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THE EFFECTS OF PHOTOPERIOD ON MEDIAN EMINENCE DOPAMINE AND TUBEROINFUNDIBULAR NEURONAL ACTIVITY IN THE MALE SYRIAN HAMSTER (*MESOCRICETUS AURATUS*)

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ABSTRACT

THE EFFECTS OF PHOTOPERIOD ON MEDIAN EMINENCE DOPAMINE AND TUBEROINFUNDIBULAR NEURONAL ACTIVITY IN THE MALE SYRIAN HAMSTER (*MESOCRICETUS AURATUS*)

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Seasonal changes in daylength affect reproductive physiology and behavior in Syrian hamsters. One of the systems affected by these changes in daylength is the tuberoinfundibular dopamine (TIDA) neuronal system. The experiments presented here examined the effects of daylength (photoperiod) on TIDA neurons to determine the mechanisms responsible for photoperiod-induced changes.

TIDA neurons are located in the arcuate nucleus of the hypothalamus and send projections to the median eminence (ME). In male hamsters, exposure to a short photoperiod (i.e., less than 12.5 hours of light/day) for 12 weeks resulted in a decrease in ME dopamine concentrations. This decrease in dopamine was not due to a decrease in dopamine synthesis or an increase in metabolism by TIDA neurons. The number of tyrosine hydroxylase producing neurons in the arcuate nucleus of male hamsters also was not affected by exposure to a short photoperiod. Time course experiments revealed that in males ME dopamine concentrations slowly decline and are

significantly decreased after 4-6 weeks of short-photoperiod exposure, again without a concurrent reduction in synthesis.

In female hamsters housed in a short photoperiod for 12 weeks, there were no changes in either ME dopamine concentrations, or in dopamine metabolism by TIDA neurons. This suggests that there may be a gender difference in the photoperiodic regulation of TIDA neurons and in the hormones these neurons modulate.

Indirect estimates of dopamine release from TIDA neurons suggest that dopamine release from the ME is increased in males exposed to a short photoperiod. An increase in the release of dopamine from the ME without a compensatory increase in dopamine synthesis may be responsible for the depletion of dopamine stores seen when males are exposed to a short photoperiod. The increased release of dopamine from TIDA neurons may also be responsible for the decrease seen in a number of hormones normally inhibited by this transmitter.

To my fellow graduate student and husband Mike who is not only an excellent scientist and collaborator, but also my number one supporter and best friend.

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KEY TO ABBREVIATIONS

Abbreviation	Term or Phrase
ANOVA	analysis of variance
Arc	arcuate nucleus
COMT	catechol-O-methytransferase
CP	caudate-putamen
cPeri	caudal periventricular nucleus
CVs	coefficients of variation
DAB	diaminobenzadine
DA	dopamine
DOPA	3,4-dihydroxyphenylalanine
DOPAC	3,4-dihydroxyphenylacetic acid
GnRH	gonadotropin-releasing hormone
HPLC-ED	high performance liquid chromatography with electrochemical detection
ICC	immunocytochemistry
i.m.	intramuscular (injection)
i.p.	intraperitoneal (injection)
LAAD	l-aromatic amino acid decarboxylase
MAO	monoamine oxidase
MBH	medial-basal hypothalamus
ME	median eminence
αMPT	α-methylparatyrosine
PB	sodium phosphate buffer
PBS	sodium phosphate buffered saline
PHDA	periventricular-hypophyseal dopamine (neurons)
SOS	sodium octyl sulfate
TBS	Tris buffered saline
TH	tyrosine hydroxylase
TH+	tyrosine hydroxylase immunopositive
TIDA	tuberoinfundibular dopamine (neurons)
TSH	thyroid stimulating hormone
Tx	Triton-X

INTRODUCTION

In the Syrian hamster, exposure to short-photoperiods (less than 12.5 hours of light per day) dramatically affects reproductive physiology and behavior. In the male these changes include decreases in circulating gonadal steroid levels (Desjardins, Ewing, & Johnson, 1971), gonadotropins (Turek, Elliott, Alvis, & Menaker, 1975), prolactin (Goldman, Matt, Roychoudhury, & Stetson, 1981) and thyroid-stimulating hormone (TSH; Vriend, 1985), as well as regression of the testes (Gaston & Menaker, 1967) and behavioral reproductive quiescence (Morin & Zucker, 1978). In the female hamster, exposure to short photoperiod results in decreases in circulating levels of estrogen and prolactin (Widmaier & Campbell, 1981), daily surges of gonadotropins (Bridges & Goldman, 1975), and anestrus (Seegal & Goldman, 1975). These photoperiod-induced changes in reproductive physiology are the result of an increase in the duration of the nocturnal melatonin rise in short photoperiod (Tamarkin, Westrom, Hamill, & Goldman, 1976). The pineal is the main source of melatonin, and pinealectomy prevents the effects of photoperiod on reproductive functions (Reiter, 1973/74).

Many of the effects of exposure to short days (e.g., changes in gonadotropin and prolactin secretion) involve the

hypothalamic-pituitary-gonadal axis. The release of the gonadotropins and prolactin from the anterior pituitary is regulated by the hypothalamus, and changes in the release of pituitary hormones in turn affect gonadal steroid synthesis and release (for review see; Everett, 1988). The purpose of the research discussed in this dissertation is to examine the effects of photoperiod on the hypothalamus, in particular, dopaminergic activity in the median eminence (ME). Dopamine released into the median eminence is carried to the anterior pituitary in the hypophyseal portal blood where it tonically inhibits the release of prolactin (Ben-Jonathan, 1985). Dopamine also can inhibit the release of gonadotropin-releasing hormone (GnRH) from the ME (Rasmussen, 1991) and TSH from the anterior pituitary (for review see; Krulich, 1982). Therefore, it is possible that the short-photoperiod induced decrease in the release of these hormones is related to changes in ME dopamine.

Dopamine Neurochemistry

Dopamine is synthesized from the amino acid precursor tyrosine which is actively transported from the blood into monoaminergic neurons where it is converted to 3,4-dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase (TH; See Figure 1). The rate at which TH converts tyrosine to DOPA (or the activity of TH) is the rate limiting step in the synthesis of dopamine (for review see; Cooper, Bloom, & Roth, 1991). In the terminals of dopaminergic neurons, DOPA is converted to dopamine by

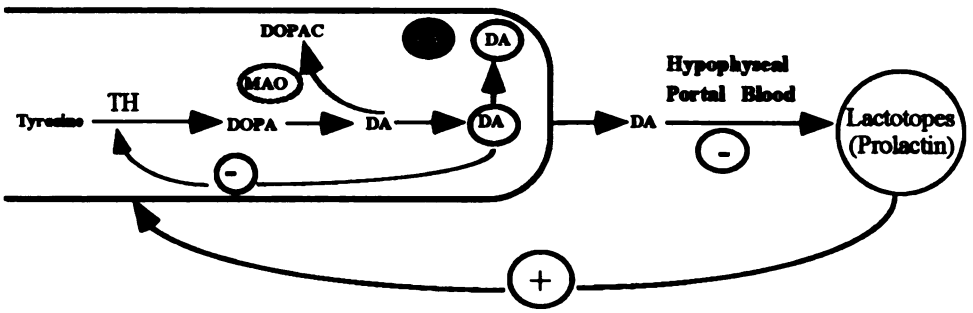

Figure 1. The synthetic and metabolic pathway of dopamine in a tuberoinfundibular dopamine (TIDA) neuron. In TIDA neurons, tyrosine is taken into the terminal and converted to 3,4-dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase (TH). DOPA is then converted to dopamine (DA) by the enzyme l-aromatic amino acid decarboxylase. Dopamine can be released into the hypophyseal portal blood, stored in releasable pools (in white) or nonreleasable pools (in black), or metabolized by monoamine oxidase (MAO) to form 3,4-dihydroxyphenylacetic acid (DOPAC). Cytoplasmic pools of DA feedback onto TH to regulate its activity (i.e., end-product inhibition). The activity of TIDA neurons is also stimulated via prolactin positive feedback onto these neurons.

L-aromatic amino acid decarboxylase (LAAD). Because the conversion of DOPA to dopamine occurs so quickly in the brain, it is impossible to measure DOPA concentrations without blocking the activity of LAAD (Carlsson, Davis, Kehr, Lindqvist, & Atack, 1972).

Dopamine synthesis is tightly regulated through a number of mechanisms. Regulation of synthesis by end-product inhibition involves the negative feedback of intraneuronal dopamine onto the enzyme TH activity. As cytoplasmic levels of dopamine are increased (either through an increase in newly synthesized dopamine or through the reuptake of released dopamine), dopamine binds to TH thereby preventing the binding of a tetrahydrobiopterin cofactor and

inhibiting the activity of the enzyme. As dopamine is released and cytoplasmic levels of dopamine are decreased, there is a decrease in dopamine binding to TH which leaves the enzyme free to bind to the tetrahydrobiopterin cofactor. Binding of the cofactor to the enzyme stimulates TH activity (for review see; Roth, Walter, Murrin, & Morgenroth, 1975). Concurrently, however, extraneuronal dopamine in the synaptic cleft acts at the D_2 autoreceptor to inhibit both dopamine release and TH activity (Wolf & Roth, 1990). It is this coupling between synthesis and release that keeps intraneuronal stores of dopamine fairly constant under basal and stimulated conditions.

Once synthesized, dopamine can be released, stored or metabolized. Newly synthesized dopamine is preferentially released over dopamine that has been stored in the terminal (Gudelsky & Porter, 1979). However, newly synthesized dopamine can also be vessicled and stored within the terminal. These stores of dopamine have been referred to as the regulatory pool of dopamine (Cooper, et al., 1991). Some of this pool is readily available for release when the neuron is activated (i.e., releasable pool of dopamine), however, some of this dopamine is stored in what has been called a nonreleasable pool. This nonreleasable pool of dopamine is not readily released, even with chronic stimulation of the neuron (Fekete, Szentendrei, Herman, & Kanyicsak, 1980). Dopamine can also be metabolized via a number of different mechanisms. First, dopamine can be

metabolized by monoamine oxidase (MAO) before it is sequestered in vesicles to form one of its major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC; Kopin, 1985). DOPAC concentrations represent the catabolism of newly synthesized dopamine, and of dopamine that has been recovered via the reuptake system (i.e., dopamine released into the synapse may be taken back into the terminal and metabolized by MAO to form DOPAC; Roth, Murrin, & Walters, 1976). Dopamine can also be metabolized extraneuronally by catechol-O-methyl transferase (COMT) to form 3-methoxytyramine, or it can be converted to homovanillic acid by the actions of MAO and COMT (Kopin, 1985).

The activity of dopaminergic systems can be assessed using a number of different techniques. One strategy involves measuring the accumulation of dopamine stores after dopamine metabolism has been blocked with a MAO inhibitor such as pargyline. Neurons which are more active should accumulate more dopamine after an injection of a MAO inhibitor (Alexiuk & Vriend, 1991). The problem with this method is that an increase in the cytoplasmic levels of dopamine will inhibit TH activity and dopamine synthesis via end product inhibition, resulting in an inaccurate estimation of what the activity of the system would have been under physiological conditions. TH activity is suppressed as early as 30 minutes after pargyline administration (Carlsson, Kehr, & Lindqvist, 1976).

