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THE ROLE OF THE SUPRACHIASMATIC NUCLEUS IN THE PHOTOPERIODIC MODULATION OF BEHAVIORAL SENSITIVITY TO STEROID HORMONES

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THE ROLE OF THE SUPRACHIASMATIC NUCLEUS IN THE PHOTOPERIODIC MODULATION OF BEHAVIORAL SENSITIVITY TO STEROID HORMONES

By

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ABSTRACT

THE ROLE OF THE SUPRACHIASMATIC NUCLEUS IN THE PHOTOPERIODIC MODULATION OF BEHAVIORAL SENSITIVITY TO STEROID HORMONES

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Syrian hamsters kept in a short photoperiod gain weight and show less sexual behavior in response to exogenous steroids (i.e., estrogen and progestersone (P)) than do their counterparts in long days. In Experiment 1, the effects of photoperiod on responses to P alone were investigated. Animals maintained in long days and given P show less aggression than those in short days similarly treated. Knife cuts between the suprachiasmatic nucleus (SCN) and the paraventricular nucleus disrupt some responses to short photoperiods, but animals with such knife cuts kept in short days maintain a decreased behavioral responsiveness to exogenous steroids. In Experiment 2, electrolytic lesions of the SCN were made in female Syrian hamsters and the effects of photoperiod on these animals were examined. SCN lesions blocked the effect of photoperiod on body weight, and animals with SCN lesions failed to show a reduced behavioral sensitivity to gonadal steroids when kept in a short photoperiod.

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General Introduction

Many species are known to have seasonal breeding cycles, in which the animal undergoes physiological and behavioral changes in response to differing amounts of light per day. Many mammalian species display this photoperiodic response, such as sheep, ferrets, gerbils, Siberian and Syrian hamsters (for examples see Bittman and Blaustein, 1990; Bissonette, 1932; Bartness and Goldman, 1989; Underwood and Goldman, 1987). Some responses to changing photoperiods (i.e. short winter days) are highly visible, such as coat color variations (Underwood and Goldman, 1987). While alterations in reproductive physiology are less visible, these changes are equally striking. Male Syrian hamsters exposed to short days (e.g. less than 12.5 hours of light/day) show testicular regression as well as reduced spermatogenesis and testosterone (T) production. In females, the uterus decreases in size, ovulation ceases and the normal four day estrous cycle is replaced by constant diestrus. These physiological responses to changes in photoperiod are mediated by hormonal changes. For example, in short-day housed females, both prolactin and estrogen (E) levels decrease while progesterone levels increase (Lisk, 1985). Also, daily surges of luteinizing hormone (LH) and follicle stimulating hormone (FSH) replace the regular 4-day surges of these hormones (Bridges and Goldman, 1975; Seegal and Goldman, 1975).

In addition to these physiological differences between animals kept in either long days or short days, changes in behavior are also evident. In both males and females, mating behavior ceases during short days. Males show a reduction in copulatory behavior and become more aggressive (Garrett and Campbell, 1980), and females fail to show lordosis in response to a male. Females also tend to be more aggressive if kept in

which the female is not receptive and will readily attack a male intruder, followed by the day of proestrus, in which the female becomes somewhat more tolerant of the male late in the day. Behavioral estrus then ensues, during which the female is most receptive (i.e. does not rebuff the male and shows lordosis) for a few hours (Kislak and Beach, 1955; Vandenbergh, 1971).

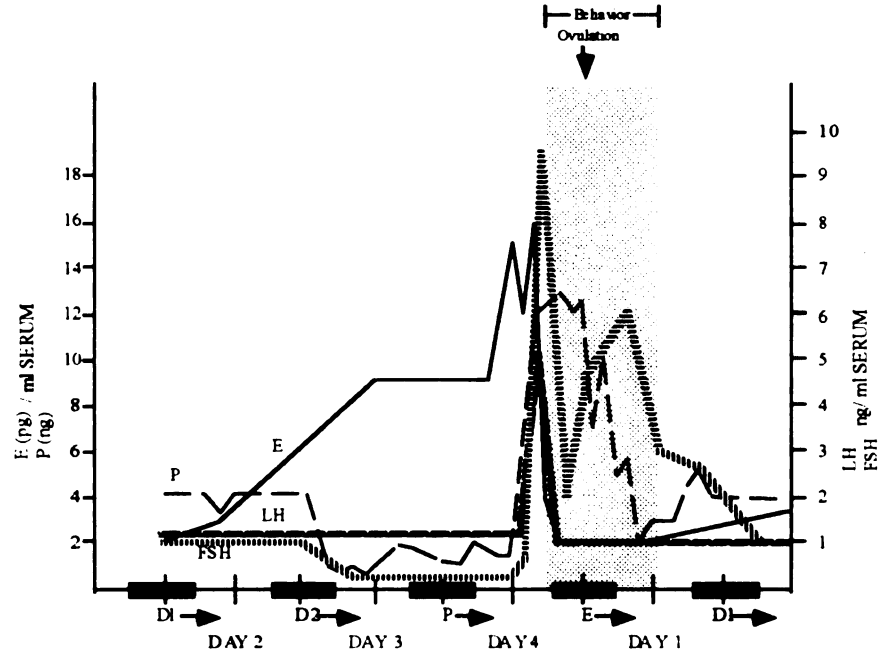

Figure 1. Serum profiles of the gonadotropins and the steroids are indicated for the estrous cycle of the hamster along with the potential period of sexual receptivity and the time of ovulation. The standard designation for each day of the rodent cycle (D1 = diestrus day 1, D2 = diestrus day 2, P = proestrus, E = estrus) is indicated and beneath this the terminology for the days of the estrous cycle found in most references on the hamster has been indicated. Serum profiles of gonadotropins are based on Bast and Greenwald (1974), estrogen on Saidapur and Greenwald (1978), and progesterone on Ridley and Greenwald (1975), with the receptivity and ovulation time based on Reuter et al. (1970). Heavy black bar, 10-hr dark period. Black line, 14-hr light period. From *The Hamster*, Ed. H. Siegel (1985).

The hormonal profile that coincides with the behaviors just described is shown in Figure 1. E slowly rises from the first day of diestrus, reaching moderately high levels prior to proestrus and peaking immediately before the onset of behavioral estrus. P levels stay relatively low until the surge which corresponds to the E peak, and then quickly diminishes. In the ovary, the rise in E and the subsequent surge of gonadotropins (LH and FSH) result in ovulation, and the rise in P levels is due to output from the corpora

lutea. Exogenous administration of E and P to an ovariectomized hamster which mimic the pattern described above can induce the receptive behavior seen in the intact animal.

Since the female hamster is very aggressive when not in behavioral estrus, the animal also provides a model for studying the effects of steroids on aggression. Aggressive behavior is also influenced by photoperiod (Garrett and Campbell, 1980; Fleming *et al.*, 1988). Fleming *et al.* (1988) showed that while intact hamsters kept in short days were more aggressive than intact hamsters kept in long days, this difference was abolished by pinealectomy. Other authors have noted that P, with and without E priming, modulates the display of agonistic behaviors. Fraile, McEwen and Pfaff (1987) have found that large doses of P without E priming inhibit aggression in hamsters of both sexes kept under a stimulatory photoperiod and tested in same-sex pairs. On the other hand, Meisel, Sterner and Diekman (1988) have shown that priming with E is necessary for a single P injection to exert an inhibitory effect on aggressive behaviors of females kept under a stimulatory photoperiod and tested with male stimuli. The same treatment failed to affect the display of aggression towards other females.

Previous work has shown that females kept under a short photoperiod are less sensitive to the activational effects of E on lordosis behavior. If ovariectomized animals are given a "threshold" dose of E (25% E in Silastic capsules subcutaneously implanted for seven days), approximately 50%-70% of animals in long days will show lordosis in response to a male, while only 10%-25% of the animals housed in short days respond with lordosis (Badura *et al*,. 1987b). Furthermore, this difference in sensitivity appears to be independent of pineal melatonin. While short day females that are pinealectomized maintain cyclicity, the surgery did not affect the reduced behavioral sensitivity to E (Badura and Nunez, 1989). Further evidence that this effect is pineal independent is provided by an experiment that utilized knife cuts between the SCN and PVN (Badura, Sisk and Nunez, 1987a). Such cuts severed the connection between the two nuclei and should have disrupted the flow of information to the pineal while leaving the gland intact.

However, behavioral differences still existed between animals kept under the different photoperiods.

When Syrian hamsters are exposed to short days, they gain weight and increase their thermogenic capacity (Wade, 1982). These effects of photoperiod on energy balance are seen even in PNX animals (for a review, see Bartness and Wade, 1985). However, afternoon injections of melatonin mimic the effect of short days on body weight, in that hamsters housed in long days that receive these injections show an increase in body weight (Bartness and Wade, 1984). These results suggest that there may be at least two mechanisms mediating the effects of short days on body weight gain, one independent of the pineal and another that depends on pineal melatonin.

The PVN may be involved in the pineal-independent control of body weight and metabolism, as well as being an important component of the pineal-dependent mechanism. Lesions of the PVN as well as the SCN block the effects of short days on body weight in Siberian hamsters (Bittman, Bartness, Goldman and DeVries, 1991), a species which shows a reduction in body weight when exposed to short days. However, different from Syrian hamsters, in Siberians hamsters effects of short days on body weight and metabolism are prevented by pinealectomy (Vitale, Darrow, Duncan, Shustak and Goldman, 1985). In Syrian hamsters, lesions of the PVN block weight gain induced by short days (Bartness, Bittman and Wade, 1985). That study, however, was complicated by the fact that in animals housed in long days, PVN lesions induced obesity when fed the high fat diet used in that experiment. Half of these "obese" animals were then switched to a short photoperiod, and no further gain in weight was observed in either group. These results do not rule out that a 'ceiling effect' was encountered, one that prevented further gains in weight following exposure to short days. The authors do not believe this to be the case, as these animals plateaued at approximately 190 g, while weights of up to 250 g for Syrian hamsters have been observed in the same laboratory. Sham operated controls that were placed in short days attained the same weight as did the

animals with PVN lesions, so instead of short day animals with lesions maintaining the weight of long day shams, long day animals with lesions showed a weight gain similar to that of short day shams.

