6 sham SCGx, 6 SCGx).

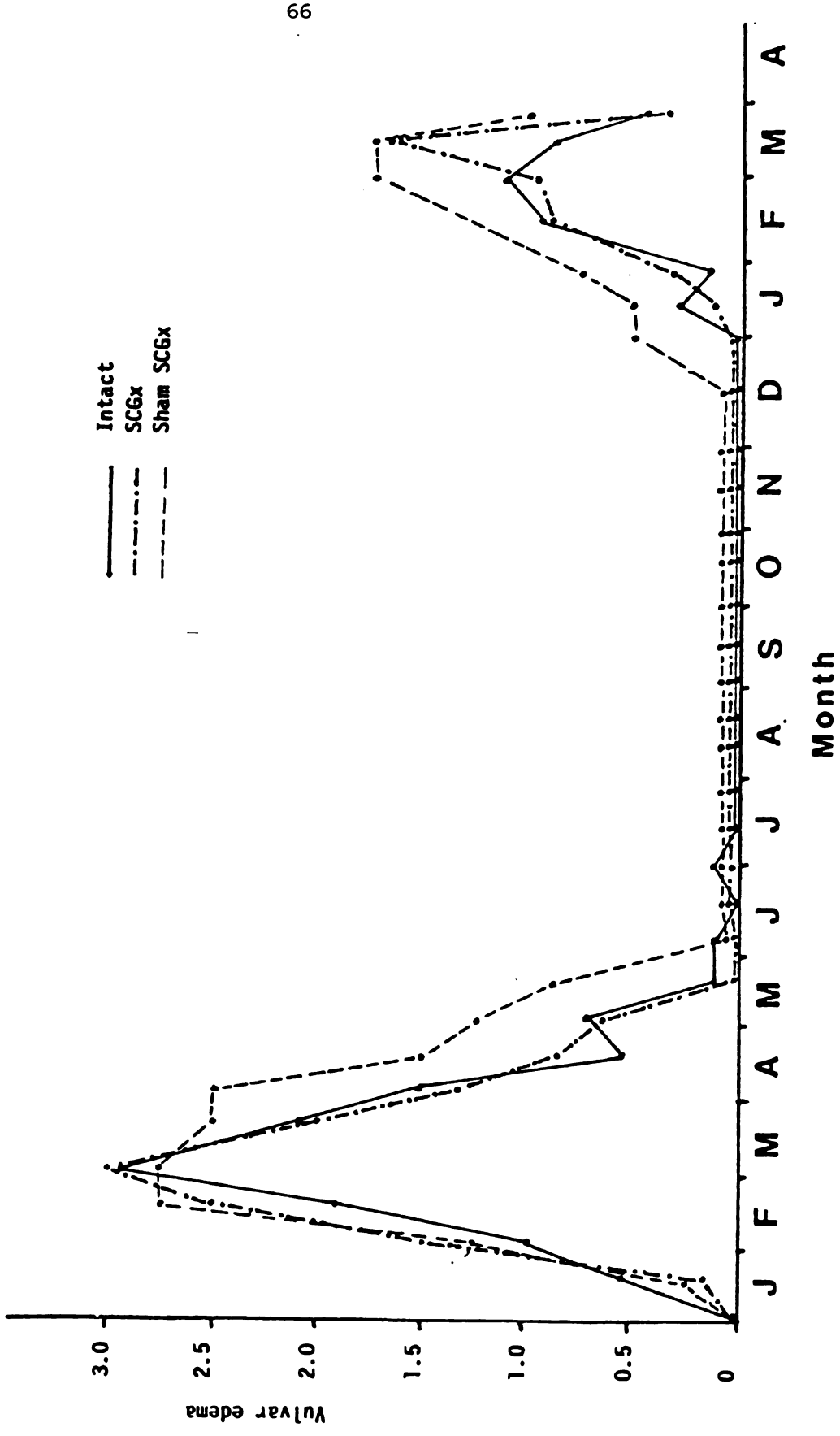
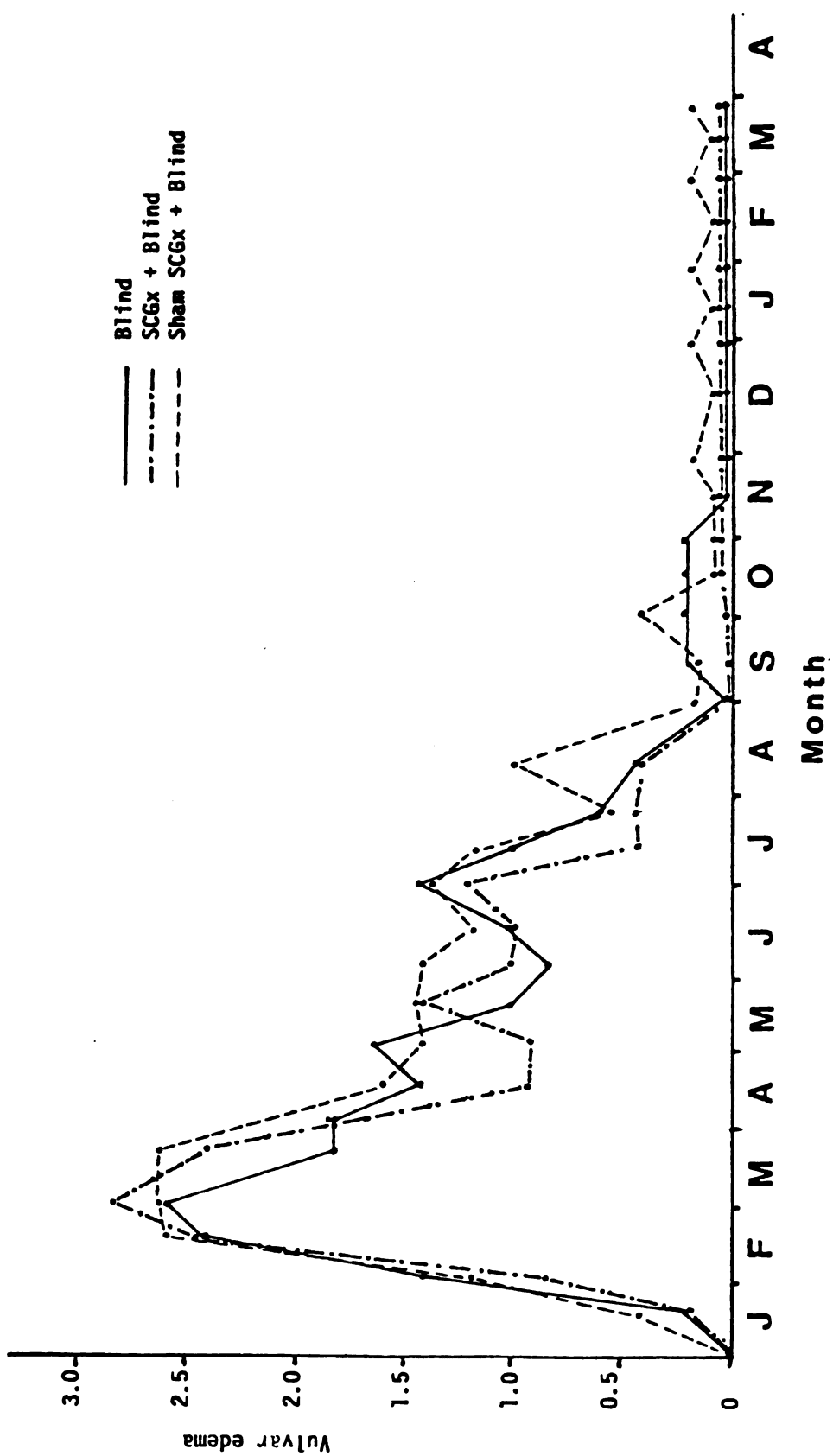