Another method used to assess dopaminergic activity is

to measure dopamine turnover, or the amount of time it takes neurons to completely replenish stores of dopamine after inhibiting dopamine synthesis. Because dopamine synthesis and release are usually tightly coupled, turnover can be estimated by measuring the decline in dopamine stores after the injection of a TH inhibitor [i.e., α-methylparatyrosine (αMPT)]. Previous work has shown that the decline of dopamine is linear up to 120 minutes after αMPT injection in whole rat brain preparations (Brodie, Costa, Dlabac, Neff, & Smookler, 1966) and in the ME (Rance, Wise, Selmanoff, & Barraclough, 1981). The slope of the linear decline in dopamine is referred to as the rate constant. To calculate turnover, the rate constant is multiplied by the concentration of dopamine in animals injected with saline and the inverse of this product serves as an index of turnover time (Brodie, et al., 1966). When this method is used to compare dopaminergic neuronal activity across groups, the initial concentrations of dopamine must be equal. If the initial dopamine concentrations are not equal, turnover should not be calculated, and rate constants should be used to compare treatment groups (Brodie, et al., 1966).

The third method which can be used to estimate dopamine neuronal activity is to measure the accumulation of DOPA after inhibiting LAAD activity with m-hydroxybenzylhydrazine dihydrochloride (NSD 1015; Carlsson, et al., 1972). Neurons which are more active will accumulate more DOPA than

neurons which are less active. DOPA accumulation can be taken as an index of TH activity (Carlsson, et al., 1972) and has been used as an index of dopamine synthesis in the ME (Demarest & Moore, 1980).

Finally, a method commonly used to assess dopaminergic activity in the rat involves measuring DOPAC concentrations in the terminal regions of dopamine neurons. Previous studies have shown that as activity increases in dopamine neurons, so do DOPAC concentrations (Karoum, Neff, & Wyatt, 1977; Lookingland, Gunnet, & Moore, 1987a; Lookingland, Jarry, & Moore, 1987b). The advantage of using this method to assess activity of dopaminergic neurons is that DOPAC can be measured without disrupting the synthetic or metabolic pathways of dopamine neurons with drugs. The disadvantage of using DOPAC concentrations to assess the activity of dopaminergic neurons is that changes in DOPAC could possibly be due to increased levels of MAO (Lookingland, et al., 1987b) rather than changes in the activity of dopaminergic neurons per se. Therefore, if DOPAC is going to be used to estimate dopaminergic activity, the results should be validated using other methods to establish that the changes in DOPAC are not just due to changes in the levels of MAO.

Several dopaminergic systems have been identified in the mammalian brain. The major groups of dopamine neurons are labelled A_{8-16} (Hökfelt, Martensson, Björklund, Kleinau, & Goldstein, 1984). The largest group of dopamine neurons make up the mesotelencephalic systems (A_{8-10}). Neurons of

these systems are located in the substantia nigra and ventral tegmentum, and send axonal projections to regions of the forebrain including the striatum and nucleus accumbens (for review see; Lindvall & Björklund, 1976), and are involved in the regulation of motor activity, reward and motivation. The A_{11} dopamine neurons are located in the caudal and dorsal portions of the sub-thalamus and send axonal projections to the spinal cord (Björklund, Moore, Nobin, & Stenevi, 1973). The incertohypothalamic (A_{13}) dopaminergic neurons are located in the medial zona incerta and send axonal projections to various regions within the rostral diencephalon including the lateral septum, the vertical and horizontal diagonal bands and the lateral preoptic and hypothalamic regions. The A_{13} dopamine neurons also project to the central amygdala, and possibly to the midbrain central gray, and the parvocellular region of the paraventricular nucleus of the hypothalamus (Eaton, Wagner, Moore, & Lookingland, 1993; Wagner, Eaton, Moore, & Lookingland, 1993). The A_{13} neurons have been implicated in the regulation of gonadotropin release (MacKenzie, Hunter, Daly, & Wilson, 1984; MacKenzie, James, & Wilson, 1988). The A_{14} or periventricular dopamine neurons are divided into two groups. The rostral periventricular neurons are located in the anterior portion of the hypothalamus. These neurons appear to synapse on other neurons within the periventricular nucleus, and they also send projections to the suprachiasmatic nucleus and the medial preoptic nucleus

(Van Den Pol, Herbst, & Powell, 1984). The caudal A_{14} neurons (or periventricular hypophyseal dopamine neurons; PHDA neurons) have cell bodies located ventral to the paraventricular nucleus (Kawano & Daikoku, 1987), and send projections to the intermediate lobe of the pituitary (Goudreau, Lindley, Lookingland, & Moore, 1992). The PHDA neurons are involved in the regulation of peptide release (e.g., αMSH) from melanotropes in the intermediate lobe of the pituitary (for review see; Millington & Chronwell, 1988). The A_{15} dopamine neurons are located in the ventral diencephalon. The most rostral A_{15} neurons are located in the ventral portion of the bed nucleus of the stria terminalis. Cell bodies from this group of neurons extend caudally to the posterior hypothalamus. The A_{16} or periglomular dopamine neurons are located in the olfactory bulb and frontal cortex (Hökfelt, et al., 1984).

The final group of dopaminergic neurons, the tuberoinfundibular dopamine (A_{12}; TIDA) neurons, are located in the arcuate nucleus of the hypothalamus and send axonal projections to the ME (Björklund, et al., 1973; Fuxe, 1963). Dopamine released by TIDA neurons is carried in the hypophyseal portal blood to the anterior pituitary where it tonically inhibits the release of prolactin from lactotropes (For review see; Ben-Jonathan, 1985). The inhibition of prolactin release by dopamine is regulated via a positive feedback loop between prolactin and TIDA neurons. As the amount of circulating prolactin is increased, TIDA neuronal

activity (synthesis and release) also increases (for review see; Moore, 1987). Dopamine from TIDA neurons might also be involved in inhibiting the release of TSH from anterior pituitary thyrotropes (for review see; Krulich, 1982) and the release of GnRH from the ME (Rasmussen, 1991).

Studies done in the rat have shown that TIDA neurons are different from other groups of dopaminergic neurons in a number of ways. Experiments measuring (^{3}H) dopamine uptake in ME fragments (Demarest & Moore, 1979b) and ME synaptosomal preparations (Annunziato, LeBlanc, Kordon, & Weiner, 1980) have shown that TIDA neurons do not have a high affinity reuptake mechanism, so DOPAC concentrations in this region are completely dependent upon the metabolism of newly synthesized dopamine (Lookingland, et al., 1987b). Because dopamine released from TIDA neurons is almost immediately picked up the hypophyseal portal blood, and little is left in the ME to be metabolized, intraneuronal DOPAC is the only measurable metabolite of dopamine in the ME (for review see; Moore, 1987). Another difference between TIDA neurons and other dopaminergic neurons is that TIDA neurons do not have D_2 autoreceptors. Studies examining the effects of dopamine agonists and antagonist show that although other systems, such as the nigrostriatal system, show rapid changes in activity in the presence of dopamine agonists and antagonists, the response of TIDA neurons to these drugs is very slow (Umezu & Moore, 1979). In hypophysectomized animals, dopaminergic agonists and

antagonists have no effects on TIDA neurons, indicating that the effects of these drugs on TIDA neuronal activity are mediated via changes in anterior pituitary hormone feedback onto TIDA neurons (Demarest & Moore, 1980).

The third major difference between TIDA neurons and other dopaminergic neurons is that although TH activity does seems to be regulated by end-product inhibition in TIDA neurons (Demarest & Moore, 1979a), synthesis and release are not always as tightly coupled in TIDA neurons as in other dopamine neurons (e.g. nigrostriatal dopamine neurons; Roth, et al., 1975). For example, in female rats, prolactin release is increased by chronic (12 days) estrogenic stimulation. In these animals, ME DOPA accumulation is unchanged, but ME dopamine concentrations are significantly reduced, indicating that under those conditions, synthesis does not compensate for the increase in release (Demarest, Riegle, & Moore, 1984). Other situations that increase prolactin release, such as suckling (Demarest, McKay, Riegle, & Moore, 1983) and morphine treatment (Alper, Demarest, & Moore, 1980) also appear to disrupt the tight coupling between dopamine synthesis and release in TIDA neurons.

Because prolactin release is inhibited by dopamine from TIDA neurons, and prolactin in turn feeds back onto TIDA neurons to stimulate their activity, it is informative to measure both variables in the same animal. Physiological circulating levels of prolactin and TIDA neuronal activity

cannot be assessed in the same animal if drugs are used to alter dopamine synthesis or metabolism because drug-induced changes in dopamine release will result in changes in circulating prolactin levels. Using DOPAC concentrations to assess TIDA neuronal activity is the only way dopaminergic activity and prolactin can be measured concurrently in the same animal.

Dopamine and Photoperiod

Numerous studies have assessed the role of dopamine and TIDA neurons in the regulation of prolactin in the rat (for review see; Ben-Jonathan, 1985), however, little is known about this system in the Syrian hamster. Because the hamster is a photoperiodic animal and photoperiod alters prolactin secretion, the dopaminergic control of prolactin is likely to be influenced by seasonal changes in day length.

A few studies in male hamsters have found that exposure to short photoperiod results in a decrease in whole hypothalamic (Steger, Bartke, & Goldman, 1982) and medial-basal hypothalamic (MBH; Benson, 1987) dopamine concentrations. However, experiments examining the effects of short-photoperiod exposure on ME dopamine concentrations, which are important for the regulation of a number of different hormones, have yielded conflicting results. In one experiment, exposure to a short photoperiod resulted in a significant decrease in ME dopamine content, but the rate constant of decline after an injection of αMPT was not

affected by photoperiod (Steger, Reiter, & Siler-Khodr, 1984) suggesting that TIDA neuronal activity was not altered in these animals. However in another experiment, Steger (Steger, Matt, Klemke, & Bartke, 1985) found that exposure to a short photoperiod did not affect ME dopamine concentrations, but that the rate constant of decline in ME dopamine after an injection of αMPT was significantly decreased in short-photoperiod housed animals, indicating that TIDA neuronal activity was decreased in these animals. These conflicting results make it difficult to determine from a review of the literature if TIDA neuronal activity is affected by photoperiod.

The effects of photoperiod on ME dopamine have not been directly assessed in the female hamster. However, one study has evaluated the role of melatonin in the regulation of biogenic amines in the female hamster. Alexiuk and Vriend (1991) found that if females are given daily afternoon injections of melatonin for 10 weeks, there is a decrease in ME dopamine content and TIDA neuronal activity. In this study, TIDA neuronal activity was assessed by measuring dopamine accumulation two hours after an injection of the MAO inhibitor, pargyline. However, as previously mentioned, dopamine synthesis is affected through end product inhibition as early as 30 minutes after pargyline injection (Carlsson, et al., 1976). Therefore, the results from this study are also difficult to interpret.

Because of the conflicting results and the

methodological limitations of the experiments cited above, the effects of photoperiod on TIDA neuronal activity in male and female hamsters are poorly understood. The main purpose of this dissertation was to examine the effects of photoperiod on ME dopamine and to determine if these changes in dopamine are the result of changes in TIDA neuronal activity. In the experiments presented here, ME DOPAC and DOPA concentrations were used as indices of TIDA neuronal activity. Preliminary work (see Appendices A and B) indicates that these measures can be used to assess TIDA neuronal activity in the hamster.