The specific mechanism by which retinal input and thus photoperiodic information alters body weight and metabolism is unknown. The gain in body weight induced by short days is primarily in the form of white adipose tissue (fat for fuel storage) and brown adipose tissue (for thermogenesis) and is achieved without a concurrent increase in caloric intake (Wade, 1982). Exposure to short days is also correlated with a decrease in the level of circulating thyroid hormones, but this decrease is prevented by pinealectomy while the increase in body weight is not (see Bartness and Wade, 1985). Similarly, prolactin (PRL) levels, which are inversely related to the growth of brown adipose tissue, are low in short days, but again, pinealectomy blocks the short day induced reduction in PRL but not in body weight gain. Therefore, changes in circulating levels of PRL or thyroid hormones are not necessary for the development of short day induced obesity.

The PVN are intimately involved with metabolic processes such as fat storage and mobilization, as these nuclei communicate with the adrenals and pancreas via both endocrine messengers and the autonomic nervous system (see Luiten, ter Horst and Steffans, 1987). The pancreas produces both insulin and glucagon, hormones that are responsible for the storage and release of energy in the form of fat and glycogen. The adrenals produce both glucocorticoids, which contribute to the breakdown and redistribution of fat, and catecholamines, which can affect insulin levels as well as thermoregulatory processes, such as heat production. In the rat, the cells in the parvocellular portion of the PVN contain corticotropin releasing hormone (CRF), and these cells project to the median eminence (Luiten *et al.*, 1987). CRF then stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary which in turn affects the discharge of steroids from the adrenals (see Luiten *et al.*, 1987). Short days may

stimulate the release of CRF from the PVN, resulting in an increased level of circulating glucocorticoids, and ultimately an increase in fat. Assays for these hormones under both photoperiodic conditions would provide evidence to test this hypothesis. The PVN send projections to the dorsal motor nucleus of the vagus and to the nucleus ambiguus in the rat (Swanson and Kuypers, 1980; see also Luiten *et al.*, 1987), which in turn provide parasympathetic input to the viscera. In the hamster, a projection from the PVN to the intermediolateral cell column (IML) of the spinal cord exists (Don Carlos and Finkelstein, 1987). The IML contains sympathetic preganglionic cell bodies associated with the outflow to the pancreas (see Luiten *et al.*, 1987) and to the various fat pads. Sympathetic stimulation of the pancreas reduces insulin production, while parasympathetic output (and therefore lack of sympathetic tone) increases insulin production. Short days, then, could be stimulating the storage of fuel as fat via insulin by reducing sympathetic tone and increasing parasympathetic tone. However, short days continue to influence body weight in diabetic Siberian hamsters (Bartness, McGriff and Maharaj, 1991) and in diabetic Chinese hamsters (unpublished observations, cited in Bartness *et al.*, 1991), suggesting that insulin may not be the key factor in short day induced changes in body weight. It is possible that a reduction in the sympathetic inputs to the fat pads directly affects fat mobilization and storage as Syrian hamsters develop seasonal obesity.

The research reported here was an effort to determine if the SCN is necessary for the display of short-day induced changes in socio-sexual behaviors and weight gain, two effects of photoperiod that are at least in part independent of the pineal gland.

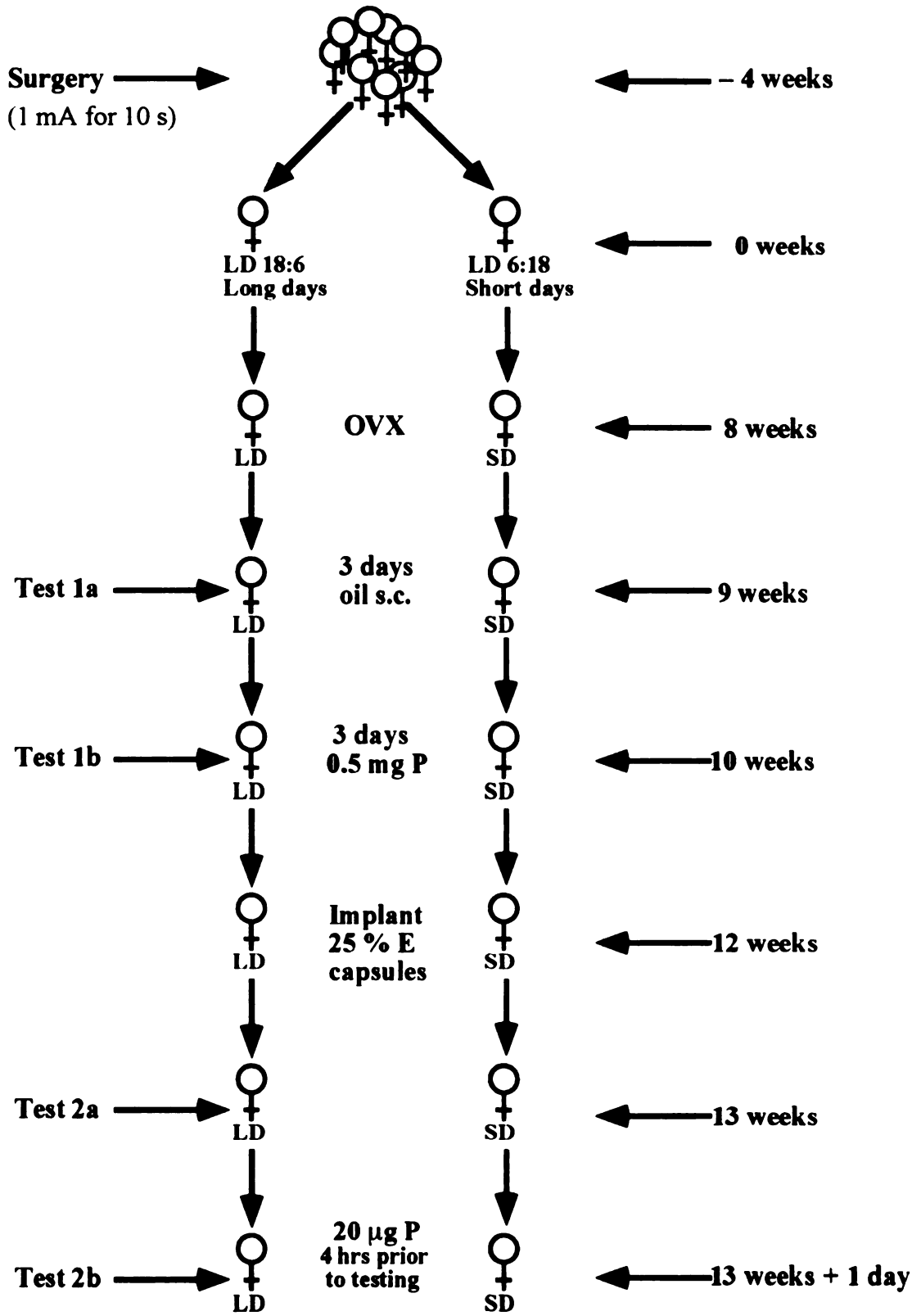

Figure 2. Flow chart for the various procedures and tests of Experiment 1.

Experiment 1

The primary goal of this experiment was to determine if the short-day induced behavioral insensitivity to ovarian hormones that is pineal independent is also independent of the SCN. In other words, does the SCN need to be intact for short day animals to show the decreased behavioral sensitivity to E? In order to answer this question, electrolytic lesions, aimed at the SCN, were made in female golden hamsters. Lesions of the SCN in female hamsters have been shown to disrupt many biological rhythms such as locomotor and drinking activity, as well as eliminating the characteristic four day estrous cycle; the animals go into persistent vaginal estrus (Stetson and Watson-Whitmyre, 1976). The presence or absence of persistent estrus as identified by inspection of the vaginal discharge (Orsini, 1961) provides a convenient bio-assay as to whether or not a lesion destroyed the SCN.

The specific hypothesis being tested was that short day animals with SCN lesions will show an increased sensitivity to E and E+P when compared to short day animals with intact SCNs. In addition, the experimental group was compared to long day animals with similar lesions, and long day shams were also monitored. A secondary goal of this research was to test the effects of photoperiod on the behavioral actions of P when this hormone is administered without previous priming with E. In this way, an effect of P that is independent of E yet susceptible to changes in photoperiod can be elucidated. Intact females housed in long days show a reduction in aggression when given P (Fraile *et al.*, 1987). Paradoxically, P levels increase in intact female hamsters housed in short days (Bridges and Goldman, 1975), but they remain aggressive (Fleming *et. al.*, 1988). Using both male and female stimuli, the effect of photoperiod on the ability of P to reduce aggression in ovariectomized females was also examined.

Although none of the lesions were effective as assessed by inspection of vaginal discharge, all animals were tested as described below. Figure 2 shows the time course of the various surgeries and hormonal treatments utilized throughout Experiment 1.

Method

Animals and housing

Adult female Syrian hamsters were obtained from Charles River Laboratories (Wilmington, MA) and weighed between 90g and 110g upon delivery. All animals were single housed in hanging wire cages (18 x 18 x 27.5 cm) and given food (Wayne Breeder Block) and water *ad lib*. All hamsters were kept in a long photoperiod (16L:8D) for approximately four weeks while half received lesions aimed at the SCN. Seven days after the last surgery, half of the animals with lesions and half of the sham animals were transferred to a short photoperiod (6L:18D). Stimulus animals consisted of 10 intact males (body weight ≈120g) and 10 ovariectomized females (body weight ≈150g) that were group-housed (n=5) according to sex in plastic cages (34.0 x 29.5 x 16.5 cm). Ovariectomized group-housed females are fairly passive (Grelk, Papson, Cole and Rowe, 1974) and thus provide a non-aggressive stimulus for the experimental animals. The stimulus animals used for the long day condition were kept under the same 16L:8D light/dark cycle as were the long day experimental animals, while the stimulus animals used for the short day condition were housed in a 16L:8D light cycle with lights out six hours after the lights-out time for the short day experimental group. This was done to coordinate the circadian time, and hence the period of maximum activity, of the stimulus animals with that of the experimental animals under the different photoperiods. Short day animals are most active approximately 6 hours after lights out, while long day animals are active immediately after lights out (Elliott and Goldman, 1981). By placing the stimulus animals for the short days group in a long photoperiod with lights out 6 hrs

after lights out for the short day experimental group, both the stimulus and experimental animals would be tested during their most active phase.