Intact
SCGx
Sham SCGx
Vulvar edema
3.0
2.5
2.0
1.5
1.0
0.5
0
J F M A M J J A S O N D J F M A
Month

Figure 10. Changes in vulvar edema over time in blinded female mink across housing (n = 4 blind, 5 sham SCGx + blind, 5 SCGx + blind).


Blind
SCGx + Blind
Sham SCGx + Blind
Vulvar edema
3.0
2.5
2.0
1.5
1.0
0.5
0
J F M A M J J A S O N D J F M A
Month

sighted males the first year and then showed no increase in T concentrations the second breeding season (Figures 11 & 12).

Estradiol ($E_2$) concentrations did not differ between sighted and blinded groups of females. There was no parallel of $E_2$ with vulvar swelling except perhaps the second breeding season in which $E_2$ titers were higher in sighted animals than in blinded animals (Figures 13 & 14).

Hair Coat

The pelage was not evaluated until about one-third of the way through the study (June 1985). It was noted that the blind animals were not shedding their winter coats and growing summer ones. Blind males and females had coats that were in much poorer condition than the sighted animals throughout the study after the first breeding season ($p< .001$). The coats improved in September and October but they never "primed." The sighted group molted normally and their coats were prime in December during the usual pelting time.

Reproductive Behavior

The first year essentially all the animals showed sexual interest but were not allowed to mate completely. The second year only the sighted males mated successfully with sighted females. Blind males showed some sexual

Figure 11. Changes in testosterone concentrations over time in sighted male mink across housing (n = 6/treatment).