The first four experiments of this dissertation used neurochemical and anatomical techniques to examine the effects of prolonged exposure to a short photoperiod on ME dopamine concentrations and TIDA neuronal activity in hamsters. In the first experiment, ME dopamine was measured in male and female hamsters to determine if photoperiod affects this system. TIDA neuronal activity was estimated by measuring ME DOPAC concentrations so that any changes in TIDA neuronal activity and prolactin could be assessed in the same animals. Because previous work has shown that prolonged exposure to a short photoperiod results in a decrease in whole hypothalamic (Steger, et al., 1982), MBH (Benson, 1987) and possibly ME (Steger, et al., 1984) dopamine concentrations, a short-photoperiod induced decrease in ME dopamine concentrations was expected. In addition, DOPAC concentrations were determined and used to

test the hypothesis that the short-photoperiod induced decrease in ME dopamine concentrations is the result of a decrease in TIDA neuronal activity. A decrease in ME DOPAC concentrations in short-photoperiod housed animals would suggest that TIDA neuronal activity is lower in these animals. Gonadally intact and castrated animals were also used in this first experiment to investigate the role of the gonads in the photoperiodic control of TIDA neurons.

In Experiment 2, ME DOPA accumulation after LAAD inhibition was used as an index of TH activity (or dopamine synthesis) in TIDA neurons. A decrease in ME DOPA accumulation in animals housed in short days would indicate that TH activity or dopamine synthesis is decrease in short photoperiod. A sustained decrease in TH activity in the ME of short-photoperiod housed animals would result in a decrease in ME dopamine concentrations.

Experiment 3 examined the effects of photoperiod on dopaminergic cell bodies in the arcuate nucleus. This experiment tested the hypothesis that the reduction in ME dopamine concentrations in short-photoperiod housed animals is due to a reduction in the number of TH immunopositive (TH+) neurons in those animals. A decrease in the number of TH+ neurons in this region would suggest that there are fewer neurons synthesizing dopamine and contributing to ME dopamine stores.

Experiment 4 tested the hypothesis that the depletion of dopamine concentrations seen in animals exposed to a

short photoperiod is due to an increase in the rate of dopamine release from the terminals of TIDA neurons. Dopamine release was estimated by injecting long- and short-photoperiod housed animals with αMPT and then measuring the rate of decline of ME dopamine. If exposure to a short photoperiod results in an increase in the release of dopamine from TIDA neurons, the rate constant of decline should be greater in short- than long-photoperiod housed animals. An increase in the release of dopamine from TIDA neurons without a compensatory increase in dopamine synthesis would result in a decrease in ME dopamine stores. Increased levels of dopamine release from TIDA neurons might also suppress prolactin release from the anterior pituitary

A second set of experiments (Experiments 5, 6 and 7) examined changes in ME dopamine and TIDA neuronal activity after various lengths of short-photoperiod exposure. These experiments were done to determine the time course of the decline in dopamine and prolactin in hamsters housed in a short photoperiod. Also, these experiments used data on ME DOPAC concentrations and DOPA accumulation to test the claim by Steger and Bartke (1991) that there is a decrease in TIDA neuronal activity after a brief exposure to short photoperiod.

Thus, in summary, the goals of the studies presented in this dissertation were: (i) to describe the effects of photoperiod on ME dopamine concentrations and TIDA neuronal activity (ii) to elucidate the mechanisms responsible for

the short-photoperiod induced decrease in ME dopamine, (iii) to document the time course of that decrease, and (iv) to explore possible effects of photoperiod on TIDA neuronal cell bodies. When technically possible, circulating levels of prolactin were determined to evaluate the relationship between TIDA neuronal activity and the reductions in prolactin seen in hamsters exposed to short photoperiod.

EXPERIMENT 1

The Effects of Short Photoperiod Exposure and Gonadectomy on TIDA Neuronal Activity and Circulating Prolactin Levels in Male and Female Hamsters

The purpose of this experiment was to determine if long-term exposure to short photoperiod (12 weeks) results in a decrease in ME dopamine concentration, and to assess the effects of photoperiod on TIDA neuronal activity in male and female hamsters. Because TIDA neuronal activity may be involved in the short-photoperiod induced decrease in prolactin, plasma prolactin was also measured. In this experiment, TIDA neuronal activity was estimated by measuring DOPAC concentrations in the ME of intact and gonadectomized male and female hamsters housed in either a long- or short-photoperiod. Gonadectomized animals were used to determine if changes in TIDA neuronal activity and prolactin were due to a direct effect of photoperiod or were secondary to the low levels of estrogen and testosterone seen in short-day animals. Dopamine concentrations in the caudate-putamen (CP; another terminal field of dopaminergic neurons) were also measured to determine if the effects of short photoperiod or gonadectomy were specific to the ME and TIDA neurons or were seen in other dopaminergic systems in

the brain.

Methods

Animals: Sixty-four adult hamsters (32 males, 32 females) obtained from Charles Rivers (Kingston, NY) were used in this study. All animals were maintained in individual hanging cages and supplied with Rodent Laboratory Chow 5001 (Purina) and water ad libitum. All animals were housed in a long photoperiod with lights on at 0400 and lights off at 2000 (16L:8D) for approximately 2 weeks, or until all females had displayed two complete estrous cycles determined by inspection of vaginal discharges (Orsini, 1961). After the two weeks in long photoperiod, 16 males and 16 females were anesthetized with Metofane (inhalant) and gonadectomized. In males, castration was done via two lateral incisions in the scrotum, and females were ovariectomized via a midline incision on the ventral surface. Immediately after surgery, half of the gonadally intact animals and half of the gonadectomized animals were transferred to a short photoperiod with lights on at 0800 hr and lights off at 1400 hr (6L:18D). The remaining animals were housed in long photoperiod until the end of the experiment. Vaginal secretions of intact females were checked daily for proestrous discharge. The testis width of intact males was measured weekly by injecting animals with ketamine (i.m., .1 to .15 cc) and measuring the width of the right testis with calipers. Animals were housed in the

respective photoperiods for approximately 12 weeks. By this time, intact females housed in short photoperiod were anestrous, and the intact males housed in short photoperiod had regressed testes as determined by a decrease in testis width (from greater than 10 mm to less than 6 mm).

Procedure. Intact females housed in long photoperiod were killed on day one of diestrus between 0800 and 1100, and intact males housed in long photoperiod were killed at corresponding times. All animals housed in short photoperiod were killed on the same day between 0800 and 1100. All animals were decapitated, the brains were removed, rapidly frozen on aluminum foil over dry ice, and stored at -70°C until sectioning. Trunk blood was collected into heparinized tubes and centrifuged at 4000 rpm for 30 minutes. The plasma was removed and frozen at -20°C until assayed for prolactin.

Neurochemical Estimation of DOPAC and Dopamine. Brains were sectioned (500μm) on a cryostat (-9°C) starting at the level of the striatum corresponding approximately to plate A2400 of the rat brain atlas (Palkovits & Brownstein, 1988) and continuing through the ME, corresponding approximately to plate P3200 of the rat brain atlas (Palkovits & Brownstein, 1988). The ME and CP were dissected using a modification of Palkovits' method (1973). A stainless steel cannula with a 1000 μm lumen diameter was used to dissect the CP from sections corresponding to plates A2400 - A1800 of the rat brain atlas and the ME was dissected from

sections corresponding to levels P3200 - P3600 of the rat brain atlas (Palkovits & Brownstein, 1988) using a cannula with a lumen diameter of 500 μm . Tissue samples from the ME and CP of each animal were placed into 0.1 M phosphate/citrate buffer (pH 2.5) containing 15% methanol and stored at -20°C until assayed for DOPAC and dopamine (Chapin, Lookingland, & Moore, 1986).

At the time the assay was run, samples were thawed, sonicated for 3 seconds (Heat Systems-Ultrasonics, Plainview, N.Y., USA) and centrifuged for 30 seconds in a Beckman 152 Microfuge. The supernatant was removed and assayed for DOPAC and dopamine by high-performance liquid chromatography with electrochemical detection (HPLC-ED). Samples (50 μl) of the supernatant were injected into a Bioanalytical System LC-304 Liquid Chromatograph (Bioanalytical Systems Inc., West Lafayette, IN) with a Waters M-45 solvent delivery system. The flow rate of the system was 0.7 ml/min. The mobile phase of the system, which consisted of a C18 reverse phase column (5 μm spheres; 250 X 4.6 mm; Biophase ODS, Bioanalytical Systems Inc.), separated the catecholamines and their metabolites within the supernatant by hydrophobicity. The mobile phase buffer consisted of 0.1 M phosphate/citrate buffer (pH 2.5) with 15% methanol to decrease the retention time of the metabolites, and 0.05% sodium octyl sulfate (SOS) to enhance ion pairing. The SOS allows the column to work in unison and increases the retention time of the catecholamines.

This column was paired with an electrochemical detector containing a TL-5 glassy carbon electrode with a potential of +0.75 volts relative to the Ag/AgCl reference electrode. Chromatographs were printed out on a Hewlett Packard Integrator, Model 3390A (for review of HPLC-ED and equipment see; Chapin, et al., 1986). A representative chromatograph depicting the detection of DOPAC and dopamine content in the ME of a hamster can be seen in Figure 2. To make sure that the measurement of dopamine and DOPAC between samples was consistent, standards containing DOPAC and dopamine were run (250 pg standards for ME and 500 pg standards for CP) after approximately every 20 samples. In this experiment, the coefficients of variation (CVs) for DOPAC and dopamine content in the 250 pg standard were 6% and 8% respectively, and for the 500 pg standard the CVs for DOPAC and dopamine were 9% and 10% respectively.

Tissue pellets from each animal were dissolved in 0.1 N NaOH and assayed for protein. The Lowry's protein assay (Lowry, Rosebrough, Farr, & Randall, 1951) was used to measure protein content of tissue samples from the CP. Because the tissue pellets from the ME were small, and the amount of protein in these pellets (6-12 μg) did not fall in the linear range (25-150 μg protein) of the Lowry's protein assay, the Bradford microprotein assay (linear range between 0.5 and 20 μg protein) was used instead (Bradford, 1976).

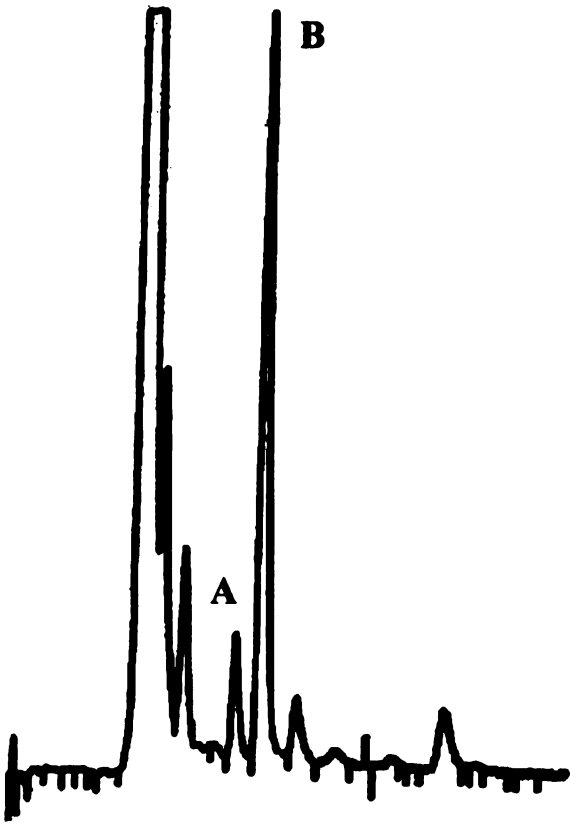

Figure 2. A sample chromatograph measuring DOPAC (A) and dopamine (B) in the median eminence (ME) of a male hamster. The peak on the left is the peak obtained from DOPAC in the sample and the peak on the right is the peak obtained from DA. By comparing the peak heights with those of the standards, the content of DOPAC and DA within the sample can be calculated. These values are then divided by the amount of protein (measured using the Bradford protein assay) in the tissue pellet from the ME to estimate the concentrations of DOPAC and dopamine.