Surgery and hormone treatments

Under Equithesin anesthesia (4.5 ml/kg), 45 of the animals received lesions aimed at the SCN, with two separate lesions made, one on each side of the midline. Using a Kopf stereotaxic instrument, the coordinates used were 1.0 mm anterior to bregma, 0.1 mm lateral to the midline, and the tungsten electrode (tip diameter ≈ 0.2 mm) was lowered 8.0 mm from the dura. The bite bar was set at -2.0 (from ear-bar zero), and a 1.0 mA current was passed for 10 seconds. Sham operations (n=20) consisted of lowering the electrode but passing no current. Seven animals were unoperated. Eight weeks after the hamsters were placed in different photoperiods, the sham-operated and unoperated animals (collectively called shams hereafter) kept under a short photoperiod had all stopped cycling. However, none of the animals with lesions had gone into persistent estrus, and these hamsters stopped cycling about the same time as did the shams. All hamsters were then ovariectomized via a single ventral incision under Metophane (methoxyflurane; Pitman-Moore Co.) anesthesia and allowed one week to recover from surgery. Each hamster then received a subcutaneous injection of sesame seed oil (0.1 ml) once a day for three days, and all animals were given a behavioral test on the evening of the third day, four hours after the injection (Test 1a; see below for testing procedures). The following week, subcutaneous injections of 0.5 mg progesterone (P; Sigma Chemical Co.) in 0.1 ml sesame seed oil were administered once a day for three days. A second behavioral test (Test 1b) was then conducted on the evening of the third day of P injections, four hours after the injection.

Two weeks after Test 1b, all experimental animals (animals with lesions and shams) were implanted with a 10 mm silastic capsule (3.175 mm o.d., 1.575 i.d.) filled with 25% estradiol (E; Sigma Chemical Co.) diluted with cholesterol. Seven days after

implantation, the hamsters were tested for behavior (Test 2a). The next day, the experimental hamsters received a subcutaneous injection of 20 μg P 4 hours prior to testing (Test 2b).

Testing procedure

The testing arena consisted of the experimental hamster's home cage set in a large plastic bin (34.0 x 29.5 x 16.5 cm) that was filled with clean bedding, with each hamster allowed an adaptation time of three minutes. In all tests, either a male or female stimulus animal (presented in a counterbalanced fashion) was placed in the experimental animal's home cage, and latencies to rear, attack or show lordosis (before a mount) were recorded on an Esterline Angus event recorder. Each test was terminated at the onset of an attack or lordosis, or after five minutes if neither behavior occurred. Long day animals were tested shortly after lights out, while the animals kept under a short photoperiod were tested six hours after lights out. All tests were conducted under dim red illumination.

A rear was scored if the experimental hamster lifted both front paws off of the floor of the cage while facing the stimulus hamster. An attack was scored when the experimental hamster pushed with the forepaws and bit the stimulus hamster. In one case the stimulus hamster was the aggressor, and that test was terminated immediately and not used in the analyses.

Histology

Of the 45 animals that received lesions, 27 died prior to testing. Four of the sham/unoperated animals also died, and the final count for each group was as follows: Short day animals with lesions, n=11; short day shams, n=13; long day animals with lesions, n=7; long day shams, n=10. After all of the data were collected, the animals with lesions were given an overdose of Equithesin and intracardially perfused with a saline rinse followed by 10% formalin. The brains from these animals were then sectioned on a freezing microtome at a thickness of 50 μm, and every other section was mounted on

glass slides coated with a subbing solution. The resulting slides were stained with cresylecht violet, cover-slipped, and the sections were evaluated for lesion placement. Six animals had large lesions, 11 were small, and 1 showed no evidence of damage. Placement of the lesions proved to be inconsistent, as 13 lesions were at the level of the SCN, 5 were posterior to the SCN, 3 were anterior and 5 were either dorsal or ventral to the nuclei (some lesions were classified as belonging to two categories, i.e. dorsal and anterior to the SCN). Figure 3 shows a representative large lesion and a small lesion and the approximate range of damage to the SCN. Lesions at the level of the SCN fell into both size categories, making it difficult to extract a group from each photoperiod with homogeneous lesions and a sizable *n* .

Statistics

Because of heterogeneity in the placement and size of the lesions, the data from the group with lesions were not analyzed. For the shams (short days, n=13; long days, n=10), the effects of photoperiod on the proportion of animals responding with attacks, rears and lordosis were evaluated using Fisher's Exact Probability Test. Separate analyses were performed for each behavior under each hormone treatment and stimulus condition (i.e. male vs. female). Within photoperiodic conditions, the effects of the sex of the intruder on the proportion of animals responding were also evaluated using Fisher's Exact Probability Test. For animals that responded, the data on latency to display each behavior were analyzed using a 2-factor analysis of variance (ANOVA) followed by post hoc comparisons utilizing the Tukey-Kramer method. For Tests 1a and 1b, latencies to show each behavior were analyzed with individual 2X2 ANOVA's (short days/long days X oil/P) within sex of the stimulus animal. The latency data from Tests 2a and 2b were subjected to similar 2X2 ANOVA's (short days/long days X E/E+P). In addition, data on latency to attack and to rear in Tests 2a and 2b were analyzed within

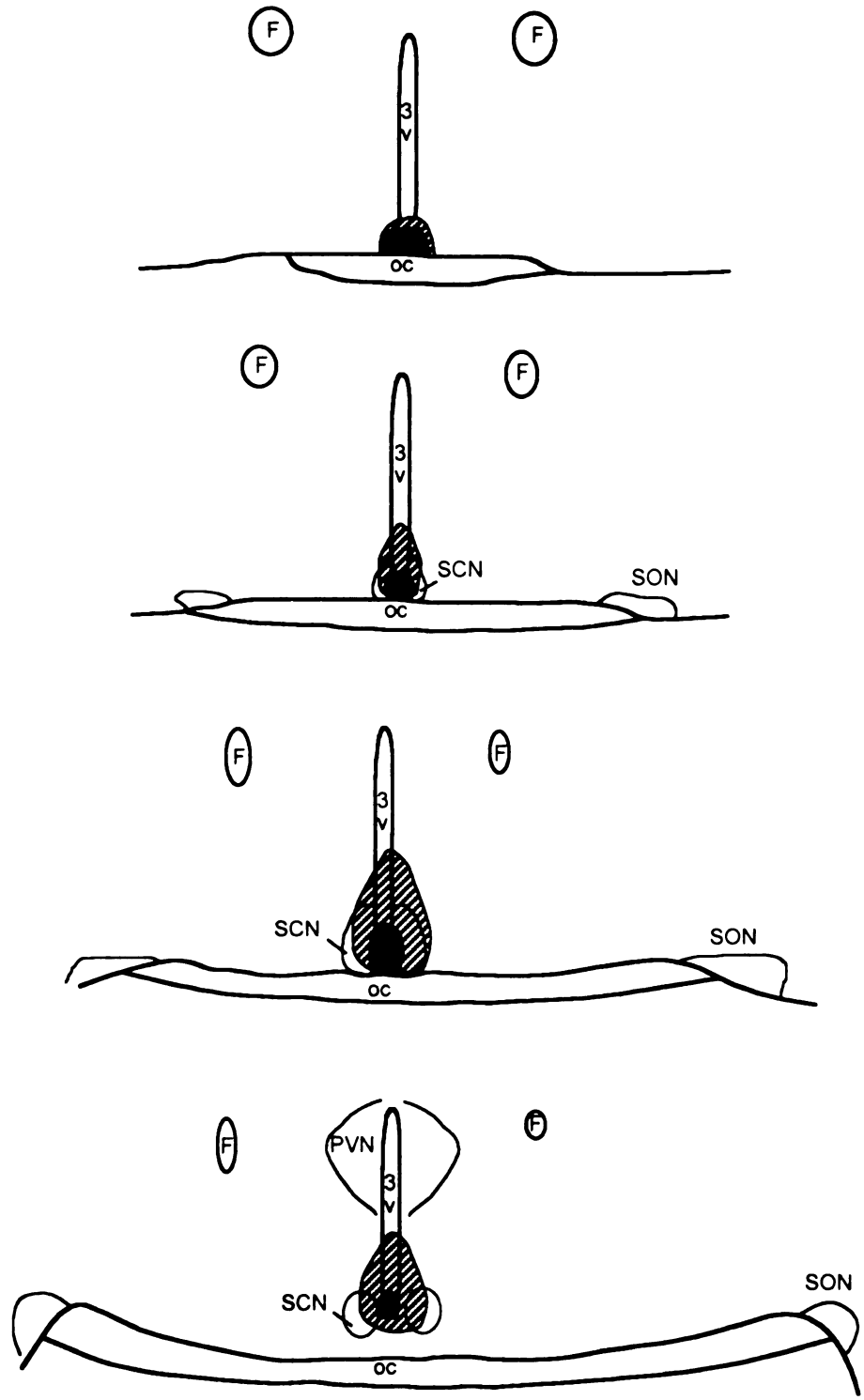

Figure 3. Representative lesions from Experiment 1; small lesion is black, large lesion is hatched. F = fornix, SCN = suprachiasmatic nucleus, 3v = third ventricle, oc = optic chiasm, SON = supraoptic nucleus, PVN = paraventricular nucleus.

photoperiodic condition to evaluate the effects of the sex of the stimulus animal. Those tests also used 2X2 ANOVA's (male/female stimulus X E/E+P) followed by post hoc comparisons.

Results

Aggression

As Figure 4 shows, the proportion of animals attacking was not significantly affected by photoperiod or sex of the stimulus animal when the experimental animals received oil injections (Test 1a). However, when the animals received the P treatment (Test 1b), female hamsters kept in long days attacked males less frequently than those kept in short days ($p<0.01$). For those that did attack, group differences in latency to respond failed to reach significance (see Table 1). All animals reared in Tests 1a and 1b, yet for latency to rear, a significant interaction between photoperiod and hormone condition (oil vs. P) was seen in tests with female stimuli ($F_{(1,40)}=4.3$, $p<0.05$).

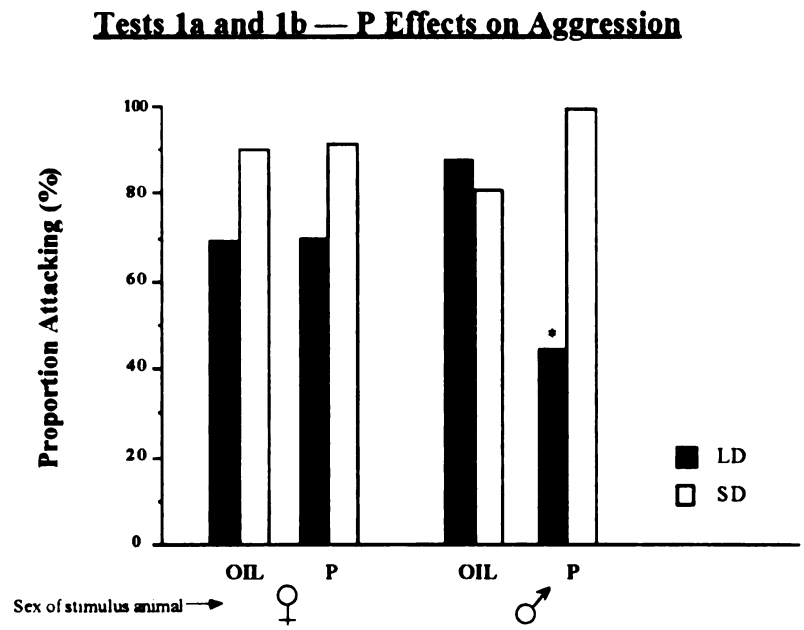

Figure 4. Only females kept in long days (LD) and treated with P attack males significantly less than do their short day (SD) counterparts. * $p<0.01$.