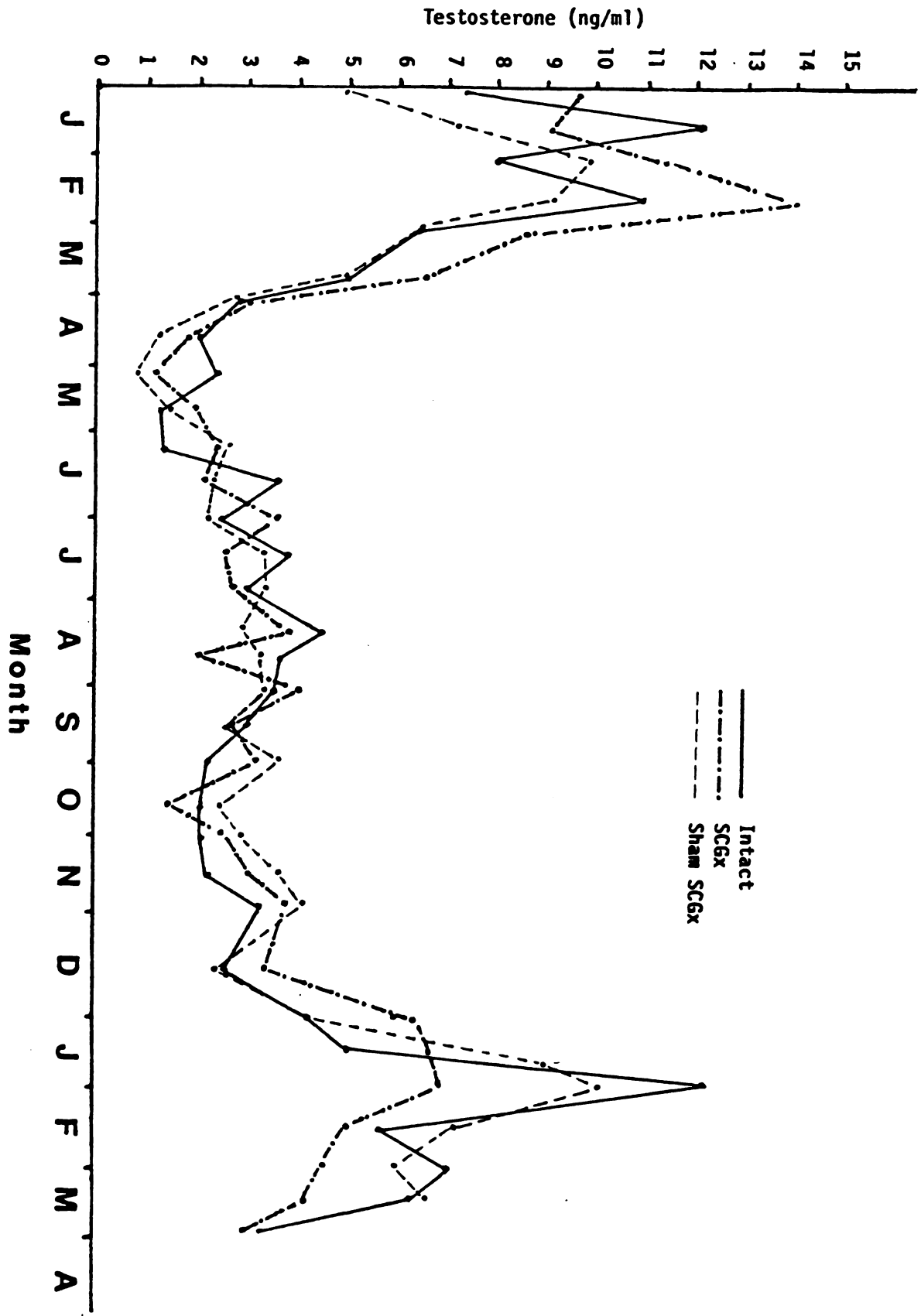

Testosterone (ng/ml)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
J F M A M J J A S O N D J F M A
Month
Intact
SCGx
Sham SCGx

Figure 12. Changes in testosterone concentrations over time in