Prolactin Assay. Prolactin was measured with a homologous hamster prolactin assay. All reagents were kindly provided by Dr. A.F. Parlow (Pituitary Hormone and Antisera Center, Harbor UCLA Medical Center, Torrance, CA). Purified hamster prolactin (AFP10302E) was used for the preparation of standards and iodination. Aliquots of plasma (50 µl or 100 µl) were diluted with 200-250 µl buffer containing 3% bovine serum albumin and 0.1% gel phosphate buffered saline (assay buffer) to make of final volume of 300 µl. The first antibody (rat anti-hamster; AFP-7472988) was used at a dilution of 1:3500 in phosphate buffered saline containing 0.05 M ethylenedinitrilo-tetra-acetic acid and 3% normal rat serum. The second antibody (anti-rat gamma globulin titre P3, Antibodies Inc, Davis, CA) was used at a dilution of 1:17 in assay buffer. The intra-assay CVs for plasma pools containing prolactin which were at 50% or 60% on the standard curve were 8% and 9% respectively. The lower and upper limits of detectability for the assay were 0.07 ng/ml and 42 ng/ml respectively and the linear range of the assay was between 0.02 and 6 ng/tube. The prolactin content in most samples fell within this linear range.

Analyses. Concentrations of DOPAC and dopamine in the ME and CP, and plasma prolactin levels were analyzed within each sex with 2(photoperiod) X 2(gonadal status) ANOVAs. Significant differences were those associated with $p \leq .05$.

One female (short-photoperiod ovariectomized) died during the experiment and therefore was not included in the

analyses. Two additional male hamsters (short-photoperiod intact) were not included in the analyses because their testes were not regressed after 12 weeks of short-photoperiod exposure. Missing values in other groups were due to the inability to obtain tissue samples from either the ME or CP of particular animals.

Results

For males, means of ME dopamine and DOPAC and plasma prolactin concentrations collapsed across photoperiod and gonadal status are listed in Table 1 and treatment means are depicted in Figure 3. In males, prolactin levels were lower in animals housed in short photoperiod than those housed in long photoperiod ($F(1,25)= 25.76$, $p \leq .05$). Photoperiod did not affect ME DOPAC concentrations, but castration did with castrated males having greater concentrations of ME DOPAC than intact males ($F(1,25)= 4.85$, $p \leq .05$). Although DOPAC was not affected by photoperiod, ME dopamine was, with males housed in short photoperiod having lower levels of dopamine than males housed in long photoperiod ($F(1,25)= 6.54$, $p \leq .05$).

For females, means of ME dopamine and DOPAC and plasma prolactin concentrations collapsed across photoperiod and gonadal status are listed in Table 2 and treatment means are depicted in Figure 4. As in males, short-photoperiod exposure resulted in a decrease in plasma prolactin levels

Table 1. Treatment means ± sems for plasma prolactin levels and concentrations of DOPAC and dopamine in the median eminence of male hamsters. These data were obtained by collapsing data across photoperiod (long vs. short photoperiod) and then gonadal status (gonadally intact vs. castrated). The asterisk (*) indicates that this mean is less than the corresponding mean from long-photoperiod animals and the plus sign (+) denotes that this mean is greater than the corresponding mean in gonadally intact animals ($p \leq .05$).

	Long-Photoperiod	Short-Photoperiod	Intact	Castrate
Prolactin	8.96 ± 1.09	2.51 ± .65 *	5.17 ± 1.24	7.08 ± 1.23
DOPAC	10.72 ± .82	9.92 ± .46	9.27 ± .41	11.38 ± .80+
Dopamine	90.48 ± 9.73	61.50 ± 1.26 *	74.69 ± 10.84	80.10 ± 7.36

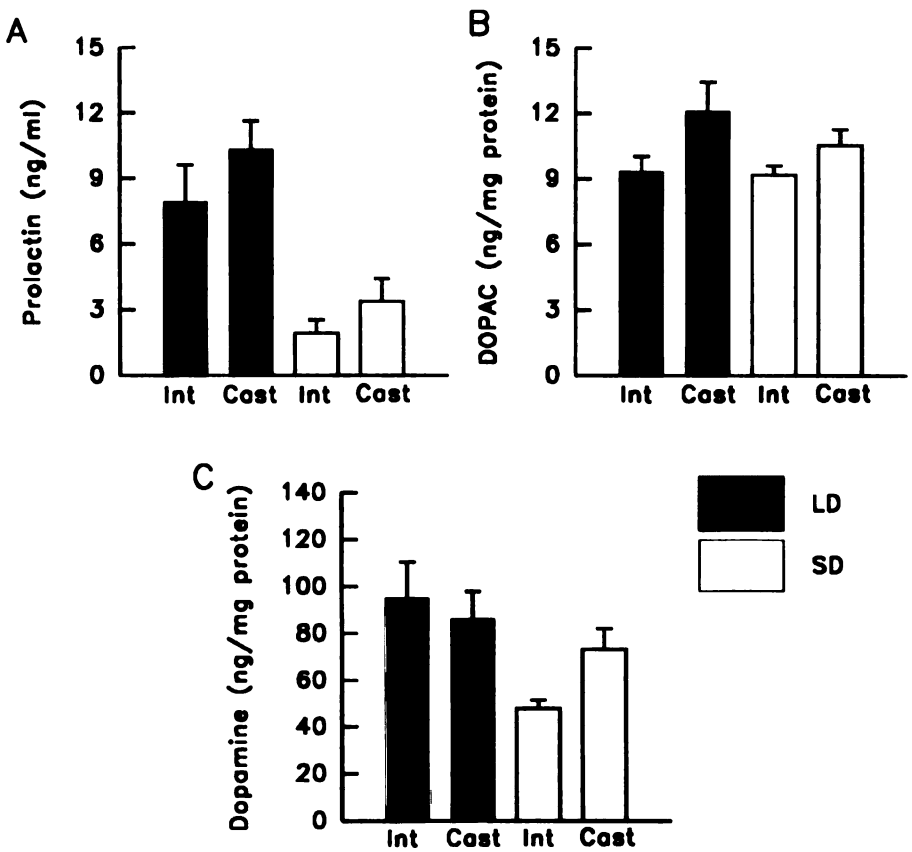

Figure 3. Mean (± sem) concentrations of circulating prolactin (A) and of DOPAC (B) and dopamine (C) in the median eminence (ME) of gonadally intact (Int) and castrated (Cast) male hamsters exposed to a long (LD) or short (SD) photoperiod. Exposure to a short photoperiod resulted in a decrease in prolactin levels and ME dopamine concentrations in both Int and Cast males (see Table 1 above). Castration did not affect prolactin or dopamine, but did result in an increase in ME DOPAC concentrations in males in both LD and SD animals.

Table 2. Treatment means ± sems for plasma prolactin levels and concentrations of DOPAC and dopamine in the median eminence of female hamsters. These data were obtained by collapsing data across photoperiod (long vs. short photoperiod) and then gonadal status (gonadally intact vs. ovariectomized). The asterisk (*) indicates that a mean is significantly less than the corresponding mean from long-photoperiod animals ($p \leq .05$).

	Long-Photoperiod	Short-Photoperiod	Intact	Ovariectomized
Prolactin	19.41 ± 3.11	6.69 ± 2.45 *	12.34 ± 4.12	13.22 ± 2.84
DOPAC	14.83 ± .84	12.95 ± 1.35	14.29 ± 1.65	12.91 ± .94
Dopamine	117.04 ± 2.25	99.45 ± 8.62	117.01 ± 8.99	100.39 ± 9.94

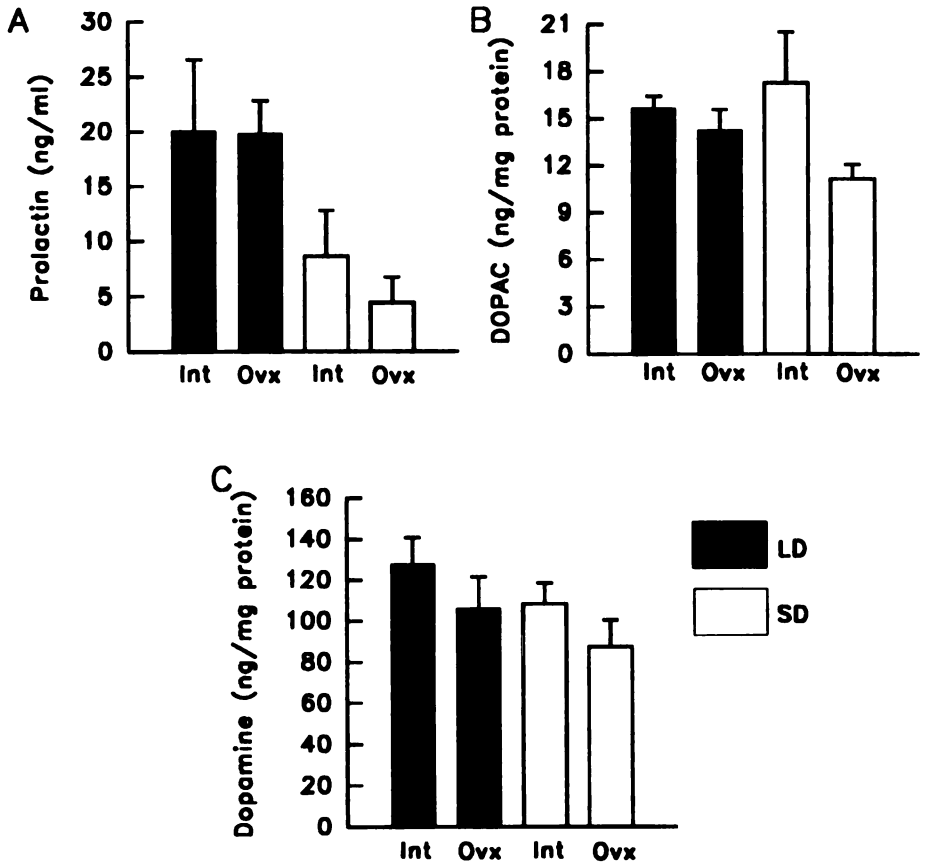

Figure 4. Mean (± sem) concentrations of circulating prolactin (A) and of DOPAC (B) and dopamine (C) in the median eminence (ME) of gonadally intact (Int) and ovariectomized (Ovx) female hamsters exposed to a long (LD) or short (SD) photoperiod. Exposure to a short photoperiod resulted in a decrease in prolactin levels in both Int and Ovx females (see Table 2 above). However, neither photoperiod nor gonadal status had an effect on ME concentrations of DOPAC or dopamine in females.

in females ($F(1,23)= 9.622$, $p \leq .05$). However this decrease in prolactin was not associated with short-photoperiod induced changes in ME DOPAC or dopamine concentrations. Ovariectomy also did not have an effect on any of the variables measured in the ME.