Table 1
Latency (seconds) to show aggressive behavior ($\bar{X}$ ± SEM) and number (n) of animals responding for each hormone condition and photoperiod for Tests 1a and 1b

SEX OF STIMULUS ANIMAL	PHOTOPERIOD	HORMONE CONDITION	
		OIL	P
		REAR	
MALE	SD	17.5±6.3 (11)	9.2±2.8 (13)
	LD	10.6±5.7 (8)	49.8±27.4 (9)
		ATTACK	
	SD	101.7±28.3 (9)	80.6±17.4 (12)
	LD	80.6±10.7 (7)	136.5±51.1 (4)
		REAR	
FEMALE	SD	15.8±7.4 (11)	6.2±1.4 (13)
	LD	7.2±1.5 (10)	14.2±2.0 (10)
		ATTACK	
	SD	103.7±29.2 (10)	53.1±11.5 (12)
	LD	62.3±17.5 (7)	96.6±26.3 (7)

Post-hoc comparisons failed to reveal significant differences between any two distinct groups, but a trend was evident in which short day animals reared more quickly when given P than without P, while long day animals reared sooner without P than with the hormone (see Table 1).

The results of Test 2a and 2b show that when treated with E or E+P, females in long days attacked males less frequently than those in short days ($p<0.01$, see Figure 5). Also, when compared to those in short days, fewer long day females attacked other females when exposed to E alone but not to E+P ($p<0.02$, see Figure 5). Within photoperiod, however, long-day females exposed to E+P attacked males significantly less

often than they attacked females ($p<0.02$, Figure 5). Analysis of latency to attack revealed that with a female as the stimulus, a main effect of photoperiod across hormone condition was present ($F_{(1,16)}=15.3$, $p< 0.001$), with the animals in short days displaying

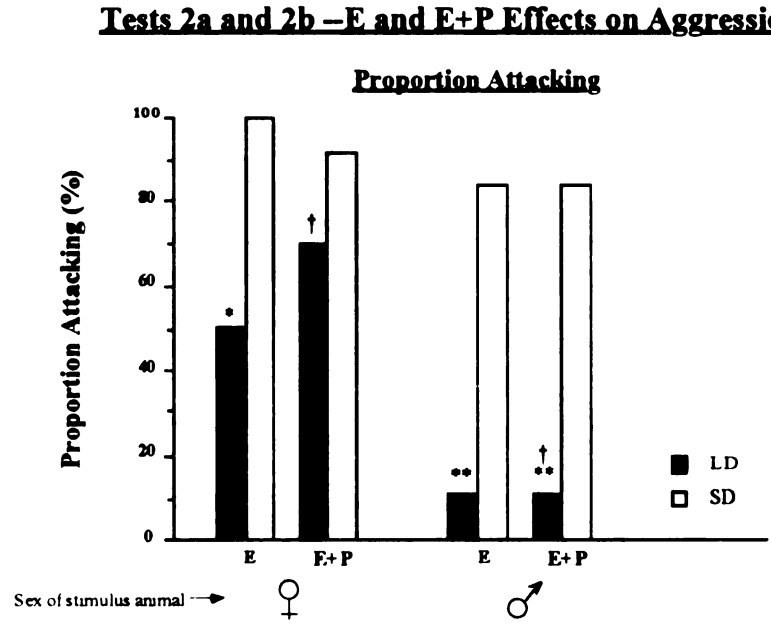

Figure 5. More short day (SD) animals attacked than long day (LD) animals in all conditions except when the female was given E+P and a female stimulus was used (*$p<0.02$, **$p<0.01$) . In the E+P condition, LD animals attacked females more frequently than males (†$p<0.02$).

shorter latencies. However, post hoc comparisons detected a significant effect of photoperiod only for the E+P condition ($p<0.01$). While short day females given E alone failed to be significantly different with respect to attack latency than those kept in long days and given only E, this difference in attack latency achieved significance when E was supplemented with P ($p<0.01$). Due to the small number of long day hamsters attacking male stimuli, no significant effect of photoperiod on latency to attack males was detected. When the latencies to attack of the short day animals were analyzed within that photoperiod condition (i.e., Hormone X Sex of Stimuli), a main effect of sex of the stimulus animal was found ($F_{(1,43)}=5.7$, $p<0.03$). As Table 2 shows, short day females treated with E or E+P attacked other females more quickly than they attacked males. For

rearing, in tests with females as stimuli, no effects of photoperiod or hormone condition on the proportion of animals responding were found (see Figure 6). However, with males

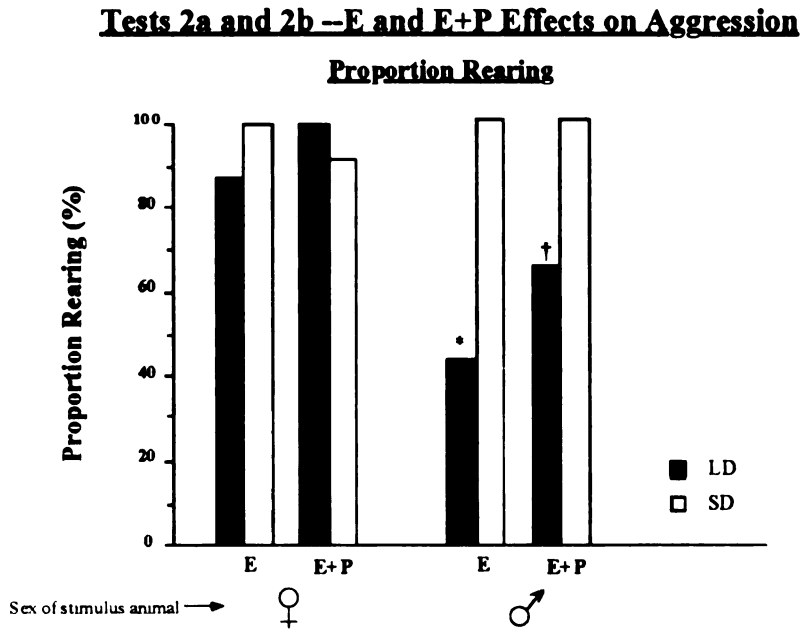

Figure 6. Fewer females housed in long days (LD) rear and a male is used as a stimulus than do short-day housed animals (SD) when exposed to E (*$p < 0.005$) or E+P (†$p < 0.03$).

as stimuli, exposure to long days significantly reduced the number of animals rearing when tested after treatment with E ($p<0.005$) or E+P ($p<0.03$; see Figure 6). A significant interaction between photoperiod and hormone group was present with respect to latency to rear in response to a female intruder ($F_{(1,38)}=6.2$, $p<0.02$). Post hoc comparisons revealed that short day and long day animals reared just as quickly when treated with E alone, but when given E+P, long day animals took longer to rear than short day animals ($p<0.01$). In addition, long day animals took longer to rear when given E+P than when treated with E alone ($p<0.05$). When rearing latencies were analyzed within each photoperiod (Hormone X Sex of Stimuli), a main effect of sex of the stimulus animal was found ($F_{(1,23)}=6.7$, $p<0.02$; $F_{(1,47)}=15.5$, $p<0.001$; long day and short day, respectively), with animals rearing more quickly in response to a female intruder (see Table 2).

Table 2

Latency ($\bar{X}$ ± SEM)to show aggressive behavior or lordosis and number (n) of animals responding for each hormone condition and photoperiod for Tests 2a and 2b

SEX OF STIMULUS ANIMAL	PHOTOPERIOD	HORMONE CONDITION	
		E_2	E_2+P
		REAR	
MALE	SD	37.3±8.5 (13)	36.4±10.1 (13)
	LD	67.5±37.7 (4)	60.5±31.8 (6)
		ATTACK	
	SD	111.6±25.5 (11)	92.1±16.1 (11)
	LD	261 (1)	216 (1)
		LORDOSIS	
	SD	42 (1)	- (0)
	LD	81.4±32.3 (5)	81.6±25.6 (5)
		REAR	
FEMALE	SD	12.6±3.1 (13)	6.5±1.7 (12)
	LD	10.4±1.4 (8)	20.8±5.5 (9)
		ATTACK	
	SD	68.2±12.9 (13)	47.9±18.2* (12)
	LD	135.4±30.8 (5)	193.5±42.5* (7)
		LORDOSIS	
	SD	- (0)	30 (1)
	LD	24.7±4.9 (3)	94 (1)

Significant differences between groups with like symbols; *$p<0.01$

Lordosis

During Tests 1a and 1b, no animal exhibited lordosis in response to any stimulus animal. However, when given E (Test 2a), females in long days showed lordosis more frequently than those in short days, regardless of the sex of the stimulus animal ($p<0.03$ for female stimuli; $p<0.02$ for male stimuli; see Figure 7). In addition, when E was supplemented with P, females kept in long days exhibited lordosis in response to males more frequently than those housed in short days ($p<0.003$), with no differences noted when a female stimulus was used. Too few animals from the short day group responded to analyze the latencies to show lordosis.

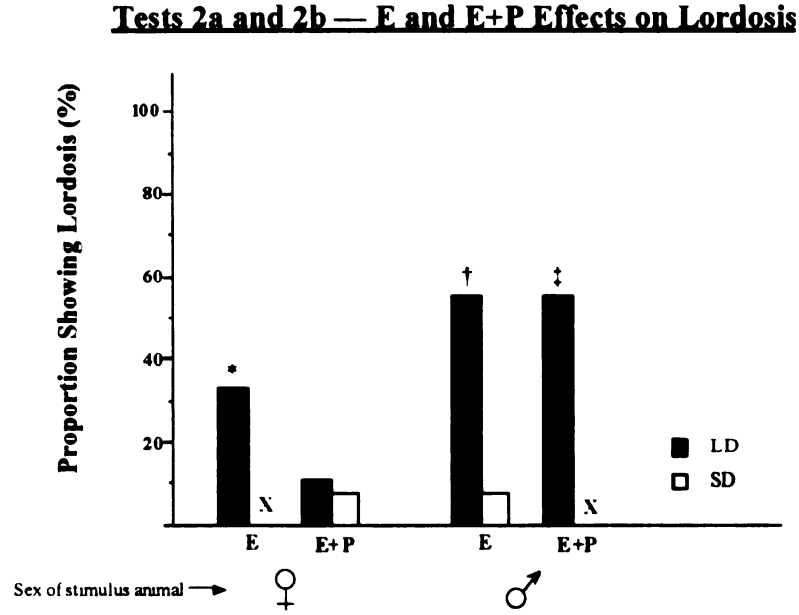

Figure 7. More females in long days (LD) given E respond with lordosis than those in short days (SD). When supplemented with P, LD females respond more than SD females to males only. *$p<0.03$, †$p<0.02$, ‡$p<0.003$.