blinded male mink across housing (n = 6/treatment).

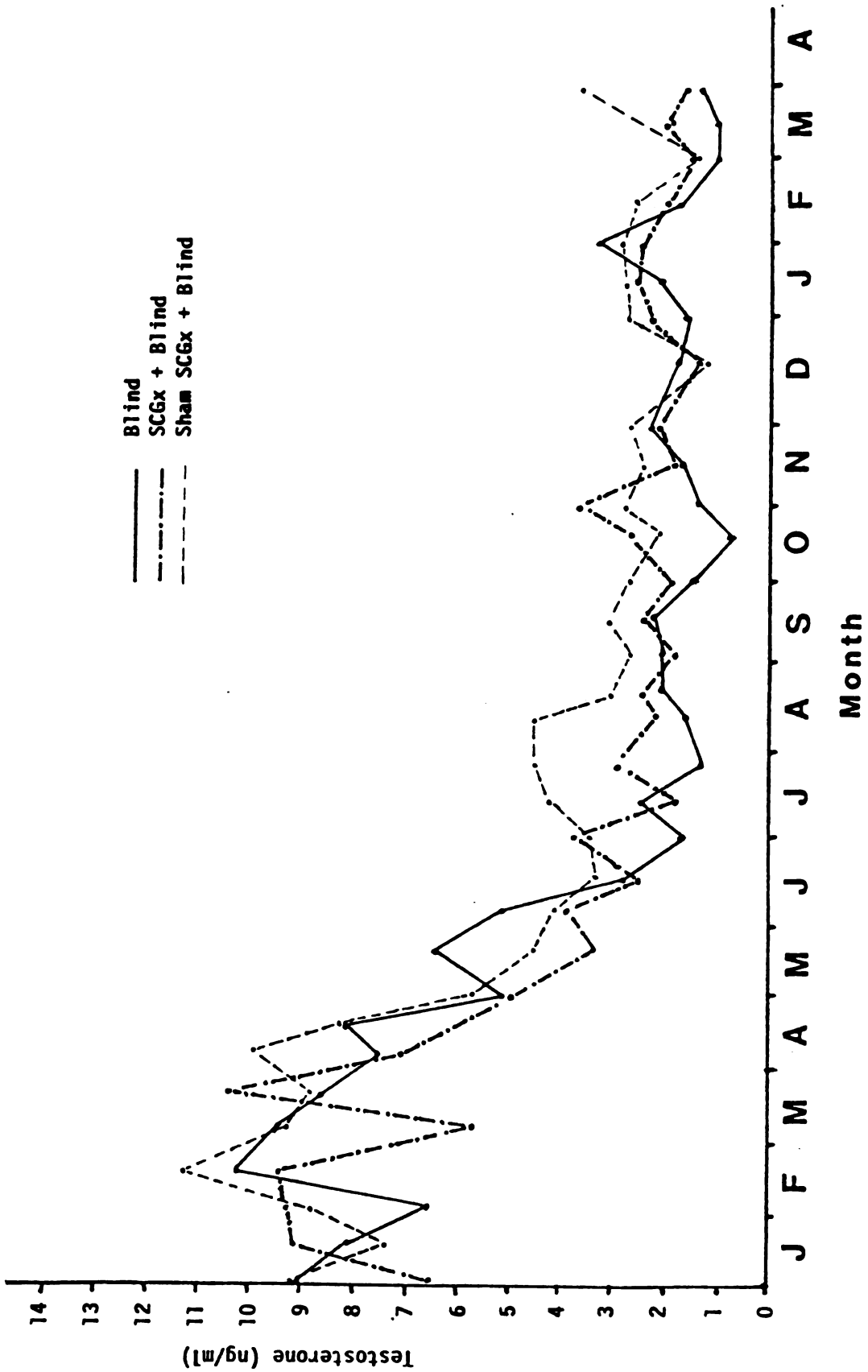

Blind
SCGx + Blind
Sham SCGx + Blind
Testosterone (ng/ml)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
J F M A M J J A S O N D J F M A
Month