DOPAC and dopamine concentrations in the CP of males are presented in Table 3. There was a main effect of castration on both DOPAC ($F(1,26)= 4.34$, $p \leq .05$) and dopamine concentrations ($F(1,26)= 15.72$, $p \leq .05$) with both measures being higher in intact than castrated animals. There was also a main effect of photoperiod on DOPAC concentrations ($F(1,26)= 11.71$, $p \leq .05$) in the CP, with DOPAC being higher in long- than short-photoperiod housed animals. This effect of photoperiod was due for the most part to a significant decline in DOPAC concentrations in castrated animals housed in short photoperiod (mean ± sem; long-photoperiod intact 26.06 ± 1.19; long-photoperiod castrate 26.09 ± 1.28; short-photoperiod intact 24.39 ± .89; short-photoperiod castrate 19.59 ± 1.10), with the interaction between castration and photoperiod close to reaching significance ($F(1,26)= 4.00$, $p = .06$)

DOPAC and dopamine concentrations from the CP of females is presented in Table 4. There was a main effect of photoperiod ($F(1,26)= 12.09$, $p \leq .05$) and of ovariectomy ($F(1,26)= 5.09$, $p \leq .05$) on dopamine concentrations. Short-photoperiod housed females had lower dopamine concentrations than long-photoperiod housed females and gonadally intact

Table 3. Treatment means ± sems for DOPAC and dopamine concentrations in the caudate-putamen (CP) of male hamsters. Comparisons were made by collapsing across photoperiod (long vs. short photoperiod) and the across gonadal status (gonadally intact vs. castrated). The asterisk (*) denotes that a mean is significantly less than the corresponding mean in the long-photoperiod animals, and a plus sign (+) indicates that a mean in less than the corresponding mean in the gonadally intact animals ($p \leq .05$).

	Long-Photoperiod	Short-Photoperiod	Intact	Castrated
DOPAC	26.02 ± .85	21.65 ± 1.00 *	25.35 ± .79	22.78 ± 1.17 +
Dopamine	84.97 ± 2.69	82.77 ± 3.70	91.72 ± 2.53	77.14 ± 2.51 +

Table 4. Treatment means ± sems for DOPAC and dopamine concentrations in the caudate-putamen (CP) of female hamsters. Comparisons were made by collapsing across photoperiod (long vs. short photoperiod) and the across gonadal status (gonadally intact vs. ovariectomized). The asterisk (*) denotes that a mean is significantly less than the corresponding mean in long-photoperiod animals, and a plus sign (+) denotes that a mean is greater than the corresponding mean in the gonadally intact animals.

	Long-Photoperiod	Short-Photoperiod	Intact	Ovariectomized
DOPAC	30.83 ± 1.47	28.27 ± 1.57	29.29 ± 1.78	29.80 ± 1.30
Dopamine	91.95 ± 2.77	80.55 ± 1.81 *	82.33 ± 2.41	90.15 ± 2.75 +

females had lower concentrations of dopamine than ovariectomized females. DOPAC concentrations in the CP of females were not affected by either ovariectomy or photoperiod in these experiments.

Discussion

Consistent with a previous report (Steger, et al., 1984), exposure to a short photoperiod resulted in a decrease in circulating prolactin levels and ME dopamine concentrations in gonadally intact males. However, the results of this study suggest that the decreases in ME dopamine and prolactin are not associated with a decrease in TIDA neuronal activity since photoperiod did not affect ME DOPAC concentrations.

Although there were no photoperiod related changes in ME DOPAC concentrations, castration resulted in an increase in DOPAC levels in castrated males under both photoperiods. These findings are similar to what has been seen in the rat (Toney, Lookingland, & Moore, 1991), and suggest that testicular hormones inhibit TIDA neuronal activity. Although there was no significant interaction between photoperiod and gonadectomy, the effect of photoperiod on ME dopamine levels was not as robust in castrated animals as it was in intact animals. Thus, the presence of the testes may enhance the effects of short photoperiod on ME dopamine levels. However, because the males in this study were

castrated for an extended period of time (i.e., 12 weeks), it is possible that the effects of castration seen in this study are not physiologically relevant, but are instead the result of the system adapting to the long-term absence of gonadal steroids. For example, in male hamsters, short-term castration results in a decrease in the number of TH immunopositive (TH+) cell bodies in the medial amygdaloid nucleus, however this decrease in the number of TH immunopositive neurons is not seen in animals castrated for longer periods of time (Asmus & Winans Newman, 1993). A study comparing the effects of long and short-term castration may better explain how the testes and gonadal steroids interact with photoperiod to regulate TIDA neuronal activity.

In intact female hamsters, exposure to short photoperiod also resulted in a decrease in prolactin, but did not affect ME DOPAC or dopamine concentrations. These results are not consistent with the findings of Alexiuk and Vriend (1991) showing that low prolactin levels in females treated with melatonin and pargyline are associated with a decrease in ME dopamine concentrations. However, in that experiment, the same melatonin treatment had only a minor effect on ME dopamine concentrations in females that were not treated with pargyline. This melatonin treatment resulted in a marked reduction in circulating prolactin concentrations. Thus, it is possible that there are sex differences in how prolactin and TIDA neurons interact in

hamsters housed in short photoperiod or treated with melatonin. There is published work suggesting that the mechanisms responsible for the photoperiod-induced decrease in prolactin may be different in male and female hamsters (Blask, Leadem, Orstead, & Larsen, 1986). In hamsters, blinding by biorbital enucleation results in a decrease in circulating prolactin concentrations. In males, pinealectomy can prevent the effects of blinding on circulating prolactin concentrations. In females however, prolactin concentrations in blinded and pinealectomized females are lower than prolactin concentrations in non-blinded females (Blask, et al., 1986).

Contrary to previous studies, ovariectomy did not cause a significant decrease in prolactin (Widmaier & Campbell, 1981). There was also no effect of ovariectomy on ME DOPAC concentrations, contrary to reports of changes in TIDA neuronal activity after ovariectomy in rats (Demarest & Moore, 1981; Gudelsky & Porter, 1981). This discrepancy may be due to the fact that in those experiments the effects of ovariectomy were assessed 1-2 weeks after surgery, whereas the females used in this experiment were ovariectomized 12 weeks before sampling. In rats, ovariectomy results in a decrease in circulating prolactin levels, which in turn results in a decrease in TIDA neuronal activity (Demarest & Moore, 1981). Because the female hamsters in this experiment did not show an ovariectomy induced decrease in prolactin, it is not surprising that TIDA neuronal activity

also was not affected in these animals.

In short photoperiod housed females however, there was a salient decrease in prolactin concentrations but no change in TIDA neuronal activity. These findings suggest that the feedback of prolactin onto TIDA neurons is altered in females housed in a short photoperiod. Low prolactin concentrations should have been associated with a decrease in TIDA neuronal activity (or DOPAC). Exposure to a short photoperiod may block prolactin feedback onto TIDA neurons, and therefore prevent the reduction in TIDA neuronal activity which follows a decrease in circulating prolactin levels. On the other hand, TIDA neurons may become more sensitive to prolactin positive feedback in short days, so that relatively low levels of prolactin become capable of maintaining high levels of TIDA neuronal activity. Nothing is known about prolactin feedback onto TIDA neurons in hamsters, thus, it is difficult to evaluate those alternative explanations.

In females, photoperiod-induced changes in prolactin were not associated with changes in TIDA neuronal activity or ME dopamine concentrations. Thus in females, the reduction in prolactin may not depend on changes in TIDA neurons. Photoperiod may regulate prolactin release in females by increasing the release of some other inhibitory factor, such as gamma-aminobutyric acid, or by inhibiting the release of a releasing factor such as vasoactive intestinal peptide (for review see; Neill & Nagy, 1994).

Further research examining the effects of photoperiod on other prolactin-regulating factors may help determine the mechanism responsible for the short-photoperiod induced decrease in prolactin in female hamsters.

In males, the effects of photoperiod and castration on DOPAC and dopamine concentrations in the CP are quite different from the effects of these manipulations on TIDA neurons. First, exposure to a short photoperiod did not affect CP dopamine concentrations. However, it produced a significant decrease in CP DOPAC concentrations which was primarily due to a decrease seen in the castrated animals. If DOPAC concentrations are compared in gonadally intact animals housed in a long or short photoperiod, no effect of photoperiod is seen. The results of Experiment 4 (see p.59) are consistent with this claim and show that in gonadally intact animals, exposure to a short photoperiod does not affect CP DOPAC or dopamine concentrations. Therefore, in intact males, photoperiod does not have a reliable effect on CP dopamine or DOPAC concentrations. The fact that dopamine concentrations were affected by photoperiod in the ME, but not in the CP, suggests that photoperiod exposure differentially affects central dopaminergic systems in the male hamster.

Although photoperiod does not seem to affect the nigrostriatal dopamine neurons of intact males, castration had a significant effect on this measure. CP DOPAC and dopamine concentrations were lower in castrates when

compared to intact males. These results suggest that the gonads (even in short-day exposed males) may stimulate nigrostriatal dopaminergic activity in male hamsters. In the present experiment, castration stimulated TIDA neuronal activity, but inhibited nigrostriatal dopaminergic activity (as measured by DOPAC). Experiments examining the effects of steroid replacement on the activity of these dopaminergic neurons may help determine how castration is regulating these two dopaminergic systems.

In female hamsters, exposure to a short photoperiod and ovariectomy did not affect ME DOPAC or dopamine concentrations. These manipulations also did not affect CP DOPAC concentrations, but did affect dopamine concentrations in the CP. The fact that ovariectomy resulted in an increase in CP dopamine concentrations but did not affect CP DOPAC concentrations in female hamsters is consistent with data collected in the rat. In ovariectomized female rats, estrogen treatment results in a decrease in CP dopamine concentrations without affecting dopamine turnover in this region (Di Paolo, Bedard, Poyet, & Labrie, 1982). Therefore, these results suggest that ovariectomy and estrogen replacement affect dopamine concentrations within the CP without affecting dopamine synthesis at these terminals. This disruption of the coupling between synthesis and release have been seen after estrogen treatment in both the CP (Di Paolo, et al., 1982) and ME (Demarest, et al., 1984; Morehead, Lookingland, & Gala, 1990) of female rats.

Short-photoperiod exposure of females resulted in a decrease in CP dopamine concentrations, but again, this was seen without effects on DOPAC concentrations. This reduction in dopamine was unexpected given the effects of ovariectomy discussed above, and the fact that short-day females have very low levels of estradiol. It is possible that the photoperiodic effects on CP dopamine concentrations are due to the time at which the animals were killed. In rats, there is a circadian rhythm in extracellular concentrations of dopamine when measured by microdialysis (Smith, Olson, & Justice, 1992) with CP dopamine concentrations being lowest approximately 6 hour after lights on and gradually increasing to peak concentrations at about 6 hours after lights off, when animals are kept in a 12:12 light/dark cycle. Therefore, if there is a circadian rhythm in CP dopamine concentrations in hamsters, it is possible that exposure to a short-photoperiod affects the entrainment pattern of this rhythm. In this experiment, the animals were killed at a common phase of their cycle using the expected onset of activity (Elliott, 1976) as the reference phase marker. However, since nothing is known about the relationship between nocturnal activity and rhythms in striatal dopamine concentrations, it remains possible that the apparent effects of photoperiod are an artifact of the sampling time used here. In order to determine if photoperiod does have an effect on CP dopamine concentrations in female hamsters, such concentrations

should be measured over the 24 hour cycle under both photoperiods.