Discussion

The results of this study provide support for the hypothesis that the ability of P to reduce aggression is modulated by photoperiod as well as by the sex of the stimulus animal. The results of Tests 1a and 1b indicate that P can decrease aggression in ovariectomized females in the absence of E_2 priming. Long-day female hamsters treated with P attacked male stimuli less often than did P-treated females kept under a short photoperiod, but even after treatment with P experimental females consistently reared and attacked female stimuli regardless of photoperiod. However, in these tests the animals in short days had shorter latencies to rear than did their long day counterparts, regardless of the sex of the stimulus animal. Therefore, photoperiod seems to affect the aggressive behavior of P-treated females towards both male and female stimuli. While Fraile *et al.* (1987) found that P given to females kept under a long photoperiod decreased attacks directed towards female intruders, that effect was not seen in the present experiment. This could be due to the amount of P used, as less than half as much was used in this study as in the earlier experiment by Fraile *et al.* (1987). It should be noted that a single injection of 500 μg P failed to affect aggression towards males or females in a previous experiment (Meisel *et al.*, 1988). Another reason for a difference in responses could be that in the experiment by Fraile *et al.* (1987), both the resident animal and the intruder were treated with P. This could change certain properties of the stimulus female, through hormonal effects on female chemosignals (Payne and Swanson, 1971) or perhaps through a difference in ultrasonic vocalizations (Matochik, Miernicki, Powers, and Bergondy, 1986), but these speculations need further research.

While photoperiod modulated the behavior of animals when exposed to P, no behavioral differences were seen between photoperiods when no gonadal hormones were present. The lack of an effect of photoperiod in the absence of gonadal steroids is in contrast to what Fleming *et al.* (1988) reported. These apparently contradictory results

could be due to methodological differences. In that earlier report, the data were presented in the form of an offensive behavior/defensive behavior ratio, whereas this study examined the frequencies and latencies of purely offensive behaviors. This method of comparing ratios takes advantage of the fact that while two groups could show equivalent amounts of offensive behavior, differences in defensive behaviors could produce significantly different ratios. In fact, if one compares the time spent engaged in offensive behaviors of short day and long day ovariectomized animals in that study, no effects of day length are seen. Therefore, the apparently "contradictory" results of effect of photoperiod are really in agreement.

It has been shown that ovariectomized females housed in a long photoperiod respond with less aggression towards intact males when treated with E and E+P (Meisel *et al.*, 1988; Takahashi and Lisk, 1985a). The present study corroborates this fact but could not extend those findings to female stimuli, as long day animals showed similar levels of aggression towards other females whether given oil, E or E+P. However, long day animals under those hormonal conditions attacked less frequently than short day animals, independently of the sex of the stimulus animal. Short day animals given E or E+P attacked female stimuli more quickly than did long day animals similarly treated. The ability of photoperiod to modulate rearing in response to E and E+P (as well as in response to P alone) was also observed in this experiment, but the sex of the stimulus animal interacted with this effect of photoperiod. These observations and the fact that short day females attacked other females sooner than males regardless of hormone condition suggest that the sex of the intruder, as well as photoperiod, plays a significant role in the response of aggression exhibited by the experimental animal.

It has been reported that P can both facilitate as well as inhibit aggression (Meisel and Sterner, 1990b). In that experiment, long day females given E followed by two P injections exhibited increased frequency of attacks towards female stimuli after the second P injection. Ciaccio, Lisk, and Reuter (1979) also showed an increase in

aggression towards male stimuli in response to prolonged exposure to P after E priming. This decreased sensitivity to the inhibitory effects of P could be due to down-regulation of P receptors after prolonged exposure to the hormone as seen in the guinea pig (Brown and Blaustein, 1985) and the rat (Parsons, McGinnis and McEwen, 1981). The results of tests 2a and 2b indicate that, for animals housed in a short photoperiod, immediate prior exposure to P is not necessary for the display of aggressive behavior. Animals given E and then a single injection of P remained aggressive to intruder animals of either sex without receiving additional P. However, since the short day animals were not ovariectomized until after they had become acyclic, one may argue that the prolonged exposure to increased levels of endogenous P due to short photoperiod exposure (Bridges and Goldman, 1975) may be enough to prevent the inhibitory effects of P on aggression.

The results of tests 2a and 2b also show that, when given E, long day animals show more lordosis in response to a stimulus animal of either sex than do short day animals, which seem fairly insensitive to the effects of E. When E was followed by P, long day animals responded with lordosis to male stimuli more than did short day animals, while neither group responded to female stimuli. In fact, only one short day animal showed lordosis to a female stimulus, and only one short day animal showed lordosis to a male stimulus. This provides further evidence that photoperiod can strongly modulate the activational effects of E and E+P, while animals kept under a long photoperiod are also sensitive to the sex of the stimulus animal.

The modulatory effect of photoperiod on sensitivity to steroids could play a significant role in the seasonal behavior of hamsters. P levels are known to increase as a hamster enters the winter months and becomes acyclic. This is presumably due to increased interstitial tissue in the ovaries (Bridges and Goldman, 1975), although Jorgenson and Schwartz (1985) reported that P levels increase even before morphological changes in ovarian tissue are evident. Bridges and Goldman (1975) also found increased levels of P similar to that of short day animals in lactating hamsters kept in long days.

Wise (1974) reported that aggression is higher during pregnancy and lactation than during the estrous cycle. Thus, the decreased sensitivity to P seen during short photoperiods may have underpinnings similar to those mechanisms responsible for the apparent decreased sensitivity to the behavioral effects of P during pregnancy and lactation. A reduced sensitivity to the behavioral effects of P may explain why animals kept in short days are aggressive even while experiencing high circulating levels of the hormone.

One mechanism responsible for the reduced behavioral sensitivity to P could be alterations in the number of P receptors. Prior exposure to P may reduce the number of P receptors as mentioned earlier or, since E is known to induce P receptors (Blaustein and Olster, 1989), one may postulate that short days may interfere with such an induction. Some researchers have found no differences in cytosolic E receptors in different species kept in either long or short photoperiods (Callard, Mak and Solomon, 1986; Baum and Schretlen, 1979), while others (Bittman and Blaustein, 1990) have found no reductions in the number of nuclear E receptors or cytosolic P receptors in the hypothalamus/POA of anestrous sheep. There have been reports that progestins implanted into different hypothalamic sites yield differential responses in terms of aggression and sex behavior (Meisel, Fraile and Pfaff, 1990a; Takahashi and Lisk, 1985a). Local alterations in P receptor levels could be occurring within the VMH, anterior hypothalamus, or POA, and these local changes may not be detected in an assay of the entire hypothalamus/POA. Another mechanism that could possibly be altered by exposure to short days is the ability of brain enzymes to metabolize P to less active molecules. Callard *et al.* (1986) reported that aromatase activity in male hamsters is decreased by exposure to short photoperiods. In the brain, P can be metabolized to 5α-dihydroprogesterone and other 5α-metabolites (Karavolas and Herf, 1971). Therefore, it is possible that enzyme action is increased by exposure to short photoperiods resulting in the break down of P into its less active metabolites. A third mechanism, as suggested by Miernicki, Pospichal and Powers

(1990b), could involve changes "downstream" of steroid receptors. In this model, a neural circuit that receives information from a steroid-sensitive area could be the substrate that is affected by a change in photoperiod. Thus, an alteration in function of this second, non-steroid sensitive circuit could produce behavioral changes that seem to be the result of changes in steroid sensitivity. As illustrated in Figure 8, a hypothetical circuit is only functional when the photoperiod-sensitive neuron is stimulated by exposure to long days. That arrangement leads to the stimulation of the target neuron, which results in reduced aggression. Exposure to short days would "turn off" the response of the photoperiod sensitive neuron, and no reduction in aggression would be observed. This model also holds for animals not exposed to steroids, as the steroid sensitive neuron would not be active, resulting in enhanced aggression.

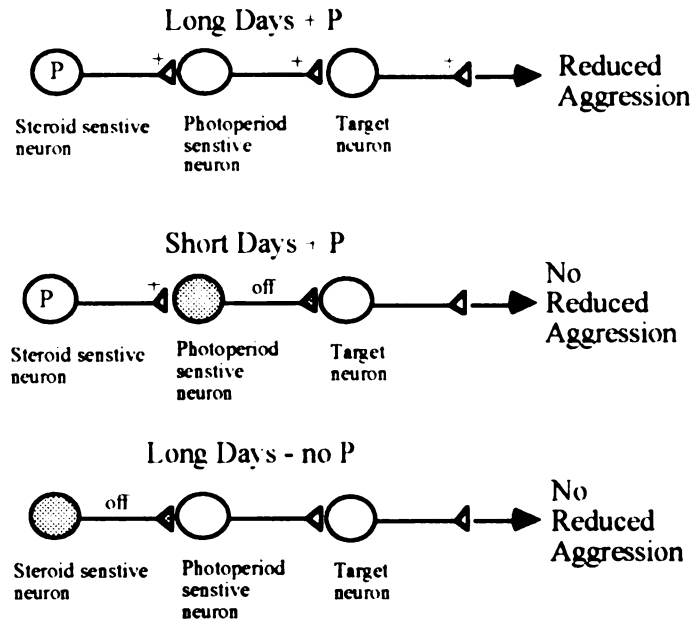

Figure 8. Cartoon illustrating how a neuron "downstream" of a steroid sensitive neuron could influence the output of a target neuron.

Since no lesions completely destroyed the SCN, the original hypothesis about the role of the SCN in the photoperiodic control of behavioral sensitivity to steroids could not be addressed. Histology revealed that some lesions were posterior to the SCN and most were too small, so the surgical parameters were modified for Experiment 2.