Figure 13. Changes in estradiol concentrations over time in sighted female mink across housing (n = 7 intact, 4 sham SCGx, 6 SCGx).

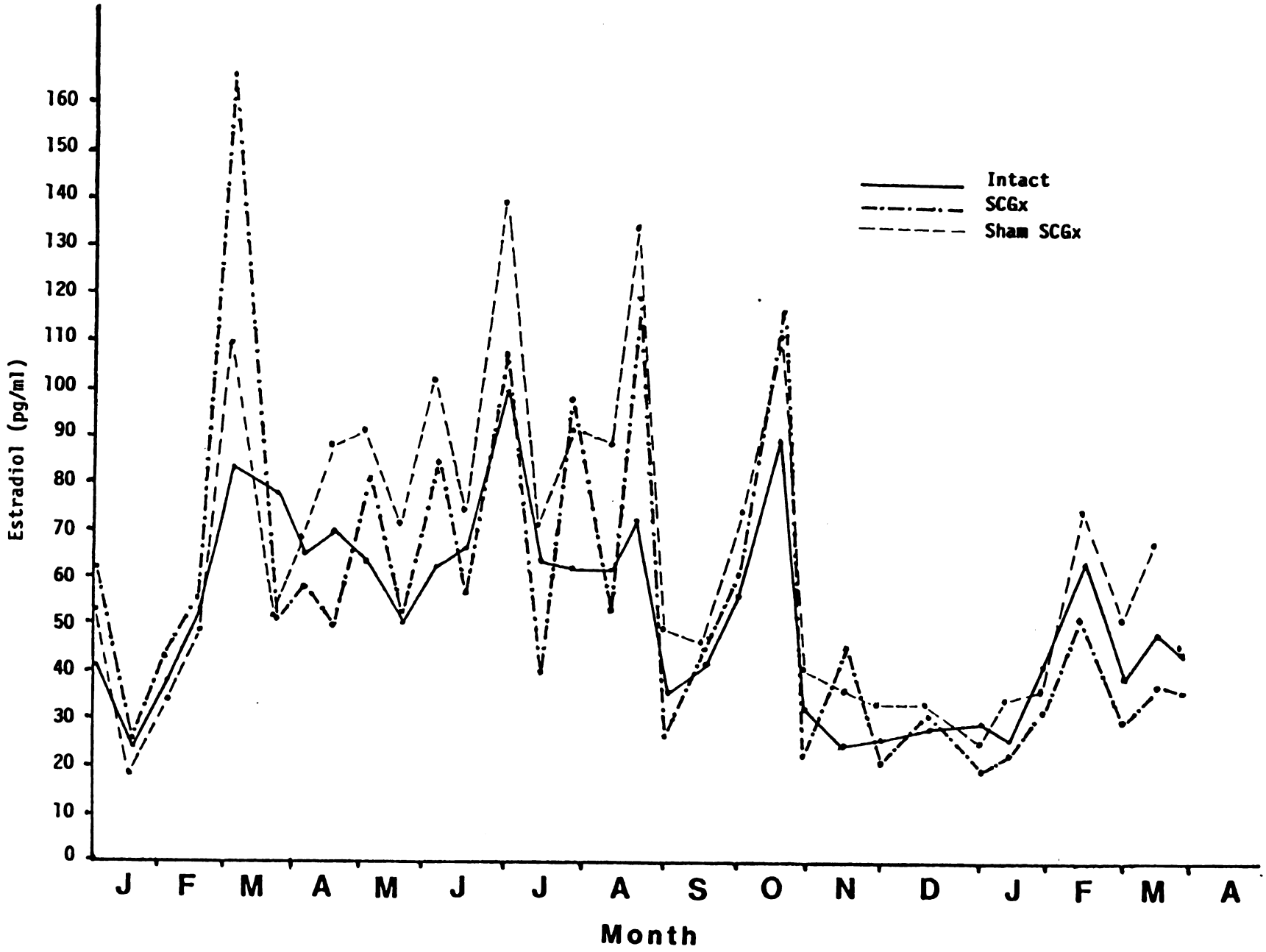
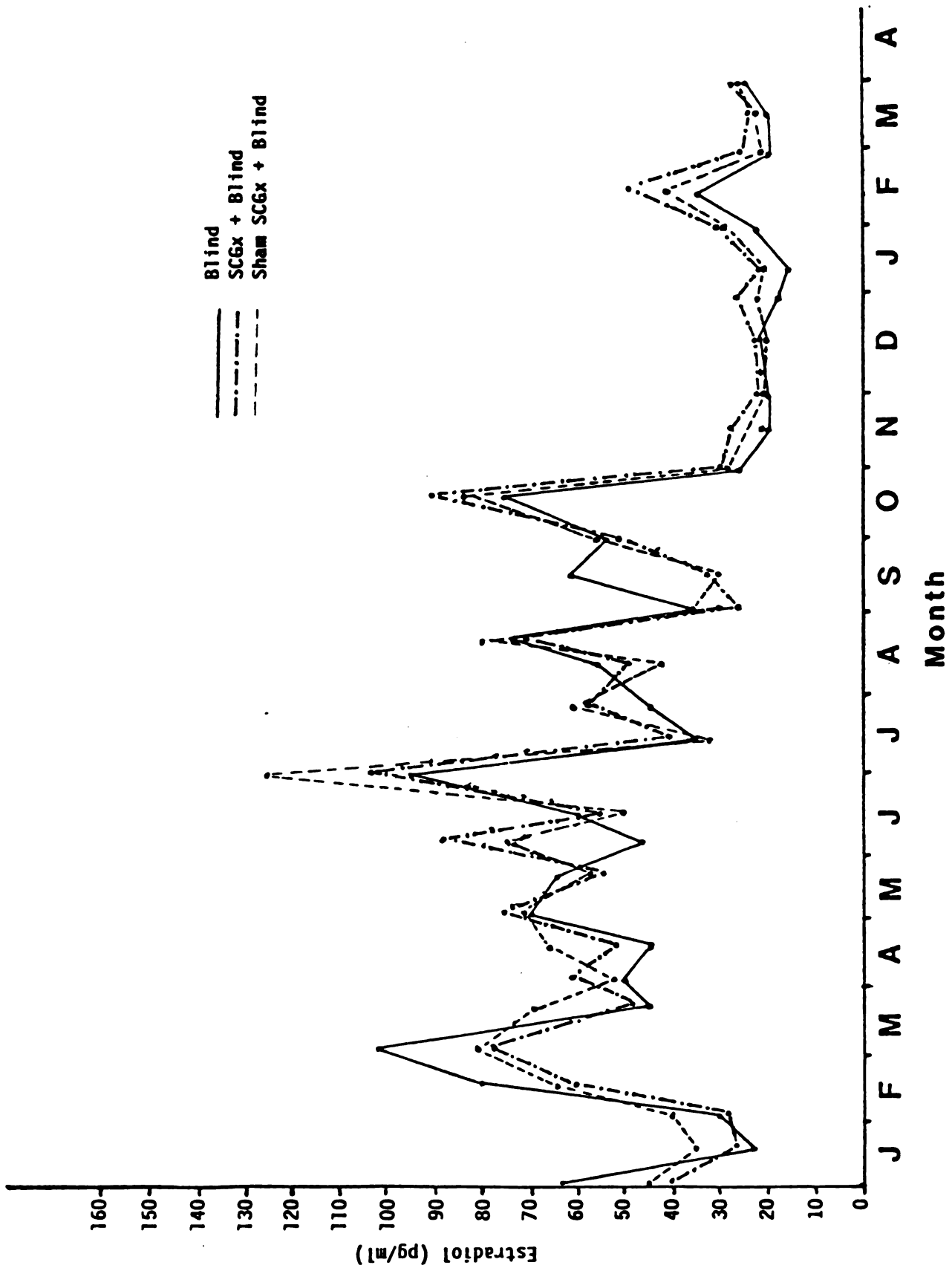

Intact
SCGx
Sham SCGx
Estradiol (pg/ml)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
Month

Figure 14. Changes in estradiol concentrations over time in blinded female mink across housing (n = 4 blind, 5 sham SCGx + blind, 5 SCGx + blind).