In summary, the results from this experiment show that exposure to short photoperiod causes a decrease in ME dopamine concentrations and circulating prolactin levels in male hamsters. Because photoperiod does not affect ME DOPAC concentrations in males, the short-photoperiod induced decrease in dopamine is probably not the result of a decrease in de novo synthesis of dopamine. However, to be sure that photoperiod does not affect dopamine synthesis, another more direct estimate of synthesis should be used to replicate the findings of this experiment. This was done in Experiment 2. Because short-photoperiod exposure did not affect ME DOPAC or dopamine concentrations in female hamsters, females were not used in the following experiments.

EXPERIMENT 2

TH Activity in TIDA Neurons in Male Hamsters Housed Under Long and Short Photoperiods.

This study was done to replicate the findings of Experiment 1 which suggest that exposure to a short photoperiod does not affect the de novo synthesis of dopamine in TIDA neurons of male hamsters. Dopamine synthesis, or TIDA neuronal activity was estimated by measuring the accumulation of DOPA in the ME after an injection of a LAAD inhibitor, NSD 1015. In the rat, manipulations that increase TIDA neuronal activity (such as estradiol treatment) result in higher levels of DOPA accumulation after an injection of NSD 1015 (Demarest & Moore, 1980). DOPA accumulation is an estimate of TH activity (Carlsson, et al., 1972) and has been used as an estimate of dopamine synthesis (Demarest & Moore, 1980) in the ME. In this experiment, DOPA accumulation was measured in the ME of gonadally intact and castrated male hamsters housed in a long or short photoperiod for 12 weeks. Castrated animals were included to determine if the testes modulate TH activity under either photoperiod.

Methods

Animals. Thirty-six adult male Syrian hamsters were used in this study. All animals were housed in a long photoperiod (lights on 0400, lights off 2000) for one week to acclimate to the laboratory. After the first week, 18 males were castrated as described in Experiment 1. Half of the intact males (n=9) and half of the castrated males (n=9) were placed into a short photoperiod (lights on 0800, lights off 1400). The other half of the animals remained in the long photoperiod. As in Experiment 1, the testis width of the intact males was measured weekly. The animals were housed in the respective photoperiod for 12 weeks, or until all the intact males housed in short photoperiod had undergone testicular regression (testis width less than 6 mm).

Procedure. On the day of the experiment, all animals were injected with NSD 1015 (100 mg/kg body weight, i.p.) thirty minutes before decapitation (see Appendix B for NSD 1015 drug time course). The brains were removed, rapidly frozen on dry ice over aluminum foil, and stored at -70°C until sectioning.

Neurochemical Estimation of DOPA Accumulation. The method used to dissect the median eminence was identical to that described in Experiment 1. ME DOPA accumulation was measured by HPLC-ED as described in Experiment 1.

Analysis Data on ME DOPA accumulation were analyzed with a 2 (gonadal status) X 2 (photoperiod) ANOVA. Differences were considered significant if $p < .05$.

Results

Two animals (1 long-photoperiod castrate, 1 short-photoperiod intact) were not included in the analysis because poor sectioning made it impossible to accurately dissect the ME. There was no effect of either photoperiod or castration on ME DOPA accumulation (Figure 5).

Discussion

Exposure to either a long or short photoperiod for 12 weeks did not affect ME DOPA accumulation in intact or castrated animals. These results are in agreement with the findings of Experiment 1 and suggest that the short-photoperiod induced decrease in ME dopamine is not the result of a change in TH activity or dopamine synthesis in these animals.

In Experiment 1, TIDA neuronal activity (or ME DOPAC concentrations) was higher in castrated animals than in gonadally intact animals. Although there was a trend for ME DOPA accumulation to be higher in castrated than in intact animals, regardless of photoperiod, this difference was not significant. These results suggest that the effects of the testes on TIDA neuronal activity may not be very

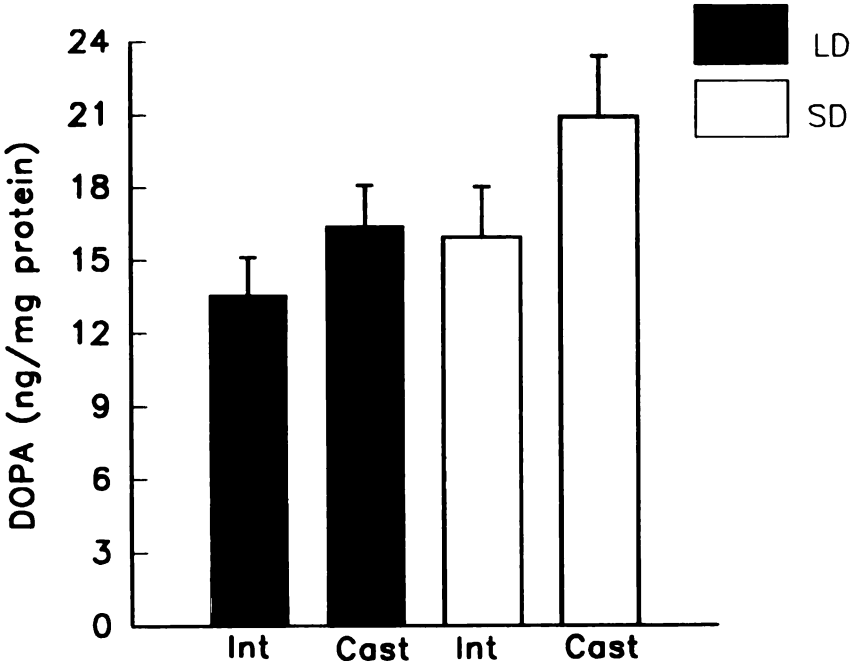

Figure 5. Accumulation of 3,4-dihydroxyphenylalanine (DOPA) 30 minutes after an injection of the LAAD inhibitor (NSD 1015) in the ME of gonadally intact (Int) and castrated (Cast) male hamsters housed in a long (LD) or short (SD) photoperiod. ME DOPA accumulation was not affected by photoperiod in Int or Cast animals. Castration also did not affect ME DOPA accumulation in either LD or SD housed animals.

robust in the hamster. Therefore, because the removal of the testes did not consistently influence the effects of photoperiod on ME dopamine or TIDA neuronal activity, castrated males were not used in further experiments.

EXPERIMENT 3

Tyrosine Hydroxylase Immunocytochemistry in the Arcuate Nucleus of Male Hamsters Housed in Long and Short photoperiod.

Experiments 1 and 2 used neurochemical techniques to examine the effects of photoperiod on TIDA neuronal terminals in the ME. These experiments suggest that the short-photoperiod induced decrease in ME dopamine is not the result of a change in de novo dopamine synthesis or in TH activity in TIDA neurons. However, these neurochemical measures estimate the effects of photoperiod on groups of terminals at the level of the median eminence. It is possible, however that photoperiod is acting at the level of TIDA neuronal cell bodies to affect ME dopamine concentrations. For example, exposure to a short photoperiod may affect either the number or population of dopaminergic neurons that are active. Experiments in the rat have shown that there is some plasticity in the TIDA neuronal system under different physiological conditions. For example, in male rats, castration results in an increase in TIDA neuronal activity as measured by DOPAC and DOPA (Toney, et al., 1991) and it also results in an increase in the number of tyrosine hydroxylase (TH+) immunopositive

neurons in the arcuate nucleus (Brawer, Bertley, & Beaudet, 1986). This castration induced increase in neurochemical and immunocytochemical measurements of TIDA neuronal activity can be reversed by testosterone replacement (Brawer, et al., 1986; Toney, et al., 1991). These findings suggest that there is some plasticity in the TIDA neuronal system and that changes in neurochemistry can be associated with changes in gene expression in these neurons. However, it is also possible to see changes in the number of neurons that are capable of synthesizing TH without seeing changes in TH activity (ME DOPA accumulation) at the terminals. In developing male rats, synthesis of dopamine in the ME stays fairly constant between 15 and 70 days of age, however the number of neurons containing TH mRNA in the arcuate nucleus significantly increases over this same period of time (Arbogast & Voogt, 1991). These results suggest that there are situations where increases in the number of TH producing neurons in the arcuate nucleus are seen with no evidence for a corresponding change in TH activity in the ME. Therefore, it may be possible that under certain physiological conditions, there are changes detected at the level of the cell bodies that escape detection when gross estimates of activity at the level of the terminals are used.

In this experiment, TH immunocytochemistry (ICC) was used to identify TH+ neurons in the arcuate nucleus of male hamsters housed in either a long or short photoperiod. The goal of this experiment was to determine if exposure to a

short photoperiod resulted in a change in either the number or population of neurons within the arcuate nucleus that were TH+. Changes in the number or population of neurons that were TH+ would suggest that the effects of short-photoperiod exposure on ME dopamine might be due to the effects of photoperiod acting at the level of individual cell bodies. TH+ cells in two other nuclei containing dopaminergic neurons [i.e., caudal periventricular (A_{14}) and medial zona incerta (A_{14})] were also counted to determine if the effects of photoperiod were specific to the arcuate nucleus or affected other dopaminergic systems within the hypothalamus.

Methods

Animals: Ten male Syrian hamsters were used in this study. All animals were maintained as described in Experiment 1. Upon arrival, all animals were housed in a long photoperiod (light on 0400, lights off 2000) for 1 week. After the first week, half of the animals were transferred to a short photoperiod (lights on 0800, lights off 1400) and the other animals remained in a long photoperiod. Testis widths were measured weekly as described in Experiment 1. Animals remained in the respective photoperiods until all males housed in short photoperiod had gone through gonadal regression (after approximately 12 weeks).

Procedure: Animals were deeply anesthetized with Equithesin (2 ml/animal) and perfused intracardially with 200 ml of 0.1 M phosphate buffered saline (PBS; pH 7.4) followed by 200 ml of 4% paraformaldehyde in PBS. The brains were removed and post-fixed in 4% paraformaldehyde in PBS and 20% sucrose for 24 hours. After post-fixing, 40 μm sections were cut through the hypothalamus using a freezing microtome. Every fourth section (approximately 30 sections/brain) was reacted in anti-mouse TH (INCSTAR INC) at a dilution of 1:10,000 in 0.1 M sodium phosphate buffer (PB) plus 0.3% Triton-X (Tx) and 2% normal horse serum for 48 hours at 4°C. Sections were then incubated in a biotinylated secondary antibody (horse anti-mouse, Vectastain) diluted 1:250 in 0.1 M PB plus 0.3% Tx for 1 hour at room temperature. Finally sections were incubated in a 1:125 dilution of the avidin-biotin HRP complex (Vectastain Elite kit) in 0.1 M PB plus 0.3% Tx for 90 minutes. TH+ neurons were visualized using 0.1% diaminobenzidine (DAB) plus 50 μl/ml H_2O_2 as a chromogen. Sections were mounted onto gelatin coated slides, counterstained with Pyronin Y (a cell membrane stain; Sigma), and coverslipped.