Experiment 2

The aim of this experiment is to investigate the role of the SCN in the photoperiodic modulation of lordosis behavior, aggression and energy balance. Given the well-established need for an intact SCN in the display of many photoperiodic responses, it is expected that complete SCN lesions will produce animals equally responsive to E and E+P treatments regardless of photoperiod. It is also expected that such lesions will prevent photoperiod-induced changes in body weight. Animals kept in short days increase their body weight and show a reduced behavioral sensitivity to steroids even after pinealectomies (Bartness and Wade, 1985; Badura and Nunez, 1989). If the results show that hamsters are able to sense changes in photoperiod without an SCN, then it will be proposed that retinal input to other areas of the brain are responsible for photoperiodic influences that are independent of the pineal gland.

Method

The method is identical to that of Experiment 1, except that the coordinates for surgery were altered to 1.1 mm anterior to bregma, 0.3 mm lateral to midline, and 7.6 mm ventral to the dura. The current was applied for 5 seconds more and raised 0.5 mA, resulting in a 1.5 mA current for 15 seconds; 47 animals received lesions. In addition, only males were used as stimuli (n=10), and the effects of P treatment in the absence of E priming were not investigated. Since previous work in the laboratory has shown no difference in sham operated animals and unoperated animals, no sham surgeries were performed, but this unoperated group will be called shams for identification purposes (n=38). The vaginal discharges from all animals were monitored daily. Three animals with lesions continued to show vaginal cycles, and those animals were excluded from all data analyses. Three weeks post-surgery, half of the animals with lesions (n=24) and half of the shams (n=19) were transferred to the short day room (8L:16D). Body weights of

all animals were recorded. Eleven weeks after the animals were transferred, all but 5 short day sham animals had stopped cycling. These 5 animals were excluded from all data analyses. All females were ovariectomized at this time, body weights were recorded, and a 20 mm portion of the left uterine horn was excised from each animal. This piece of tissue was stretched, pinned down, and all excess fat (and attached ovary) was removed. The stretched tissue was trimmed to exactly 15 mm and weighed wet. Any animals that were still cycling (i.e. long day sham animals) were ovariectomized on diestrus. One week after ovariectomy, all females were implanted with 25% E capsules and tested 7 days later at the appropriate times (test 1). The following day, all females received an injection of 20 μg P four hours prior to testing (test 2). These tests comprise the replacement paradigm. In addition, a different hormonal paradigm was utilized. Three weeks after the previous tests were complete (four weeks since initial implantation of the E capsules), the capsules were removed and the animals were tested three days later (3 days w/o E_2). The next afternoon, the animals were given a 20 μg P injection and retested (4 days w/o E_2(+P)). After all of the data were collected, the animals were perfused and lesion placement evaluated as in Experiment 1.

Statistics

The effects of SCN lesions on body weight were analyzed with a 2-factor repeated measures ANOVA (Group X Time), and uterine weights were analyzed with a 2-factor ANOVA (Surgery X Photoperiod). Comparisons following significant F-ratios were made utilizing the Tukey-Kramer method. The effects of these lesions on the proportion of animals responding with attacks, rears and lordosis were evaluated using Fisher's Exact Probability test. Separate analyses were performed for each behavior under each photoperiod (animals with lesions vs. shams) and hormone condition. Within surgical condition, the effects of photoperiod on the proportion of animals responding were also evaluated utilizing Fisher's Exact Probability test.

For animals that responded with behavior (attack, rear and lordosis), the latencies to display each behavior were analyzed within hormone condition using a 2-factor ANOVA (Surgery X Photoperiod) followed by post hoc comparisons using the Tukey-Kramer method. Some animals died before the replacement paradigm tests and more died before the hormonal withdrawal paradigm tests. In addition, animals with incomplete lesions (as described in *Histology* below) were excluded from the data set. The numbers of animals used in the analyses were as follows. For the replacement paradigm tests: Short day lesion group (SDL), n=12; Short day shams (SDS), n=19; Long day lesion group (LDL), n=11; Long day shams (LDS), n=17. For the hormonal withdrawal paradigm tests: SDL=11, SDS=19, LDL=9, LDS=16.

Results

Histology

A lesion was considered to be complete if no clusters of neurons were seen where the SCN would be normally. The lesions that completely destroyed the SCN were homogeneous in size, as smaller lesions resulted in incomplete destruction of the SCN. The lesions typically ranged from the caudal pole of the POA (where the SCN normally begins) through the SCN to the retrochiasmatic region. The lesions encroached upon the optic chiasm ventrally, often damaging the dorsal portion of the chiasm and occasionally severing it, while the dorsal extent of a typically lesion was midway between the SCN and the ventral border of the PVN. Damage to the anterior hypothalamus was limited to the area immediately adjacent to the SCN. Figure 9 illustrates the range in size of the lesions and the typical damage imparted by the lesions. Seven short day animals and nine long day animals were excluded from analyses due to incomplete destruction of the SCN.

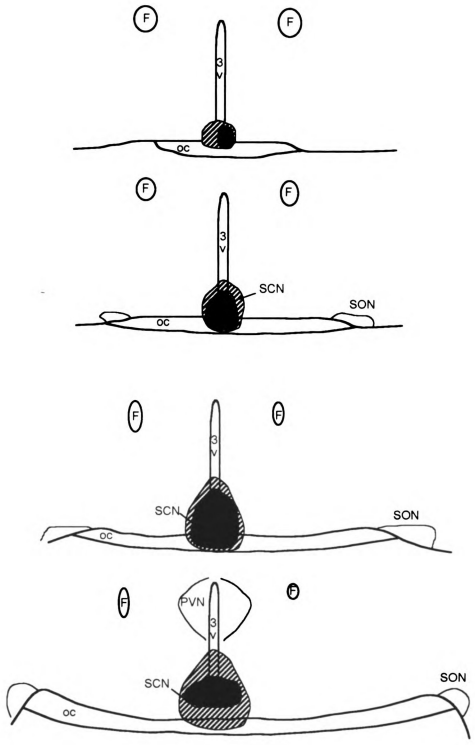

Figure 9. Representative lesions from Experiment 2; small lesion is black, large lesion is hatched. F = fornix, SCN = suprachiasmatic nucleus, 3v = third ventricle, oc = optic chiasm, SON = supraoptic nucleus, PVN = paraventricular nucleus.

Body Weight and Uterine Weight

Complete lesions of the SCN abolished the photoperiod-induced body weight gain seen in animals housed in short days (see Figure 10). The analysis revealed a significant interaction between group (SDS, LDS, SDL or LDL) and time of sampling, and post hoc tests showed that the SDS group gained significantly more weight than any other group ($F_{(3,58)}=4.04$, $p<0.02$). In addition, complete lesions of the SCN prevented short day induced uterine regression (see Figure 11). The analysis of those data showed a main effect of both surgery ($F_{(1,58)}=5.03$, $p<0.03$) and photoperiod ($F_{(1,58)}=12.95$, $p<0.001$), and a significant interaction of those two factors ($F_{(1,58)}=4.34$, $p<0.05$). Post hoc tests showed that only sham animals housed in short days showed uterine regression ($p<0.0001$), with all other groups maintaining similar uterine size.

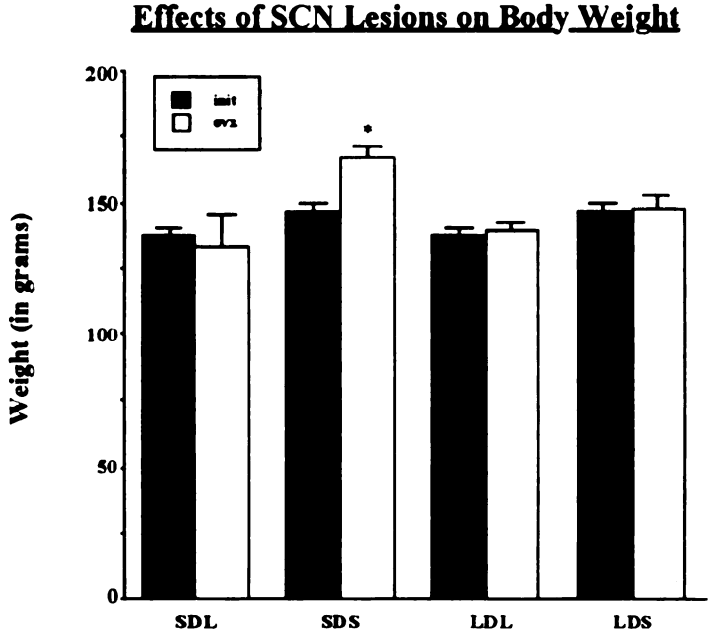

Figure 10. Sham animals kept under a short photoperiod showed a significant weight gain over the 10 week period (*$p<0.02$). Lesions of the SCN blocked this effect of photoperiod. No differences were seen in either group kept under a long photoperiod.

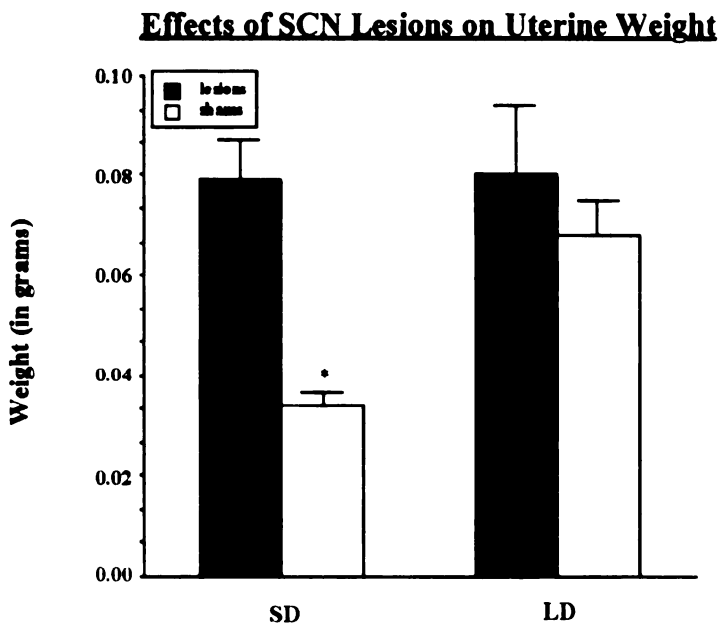

Figure 11. Animals kept in short days had a lower uterine weight (per unit length) than did those kept in long days. Lesions of the SCN abolished this difference between groups under different photoperiods (*significantly different from all other groups; $p<0.0001$).