Blind
SCGx + Blind
Sham SCGx + Blind
Estradiol (pg/ml)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
J F M A M J J A S O N D J F M A
Month

interest even though their testes were very small but the interest was more aggressive in nature. The blind females had some interest in the males the first year and none the second year.

The sighted males housed outdoors had more successful matings as verified by vaginal post-coital sperm checks than those housed indoors. The group that had the largest number of matings was the SCGx males with a total of 21. The intact males were next with 6. The sham SCGx males had no verified good matings.

All the sighted females were mated, and of them 66% whelped. Those not producing litters were evenly distributed across the three sighted groups. The average litter size at birth was 5.80 for intact females, 3.25 for SCGx, and 2.66 for sham SCGx. There were too few females in each group to determine any statistical significance.

## DISCUSSION

### Body Weight

The cyclical pattern of weight gain and loss in the sighted mink closely parallel the annual changes in daylength. As the days lengthen the animals lose weight which they then gain as the days shorten. Ambient temperature also follows an yearly cycle, increasing when days lengthen and decreasing as they shorten. However, a role of temperature changes in circannual weight changes can be discounted since: 1) blinded animals showed no such pattern, although the females showed a trend in that direction, and they were exposed to the same temperature conditions, and 2) the body weight changes also occurred in mink housed indoors, sheltered from the stress of large shifts in temperature. The importance of photoperiod over ambient temperature has been reported in cattle (Petitclerc et al., 1983a), hamsters (Steinlechner et al., 1983; Bartness and Wade, 1984; Vitale et al., 1985) and mice (Hutchinson and Hart, 1984; Andrews and Belknap, 1985).

It is not clear why the indoor SCGx females were so much heavier than the other sighted female groups. It was not due to genetics *per se*, since their brothers in the male SCGx group were not heavier than other sighted males. It is possible that the combination of the surgery and housing synergized within a naturally-occurring female

annual cyclicity to cause those animals to gain more weight.

External Genitalia

Clear evidence of the importance of photoperiod in controlling the annual breeding season is demonstrated by the changes in testes size of the sighted and blinded males. Not only does photoperiod control the onset of testes growth but also appears to control its regression after the breeding season since the blinded animals had a slower rate of testes decline than sighted males. The decline in testes size seems inevitable and the lengthening photoperiod only hastens it. However, recrudesence needs to be stimulated by decreasing daylength (Onstad, 1967). Those animals unable to visually detect light do not spontaneously prepare for the breeding season.

The SCG may have an inhibitory or modulatory role in testes regrowth,since the SCGx males began testes enlargement over one month sooner than the other ganglia-intact animals. This effect has not been described previously, although SCGx animals do maintain large testes longer (Lincoln, 1979).

The pattern of vulvular size changes in the females is very similar to the testes size changes of the males. The blind females did not exhibit estrual swelling at all the second year. The sighted females showed swelling both

years but not as much the second year. This could be due to the repeated stress of handling, anesthesia, and blood collection, or it could be the result of copulation which was permitted that season. The swelling is positively correlated with blood estradiol concentrations (Travis et al., 1978) and since mink are induced ovulators, mating would cause ovulation and reducted synthesis of estradiol.

Based on genital changes both sexes achieved puberty whether sighted or blinded. They could have very well gotten their critical photoperiodic cues for puberty before the surgical treatment. It is very difficult to prevent puberty totally. It may occur but then the animal become secondarily infertile. This has been noted in heifers on restricted intake diets (Petitclerc et al., 1983a), lambs (Foster and Yellon, 1985), and rats (Holehan and Merry, 1985; Bronson, 1986). It can also be delayed but not prevented by certain social interactions in mice (Coppola and Vandenbergh, 1985) and voles (Rissman and Johnston, 1986), and by alterations of central nervous system activity (Reiter and Ellison, 1970; Ojeda et al., 1983b; Rivest et al., 1985).