To test for non-specific binding of the secondary antibody, sections from one male were processed for ICC as described above, except that the primary antibody was deleted. There were no labelled cells or fibers in any of the control sections. Specificity of the primary antibody

has been demonstrated by Western blot analysis (Wolf, Zigmond, & Kapatos, 1989).

Analysis All the slides were coded so that the analysis was done blind to the experimental conditions. Sections containing the arcuate (Arc) and caudal periventricular (cPeri) nuclei, and the medial zona incerta, (MZI) were identified using a rat brain atlas (Paxinos & Watson, 1982). The number of TH+ neurons in sections containing these nuclei was determined by counting the number of stained cells bilaterally within each region at a magnification of 400x. Labelled cells with their processes were stained dark brown, and were easily identified against the red counterstaining. The number of TH+ neurons/section within each region was compared between long- and short-photoperiod animals using Students *t*-tests. Differences were considered significant if $p < .05$. The location of TH+ neurons within the arcuate nucleus was drawn onto *camera lucida* drawings of the arcuate nucleus. The distribution of TH+ immunopositive neurons in the arcuate nucleus of long- and short-photoperiod housed animals was compared to determine if there were any obvious differences in the location of the labelled neurons.

Results

Figure 6 is a photomicrograph showing TH+ neurons within the arcuate nucleus. Photoperiod did not have an effect on the number of TH+ neurons in the Arc (Figure 7A),

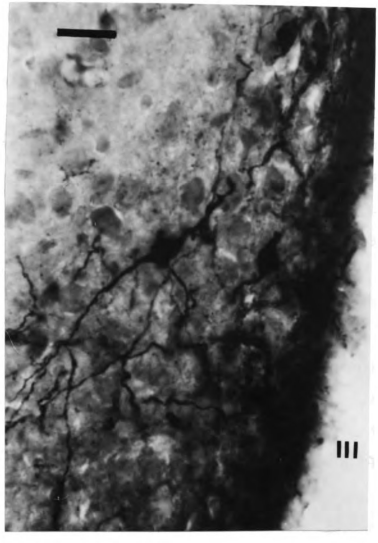

Figure 6. A photomicrograph of tyrosine hydroxylase immunopositive (TH+) neurons in the arcuate nucleus of a male hamster. TH+ neurons and their processes were easily identified from background because of their dark brown staining. Abbreviations; third ventricle (III), Bar = 30 μm.

the cPeri (Figure 7B) or the MZI (Figure 7C). There also was not an effect of photoperiod on the distribution of TH+ neurons within the arcuate nucleus. The majority of TH+ neurons were located in the dorsomedial portion of the arcuate nucleus, adjacent to the third ventricle (Figure 8).

Discussion

The results from this experiment suggest that the short-photoperiod induced decrease in ME dopamine concentrations is not the result of a change in the number of neurons synthesizing dopamine or in the population of active neurons. Therefore, the results obtained by measuring DOPA in the terminals (Experiment 2) and TH+ neurons in the arcuate nucleus are in agreement. These data suggest that the short-photoperiod induced decrease in ME dopamine is not the result of a reduction in dopamine synthesis at the terminals or due to a decrease in the number of active TIDA neurons in the arcuate nucleus.

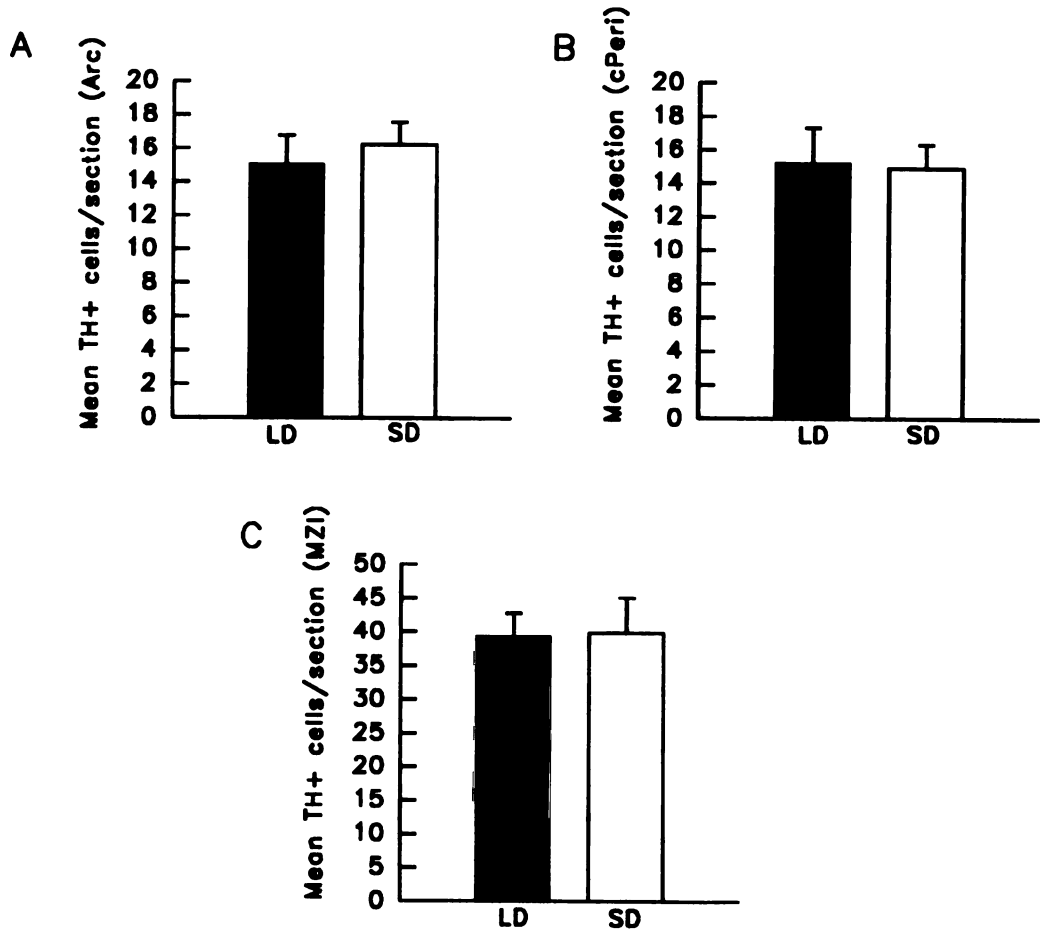

Figure 7. Mean (± sem) tyrosine hydroxylase immunopositive (TH+) cells/section in the arcuate nuclei (A; Arc), caudal periventricular nuclei (B: cPeri) and medial zona incerta (C; MZI) in male hamsters exposed to a long (LD) or short (SD) photoperiod. There was no effect of photoperiod on the number of TH+ neurons in any of these regions.

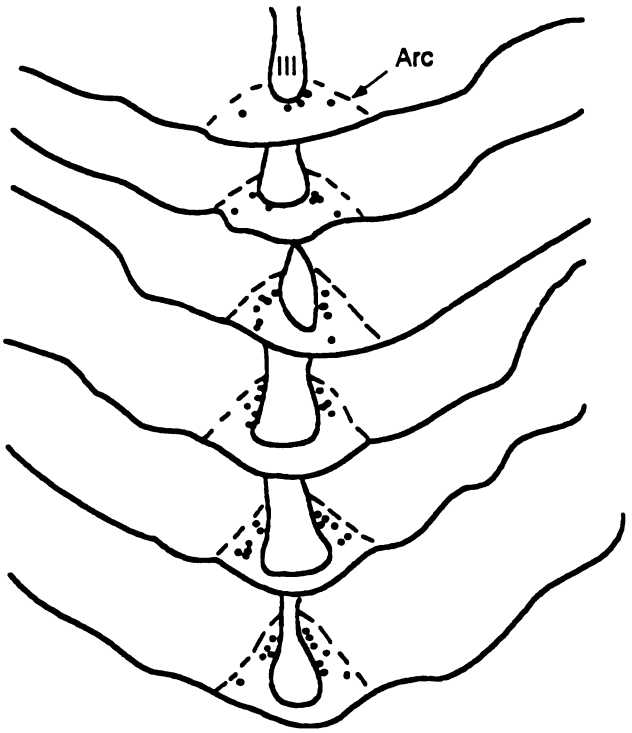

Figure 8. Camera lucida drawings showing the distribution of tyrosine hydroxylase immunopositive (TH+) neurons in the arcuate nucleus of a male hamster. This diagram shows 6 representative drawings of the arcuate nucleus of a male hamster from its rostral (top) to caudal (bottom) extent. The dashed lines indicate the boundaries of the arcuate nucleus and each dot represents two TH+ neurons. The majority of TH+ neurons in both long- and short-photoperiod housed males were located in the medial portion of the nucleus, near the third ventricle (III).

EXPERIMENT 4

Dopamine Release in Male Hamsters Housed in Long and Short Photoperiod.

The results from Experiments 1-3 indicate that the short-photoperiod induced decrease in ME dopamine is not associated with a change in dopamine synthesis or TH activity, or with a reduction in the number or distribution of TH+ neurons in the arcuate nucleus. Therefore, it is possible that this decrease in ME dopamine is the result of an increase in the release of dopamine from TIDA neurons. Because synthesis is not increased in short photoperiod (no change in DOPAC or DOPA accumulation), an increase in release would result in a decrease in dopamine stores. An increase in the release of dopamine without a compensatory increase in synthesis would indicate that photoperiod is interrupting end-product inhibition in these neurons. However, there is evidence which suggests that there may be a change in the coupling between synthesis and release of dopamine in TIDA neurons under various physiological conditions (see Introduction). A short-photoperiod induced increase in the release of dopamine from TIDA neurons could be responsible for the sustained decrease in circulating prolactin levels seen in short-photoperiod housed males and

could inhibit the synthesis of prolactin within anterior pituitary lactotropes (Ben-Jonathan, 1985).

The purpose of this study was to compare dopamine release from TIDA neurons in long- and short-photoperiod housed males. The hypothesis tested in this experiment was that the short-photoperiod induced decrease in ME dopamine is the result of an increase in transmitter release. To directly measure dopamine release, dopamine must be measured in the portal blood (Gudelsky & Porter, 1979). Because it would be extremely difficult to cannulate the portal blood system of a hamster, release was measured indirectly. In this experiment, the rate of decline of dopamine after an injection of αMPT was used as an estimate of the rate of dopamine release. αMPT blocks TH activity, thereby inhibiting dopamine synthesis. Therefore, as dopamine is released, synthesis cannot replenish the released stores, and this decline in dopamine is an estimate of release. Because the initial concentrations of dopamine were expected to be different in long- and short-photoperiod animals (Experiment 1), turnover was not calculated, and instead the change in ME dopamine concentrations after αMPT injections was used as an index of release.

Methods

Animals. Sixty adult male hamsters were obtained from Charles Rivers (Kings, NY). Animals were maintained as described in Experiment 1. Upon arrival, all animals were

housed in a long photoperiod (lights on 0400 light off 2000) for one week. After the first week, half of the animals were transferred to a short photoperiod (lights on 0800 lights off 1400). Testis widths were measured weekly as described in Experiment 1. Animals were housed in their respective photoperiods for 12 weeks, by which time all the males housed in short photoperiod had undergone testicular regression (i.e., testis width less than 6 mm).