Socio-sexual Behaviors:

Replacement paradigm

Significantly fewer SDL animals reared than did the SDS animals under both hormonal conditions of the replacement paradigm (E, $p<0.01$ and E+P, $p<0.043$; see Figure 12). No significant differences in the proportion of animals rearing were seen between the two sham groups nor between shams and animals with lesions kept in long days. The latencies to rear are shown in Table 3. No significant group differences were found under either hormone condition. Figure 13 shows the proportion of animals attacking. Again, no significant differences were seen between groups in either hormone condition. Too few animals responded to analyze the data on latency to attack (see Table 3). No statistically significant differences were seen in the proportion of animals exhibiting lordosis when treated with E (see Figure 14). When exposed to P after E priming, however, the difference between SDL and SDS was significant ($p<0.012$).

Replacement Paradigm

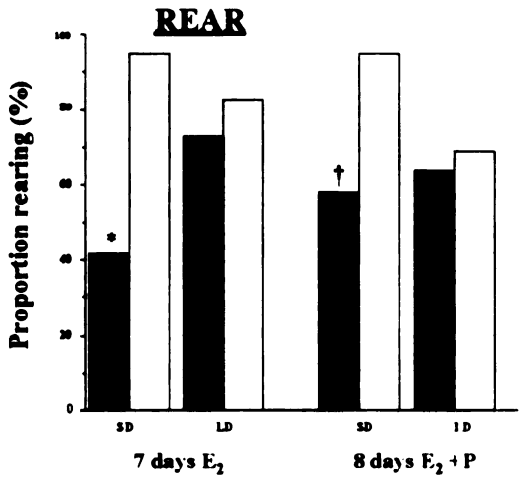

Figure 12. Proportion of animals rearing after 7 days of 25% E_2 (implanted subcutaneously), or after eight days of E_2 followed by an injection of P (20μg). *SDL vs. SDS, $p<0.01$; †SDL vs. SDS, $p<0.03$.

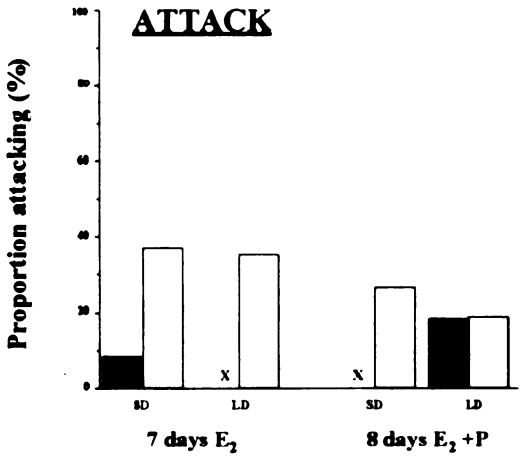

Figure 13. Proportion of animals attacking after 7 days of 25% E_2 (implanted subcutaneously), or after eight days of E_2 followed by an injection of P (20μg). **X** = no respondents.

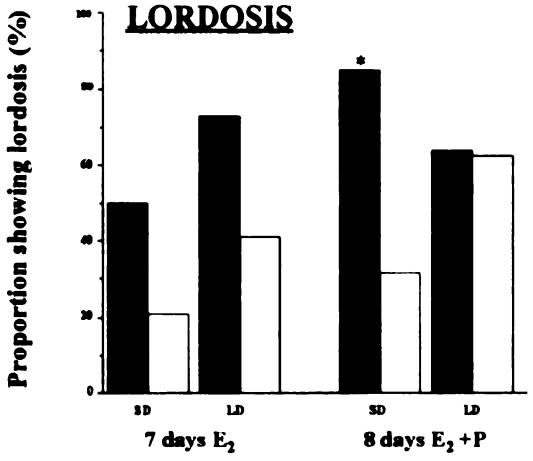

Figure 14. Proportion of animals showing lordosis after 7 days of 25% E_2 (implanted subcutaneously), or after eight days of E_2 followed by an injection of P (20μg). *SDL vs. SDS, $p<0.012$.

Table 3

Latency ($\bar{X}$ ± SEM) to show aggressive behavior or lordosis and number (n) of animals responding for each hormone condition and photoperiod for the hormone replacement tests

PHOTOPERIOD	HORMONE CONDITION	
	E_2	E_2+P
	REAR	
SDS	19.2±7.6 (18)	22.9±4.1 (18)
SDL	18.8±6.0 (5)	57.0±23.1 (7)
LDS	21.9±6.4 (14)	29.5±10.8 (11)
LDL	21.3±6.4 (8)	22.0±8.7 (7)
	ATTACK	
SDS	149.0±28.5 (7)	86.2±16.9 (6)
SDL	246.0 (1)	- (0)
LDS	153.3±30.0 (6)	64.6±21.1 (12)
LDL	- (0)	60.7±20.5 (5)
	LORDOSIS	
SDS	48.8±13.5 (4)	86.2±16.9 (6)
SDL	61.3±23.9 (6)	118.3±32.2 (10)
LDS	40.0±5.8 (7)	64.6±21.1 (10)
LDL	112.6±29.9 (8)	60.7±20.5 (7)

None of the differences between groups in latency to show lordosis were statistically significant (see Table 3).

Withdrawal paradigm

Three days after the E2 capsules were removed, proportionately more shams in short days reared than did shams in long days in response to a stimulus animal ($p<0.01$, see Figure 15). When P was given the following day, more SDS's reared than did SDL's ($p<0.002$). For those that did rear, no significant differences in latency were observed under either hormonal condition (see Table 4). The proportions of animals that attacked are shown in Figure 16. It was found that fewer animals in the SDL group attacked than did those in the SDS group under both hormonal conditions. Because only 2 animals from each lesion group attacked, the latency data were not analyzed. Table 4 lists the latencies to attack of those that showed the behavior. Under the 4 days w/o E_2 (+P) condition, a main effect of surgery was found ($p<0.01$), as fewer animals with lesions attacked than did shams. No effects of photoperiod were seen in this condition.

Figure 17 summarizes the proportions of animals displaying lordosis. Under the 3 days w/o E_2 condition, more animals in the SDL group responded with lordosis than did those in the SDS group. In addition, a photoperiodic difference was noted as SDS's responded less than did LDS. No difference was seen between the two groups with lesions, nor between the LDL's and LDS's. Under the 4 days w/o E_2 (+P) condition, more SDL's showed lordosis than did SDS's ($p<0.002$), and a lesion effect is suggested, as those animals with lesions housed in long days were more apt to show lordosis than were shams, although the difference was not significant. Table 4 shows the latencies to respond of those that displayed lordosis; too few shams showed the behavior, therefore the data were not analyzed.

Withdrawal Paradigm

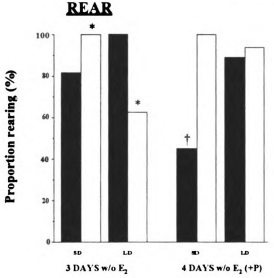

Figure 15. E_2 capsules were removed and animals tested 3 days later. The next day, animals were given an injection of progesterone, and tested 4 hr later. *SDS vs. LDS, $p<0.01$; †SDS vs. SDL, $p<0.002$.

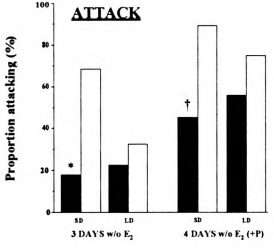

Figure 16. E_2 capsules were removed and animals tested 3 days later. The next day, animals were given an injection of progesterone, and tested 4 hours later. *SDL vs. SDS (3 days), $p<0.01$; †SDL vs. SDS (4 days), $p<0.002$.

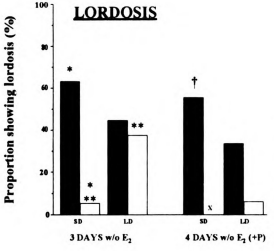

Figure 17: E_2 capsules were removed and animals tested 3 days later. The next day, animals were given an injection of progesterone, and tested 4 hours later. *SDL vs. SDS, $p<0.002$; **SDS vs. LDS, $p<0.05$; †SDL vs. SDS, $p<0.002$. **X** = no respondents.

Table 4
Latency ($\bar{X}$ ± SEM) to show aggressive behavior or lordosis and number (n) of animals responding for each hormone condition and photoperiod for hormone withdrawal tests

PHOTOPERIOD	HORMONE CONDITION	
	3 Days w/o E_2	4 Days w/o E_2 (+P)
	REAR	
SDS	44.9± 9.1 (19)	30.6±11.4 (19)
SDL	43.1±15.0 (9)	40.8±18.1 (5)
LDS	55.0±16.6 (10)	65.7±17.7 (15)
LDI	34.3±5.32 (9)	44.4±9.2 (8)
	ATTACK	
SDS	135.6±14.5 (13)	117.5±18.1 (17)
SDL	287.0±3.0 (2)	213.4±32.2 (5)
LDS	237.8±15.7 (5)	127.3±19.5 (12)
LDL	107.0±24.0 (2)	185.4±31.4 (5)
	LORDOSIS	
SDS	209 (1)	(0)
SDL	79.4±22.8 (7)	59.7±10.3 (6)
LDS	45.8±17.8 (6)	93 (1)
LDL	140.3±42.6 (4)	156±64.2 (3)

Discussion

Body Weight & Uterine Weight

Intact Syrian hamsters show an increase in body weight when exposed to a short photoperiod (Bartness and Wade, 1984). Ablation of the SCN prevented short day-induced body weight gain and also prevented uterine regression. While uterine regression was most likely blocked by disrupting information about photoperiod to the pineal gland, the effect of SCN lesions on body weight requires an alternative explanation, since an increase in body weight in pinealectomized animals housed in short days has been reported (see Bartness and Wade, 1985, for a review). Thus, SCN lesions may have prevented short-day induced obesity independently of any effects on the regulation of the pineal gland. The results of Experiment 2 show that when fed laboratory chow, animals with SCN lesions kept in short days do not gain weight and that animals with similar lesions housed in long days do not become obese. These data therefore provide evidence that the effect of photoperiod on body weight can be removed by SCN lesions without the complication of the obesity encountered in animals with PVN lesions.