Steroid Hormones

There was a very close relationship of testosterone (T) concentrations in serum and testicular size independent of surgical treatment. It is interesting that changes in the

T levels preceded measurable changes in testes volume. This could be explained by the fact that testosterone is needed to stimulate the whole spermatogenic apparatus and thus there would be a lag between the time T titers begin to rise and the semineferous tubules enlarge (Onstad, 1967; Sundquist et al., 1984). Also the testosterone radioimmunoassay is more sensitive to changes than are calipers.

Although the females' genitals changed with photoperiod and proximity to the breeding seasons, serum estradiol ($E_2$) did no follow the external changes in an obvious manner. At first there appeared to be no difference in concentrations of $E_2$ between the blinded and sighted females although the sighted group seemed to have a higher baseline and mean level the first 10 months (approximately 70 vs. 55 pg/ml). Both groups showed a nadir from November to January then increase in February and March. The sighted females showed a greater increase in concentrations of $E_2$ than the blind ones. Why there is higher $E_2$ during the summer and fall is unknown. This observation has not been reported before. The radioimmunoassay for $E_2$ had an interassay variation of 42%. Consequently the estradiol concentrations described here may not be as reliable as would be desired.

Hair Coat

Examination and evaluation of pelage was not part of the original design and was only begun because it was noted that the blind animals were not shedding their winter coats in June like the controls. The appraisal of the coat condition was subjective but was based on the experience of two knowledgable mink researchers. However, the results show definative differences between the sighted and blinded groups.

Hair coat quality is not a direct measure of reproduction, but it is further evidence for the importance of optic photoreceptors as daylength detectors in the central processing of photoperiodic information. The blind animals' coats were "normal", in phase with control animals, until after puberty and then they began to molt in abnormal patterns and incompletely. Usually the molt or priming of the fur begins with the tail and proceeds anteriorily with the head molting or priming last. The blind mink had mottled, blotchy hair growth with some patches which never shed.

Reproductive Behavior

Behavior was used to determine if the changes in genital sizes were true indicators of sexual competence which includes the desire to mate or to be mated. The enlarged testes of all the males the first year were

accompanied by sexual interest but not the second year. Surprisingly, even the blinded males whose testes were small and abdominal went through some of the copulatory behaviors such as "chuckling", grasping the back of the female's neck, dragging her around the cage, attempting to position her for coitus (MacLennon and Bailey, 1972). They did not successfully mate, though, and usually ended up in a serious fight with the female.

The sighted males housed indoors were not as vigorous breeders as those kept outside. Only one indoor male had a successful mating. A successful mating was determined post-coitally by vaginal swabbing for sperm checked under a microscope (Sundquist and Gustafssen, 1983). The SCGx males had the greatest number of good matings, the intact males had less, and the sham SCGx males had none. This latter group contained two mink from one litter that had only one testis descended and this might have had a negative effect on their breeding performance (Hartung and Seffner, 1983). The SCGx group had testes enlargement significantly earlier than the other groups and it was suspected that they may be out of reproductive condition during the normal breeding season. This was not the case as they were the most vigorous breeders.

The reproductive performance of females was actually better than for males. The first year all the females would have permitted mating, while the second year only the

sighted females mated. All females within the sighted group were mated at least once during the second season and 66% of them whelped. This is lower than average reproduction for a commercial mink ranch but these animals had not been mated the previous year and it can be surmised that this might have had a detrimental effect on their reproductive performance. Numerically, intact females had the largest average litter size, there were too few litters to make any valid conclusions.

## CONCLUSIONS

In this study it is the eyes that are the most crucial component in the eye-SCG pathway to the pineal in mink since without them the animals became desynchronous with the photoperiod despite other treatments. There was no spontaneous regeneration of the gonads and the post-breeding season atrophy of the testes was slowed in blinded animals indicating that mink require the photoperiod to stimulate reproductive function as well as to hasten its end.

The SCG may have an inhibitory role in gonadal recrudesence since without it the testes of the male became larger sooner than in intact males. However, the effect of the eyes overrode that of the ganglia since blind + SCGx animals responded the same as blinded-only animals. The SCGx females responded the same as other sighted females so the role of the ganglia in sighted mink is not well defined.

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