Since lesions of the SCN block the effect of short days on body weight gain and do not induce obesity as do PVN lesions, the effect of SCN lesions could be explained by at least two models for an anatomical substrate. First, fibers from the retina that terminate in the SCN could be relaying photoperiodic information via SCN efferents that project to the PVN and to other hypothalamic regions (Stephan *et al.*, 1981) known to play a role in the control of metabolism and energy balance (see below). Lesions such as the ones made in Experiment 2 would remove the influence of retinal information as interpreted by the SCN. Alternatively, fibers from the retina may pass through the SCN and terminate in or near the PVN, providing data about the prevailing photoperiod directly. Retinal fibers have been shown to project to the parvocellular portion of the

PVN, as well as to periventricular regions caudal to the SCN (Youngstrom *et al.*, 1991). SCN lesions could interrupt all retinal projections to the PVN. In future experiments, verification of the removal of retinal input could be made with a tract-tracing tool such as horseradish peroxidase (HRP). HRP injected into the eye after such lesions would provide evidence for removal of direct retinal input to the PVN by SCN lesions if no labeling were to be seen in the PVN. Likewise, the application of HRP to the PVN should label retinal ganglion cells, and thus would provide and assessment of damage to fibers of passage of retinal origin by SCN lesions.

Because the PVN can regulate body weight via endocrine and autonomic mechanisms (see General Introduction), and because the PVN receives information about the prevailing photoperiod either directly or via SCN efferents, lesions such as those made in Experiment 2 may have blocked short day-induced body weight gain by disrupting retinal information to the PVN. Alternatively, the lesions may have interrupted outputs from the PVN that may modulate body weight in response to photoperiodic information. In the hamster, some descending fibers from the PVN to sympathetic preganglionic cell bodies take a ventromedial trajectory along the third ventricle (Youngstrom and Nunez, 1992). The lesions made in this study most likely disrupted this pathway, and such damage may have contributed to removal of the effect of short days on body weight gain. So even if retinal information had reached the PVN through a route other than that through the SCN area (which was destroyed by these lesions), any subsequent information that had exited the PVN via the ventromedial fiber bundle would have been interrupted. Since short photoperiods alter energy metabolism via a pineal-independent process (Bartness and Wade, 1984), measures of energy metabolism could be another endpoint used to assess further the role of the SCN in pineal-independent phenomena.

Behavior

The behavioral data from the replacement paradigm of Experiment 2 failed to replicate the effects of photoperiod on lordosis and aggression seen previously (see Experiment 1, also Badura and Nunez, 1989; Badura *et al.*, 1987b), as the sham animals from opposing photoperiods did not behave differently when exposed to exogenous steroids. This could be due to the amount of E given, which was a "threshold" dose to which approximately 50% of long day- housed animals and 10% of short day-housed animals responded in other experiments (Badura and Nunez, 1989). This amount of E could have been too low for this particular group of hamsters, but such a dose was utilized in order to prevent a masking of photoperiodic effects by high levels of E. Since the testing of the original hypothesis depended upon detecting a difference between intact animals in long days and short days, any difference between short day animals with lesions and short day shams cannot be attributed solely to a removal of photoperiodic influences. Perhaps by increasing power (i.e. increasing the number of animals in each group) or by increasing the concentration of E in the implanted capsules, a significant difference between shams in opposing photoperiods would be observed, allowing the hypothesis to be tested under a hormone replacement paradigm.

Under the hormone withdrawal paradigm, a robust photoperiod effect on the display of lordosis was seen in the 3 days w/o E_2 condition, and lesions blocked the inhibitory effect of short days. In addition, aggressive behaviors were attenuated in short day animals with lesions. These data by themselves suggest that an SCN-dependent mechanism is necessary for photoperiod to modulate the behavioral sensitivity to steroids. However, this relatively simple interpretation must be revised when the data from the 4 days w/o E_2 + P condition are considered. Once again, no difference between shams in opposing photoperiods was observed in the proportion that showed lordosis (Figure 17). Yet, animals with lesions in short days still show an increase in behavioral responsiveness when compared to shams, and animals with lesions in long days show a

trend in that direction. It is most likely, then, that the lesions used here further facilitated lordosis in addition to removing any effects of photoperiod upon that behavior.

Results of lesion studies suggest that electrolytic lesions of the POA and anterior hypothalamus can increase sexual behavior and lesions of the POA can decrease aggression (Rodriguez-Sierra and Terasawa, 1979; Hammond and Rowe, 1976). The lesion effect on lordosis could be caused by disruption of fibers from the POA to the VMH. It is known that E_2-concentrating neurons in the POA project to the VMH (Corodimas and Morrell, 1990), and that stimulation of the POA suppresses lordosis (Malsbury, Pfaff and Malsbury,1980), while ablation of the POA in E-primed rats facilitates lordosis (Powers and Valenstein, 1972). The VMH is intimately involved in the lordosis response in female hamsters, as lesions of the VMH eliminate the lordosis response (Malsbury, Kow and Pfaff, 1977), while implants of E in the VMH stimulate lordosis (Floody, Blinn, Lisk and Vomachka, 1987; Takahashi and Lisk, 1985b; DeBold, Malsbury, Harris and Malenka, 1982). Some fibers from the POA to the VMH pass near the SCN in the anterior hypothalamus (Malsbury *et al.*, 1980), and thus could have been damaged by the lesions made in this experiment. It appears as if these fibers run rostrocaudally in a near-horizontal plane, since horizontal knife cuts between the SCN and PVN did not produce a similar facilitation of lordosis (Badura *et al.*, 1987a). Anterior hypothalamic lesions in rats have been reported to both enhance sexual behavior in response to E (Rodgers and Schneider, 1978) and have no effect (Mathews and Edwards, 1977). However, any facilitation of lordosis as a result of anterior hypothalamic lesions reported in the former was thought to be due to the disruption of fibers from the POA to the VMH.

Lesions restricted to the septum in hamsters have also been reported to enhance the effects of large doses of E followed by P on the display of lordosis (Vomachka, Richards and Lisk, 1982). Since electrical stimulation of the septal area suppresses the display of lordosis (Zasorin, Malsbury and Pfaff, 1975), it is thought that lesions in this

area remove a tonic inhibition of the lordosis response. Ovarian steroids would then suppress the inhibitory signal from the septum, resulting in a facilitation of lordosis. In the rat, fibers from the septum have been shown to project to the VMH as well as to the POA (Fahrbach, Morrell and Pfaff, 1989; Kita and Oomura, 1982; see also Luiten *et al.*, 1987). These studies have utilized the injection of HRP into the VMH and POA to identify cell bodies in the lateral septum. Lesions made in Experiment 2 might damage fibers from the septum to the VMH, contributing to the 'lesion effect' seen in this study. However, the septum could be acting through neurons in the POA, in which case the SCN lesions would not be disrupting septal fibers directly, but could affect the display of lordosis by damaging POA neurons as previously described.

Lesions may affect steroid-dependent behaviors by altering the metabolic fate of the hormones. For example, rats that had received retrochiasmatic lesions showed elevated serum E_2, and metabolized ^3H-E_2 at a slower rate than did intact rats (Rodgers and Chatterton, 1978). In ovariectomized rats given estradiol cypionate (EC), liver tissue from neurally intact animals showed a similar rate of steroid uptake to that of rats with retrochiasmatic lesions. However, more EC was metabolized in the former (Rodgers and Chatterton, 1978). The lesions made in Experiment 2 could have the same effects as these knife cuts, in which case E would be metabolize more slowly than than in intact animals, and thus the animal is exposed to E for a longer period of time. Regrettably, levels of circulating E were not measured in this experiment. In the future, measurements of E in the serum could be used to test this hypothesis as well as to monitor the amount of E actually maintained by the 'threshold dose' that was utilized. It is also possible that the lesions are causing the effects of E to linger as opposed to E itself . For example, E is known to induce P receptors (Blaustein and Olster, 1989), and lesions of the SCN area could cause a prolonged up-regulation of P receptors. Similarly, the lesions could be preventing the degradation of P receptors. The latter theory seems to be most likely, as lordosis was observed in animals with lesions 4 days after E had been removed.

The ability of SCN lesions to block the effect of photoperiod on the display of lordosis, as seen in the 3 days w/o E condition, could be due to the diruption of retinal fibers passing through the SCN to other hypothalamic areas. In the hamster, fibers from the retina do project to other areas of the hypothalamus, including the anterior hypothalamus, the VMH, and the POA (Youngstrom *et al.*, 1991). While none of the lesions made in Experiment 2 significantly damaged the POA, retinal fibers to the POA travel through the SCN before continuing rostrally (Youngstrom *et al.*, 1991). Thus, lesions confined to the SCN could still disrupt retinal information to more rostral areas. In the rat, efferents of the SCN have been shown to project to the VMH (Swanson and Cowan, 1975) and the POA (Watts, Swanson and Sanchez-Watts, 1987). If the efferents to the VMH are involved in an inhibitory action to the lordosis response, then lesions in the SCN would result in facilitation of sexual behavior. Likewise, if the efferents to the POA are involved in and excitatory circuit, then removal of their influence would lessen the inhibition of the VMH by the POA. The effect of a short photoperiod on these efferents would then be stimulatory.

In order to get around the problem of disrupting fibers of passage with lesions of the SCN, one could employ chemical lesions in areas of the brain that may in turn send projections through the SCN area. For example, axon-sparing lesions could be made in the POA, the septum or other areas in animals kept under different photoperiods. These chemical lesions would spare fibers of passage from areas further upstream, and thus one could tease out the effects of photoperiod on animals without a certain population of steroid-concentrating neurons. In this manner, one could differentiate the role of fibers that pass through the SCN and the role of cells and their projections that originate in the SCN. Unfortunately, neurons (and glia) of the SCN seem to be resistant to excitatory neurotoxins such as n-methyl aspartic acid (Hastings, Roberts and Herbert 1985) and kainic acid (Peterson and Moore, 1980), which precludes the use of axon-sparing lesions in the SCN proper.

Summary and Future Research

While the original hypothesis could not be tested in these two experiments due to misplaced lesions or to the lack of an effect of photoperiod on the neurally intact animals, new information was obtained. The results of Experiment 1 indicate that the effects of P without E priming on aggressive behavior are modulated by photoperiod. From Experiment 2, the results show that SCN lesions block the effect of photoperiod on body weight, and that animals with SCN lesions fail to show a reduced behavioral sensitivity to gonadal steroids when kept under a short photoperiod.

None of these results, however, can address the phenomenon of spontaneous recrudescence, in which animals in short days will return to a state of reproductive competence after being in a short photoperiod for approximately 20 weeks. Interestingly, sexual behavior returns before the gonads are functional (Honrado *et al.*, 1991). Short day-induced body weight gain is also subjected to "recrudescence" after 15-17 weeks (see Bartness and Wade, 1985). It is probable that the reduction in sensitivity to P alone also becomes refractory after extended exposure to short days. Perhaps one could exploit the mechanisms of behavioral recrudescence to understand the original phenomenon of behavioral regression.

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