Procedure. On the day of the experiment 10 animals from each photoperiod were injected with αMPT ester hydrochloride (Sigma; 250 mg/kg; i.p) 30 or 60 minutes prior to decapitation. These time points were selected because in the rat, the decline in ME dopamine concentrations occurs 30, 60 and 120 minutes after injection (Rance, et al., 1981). We also chose these time points because a preliminary study indicated that there was no decrease in ME dopamine at an earlier time point (15 minutes; See Appendix C) in the hamster. The 10 control animals from each photoperiod were injected with saline vehicle 60 minutes prior to decapitation. After decapitation, the brains were removed, frozen on dry ice over aluminum foil, and stored at -70°C until they were sectioned. Plasma was collected from trunk blood as described in Experiment 1 and stored at -20°C until assayed for prolactin.

Neurochemical Estimation of Dopamine. The methods used to dissect the ME and CP and measure DOPAC and dopamine concentrations are described in detail in Experiment 1. The CP was dissected to serve as a control for the effects of the drug and to replicate the findings of Experiment 1 showing that photoperiod differentially affects distinct dopaminergic systems within the brain.

Prolactin Assay. Plasma prolactin was measured as described in Experiment 1. The intra-assay CV for a plasma pool containing prolactin which bound 68% of the iodinated hormone was 8%.

Analysis. Normally, the release of dopamine is estimated by using linear regression to calculate the slope of the linear decline in dopamine after an injection of αMPT. However, in this experiment, the decline in ME dopamine was not linear up to 60 minutes after an injection of αMPT in the long-photoperiod group (see Results, below), so the rate constant of decline could not be calculated. Therefore, the decline in dopamine between controls and the 30 minute group in both the ME and CP was analyzed using a 2(photoperiod) X 2(control vs. 30 minutes) ANOVA. Significant differences between individual groups were compared using a Tukey-HSD. Differences in DOPAC within the ME and CP and plasma prolactin concentrations were also analyzed with a 2(photoperiod) X 2(control vs. 30 minutes) ANOVA to keep the comparisons consistent. Animals from which accurate tissue samples could not be obtained, were

not included in the analyses. The data from animals killed 60 minutes after an injection of αMPT were dropped from all analyses because ME dopamine concentrations in long-photoperiod animals had returned to baseline levels by this time.

Results

Figure 9 depicts ME dopamine concentrations in long- and short-photoperiod housed animals in the control group, and those killed 30 and 60 minutes after an injection of αMPT. Although the data from the 60 minute time point were not analyzed, the data are presented for reference purposes. The 2(photoperiod) X 2(control vs. 30 minutes) ANOVA revealed a significant main effect of photoperiod ($F(1,31)= 7.85$, $p < .05$) and injection ($F(1,31)= 4.43$, $p < .05$). Short-photoperiod animals had lower ME dopamine concentrations than long-photoperiod animals (mean ± sem; 38.94 ± 7.29 and 66.65 ± 6.70 respectively) regardless of whether they received an injection of αMPT or not. Pairwise comparisons were done to examine the effects of photoperiod in the control groups and in animals killed 30 minutes after an injection of αMPT revealed that in the control groups, ME dopamine concentrations were higher in long- than short-photoperiod housed animals ($p < .05$), however in animals killed 30 minutes after injection, there was no effect of photoperiod on dopamine concentrations. The main effect of injection was the result of control animals having higher

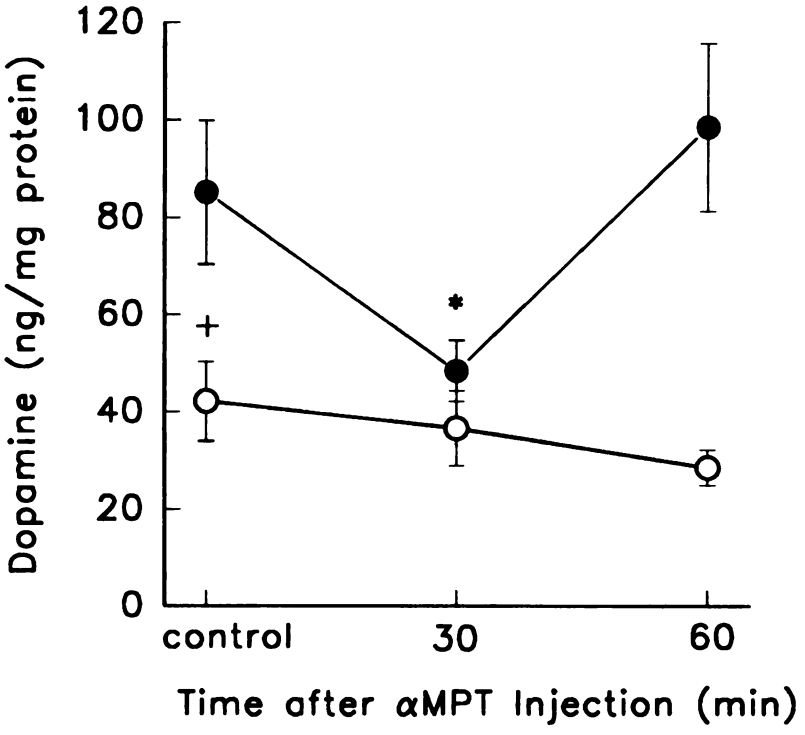

Figure 9. Mean (± sem) concentrations of median eminence (ME) dopamine in long- (LD; solid dots) and short-photoperiod (SD; hollow dots) housed male hamsters killed after an injection of saline (controls) or 30 or 60 minutes after an injection of a tyrosine hydroxylase inhibitor, αMPT. Although data from the 60 minute time point is shown for reference, it was not included in the analyses (see Analysis section above). Groups which are different from controls ($p < .05$) are marked with an asterisk (*) and differences between LD and SD are denoted with a plus sign (+). ME dopamine concentrations were higher in LD controls than in SD controls. However, this difference was no longer apparent in animals killed 30 minutes after an injection of αMPT. In LD animals, ME dopamine concentrations were decreased 30 minutes after an αMPT injection however, αMPT did not affect ME dopamine concentrations in SD animals.

concentrations of ME dopamine than animals killed 30 minutes after an injection of αMPT (mean ± sem; 63.21 ± 7.08 and 42.39 ± 6.91). Pairwise comparisons revealed that the decrease in ME dopamine concentrations between control and the 30 minute group was significant in long-photoperiod ($p < .05$) but not in short-photoperiod housed animals.

ME DOPAC concentrations are depicted in Figure 10A. The ANOVA revealed a significant main effect of injection on ME DOPAC concentrations ($F(1,31)= 15.33$, $p < .05$) with control animals having higher concentrations of ME DOPAC than animals killed 30 minutes after an injection of αMPT (mean ± sem; 35.16 ± 2.75 and 20.16 ± 2.68 respectively). This decrease in DOPAC between controls and the 30 minute group was significant in both the long- and short-photoperiod ($p < .05$) housed animals. There was no effect of photoperiod on DOPAC concentrations. Plasma prolactin concentrations are depicted in Figure 10B. There was a significant interaction between photoperiod and injection on plasma prolactin concentrations ($F(1,30)= 15.37$, $p < .05$). Analysis of the simple main effects indicated that prolactin levels were lower in short-photoperiod than long-photoperiod housed animals in both the control group and in animals killed 30 minutes after an injection of αMPT ($p < .05$). Prolactin concentrations were also higher in animals killed 30 minutes after an injection of αMPT than in control animals ($p < .05$), regardless of photoperiod.

Dopamine and DOPAC concentrations in the CP of controls

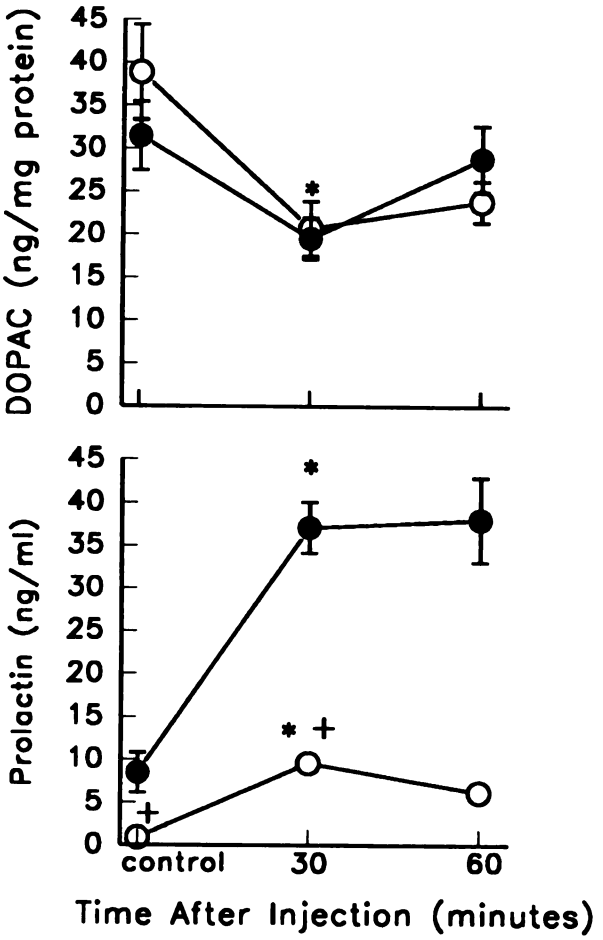

Figure 10. Mean (± sem) concentrations of DOPAC (A) in the median eminence (ME) and plasma prolactin (B) in long- (LD; solid dots) and short-photoperiod (SD; hollow dots) housed males killed after an injection of saline (controls) or 30 or 60 minutes after an injection of a tyrosine hydroxylase inhibitor, αMPT. Although the data from the 60 minute time point is shown for reference, it was not included in the analyses (see Analysis section above). Groups which are different from controls ($p < .05$) are marked with an asterisk (*) and differences between LD and SD are denoted with a plus sign (+). ME DOPAC concentrations were not affected by photoperiod. However, DOPAC was reduced 30 minutes after αMPT injection in both LD and SD animals. Plasma prolactin concentrations were higher in LD than SD animals in both controls and in animals killed 30 minutes after αMPT injections. However, plasma prolactin levels were increased 30 minutes after αMPT injections in both LD and SD animals.

and animals killed 30 and 60 minutes after an injection of αMPT are depicted in Figures 11A and 11B. The 2 X 2 ANOVA indicated that there was no effect of photoperiod on dopamine concentrations in the CP, however, there was a main effect of injection ($F(1,29) = 5.16$, $p < .05$) with control animals having higher concentrations of dopamine than animals killed 30 minutes after an injection of αMPT (mean ± sem; 97.32 ± 5.60 and 80.54 ± 4.82 respectively). Neither photoperiod nor injection had an effect on DOPAC concentrations within the CP.

Discussion

In the present experiment, exposure to a short photoperiod resulted in a decrease in ME dopamine concentrations in control animals. This short-photoperiod induced decrease in dopamine in control animals was not associated with a photoperiod-induced change in ME DOPAC concentrations. These findings are consistent with the findings of Experiment 1 indicating that the short-photoperiod induced decrease in ME dopamine concentrations in male hamsters is not the result of a change in the de novo synthesis of dopamine in TIDA neurons (as measured by DOPAC).

As was expected, injecting animals with αMPT resulted in a significant decline in ME dopamine concentrations 30

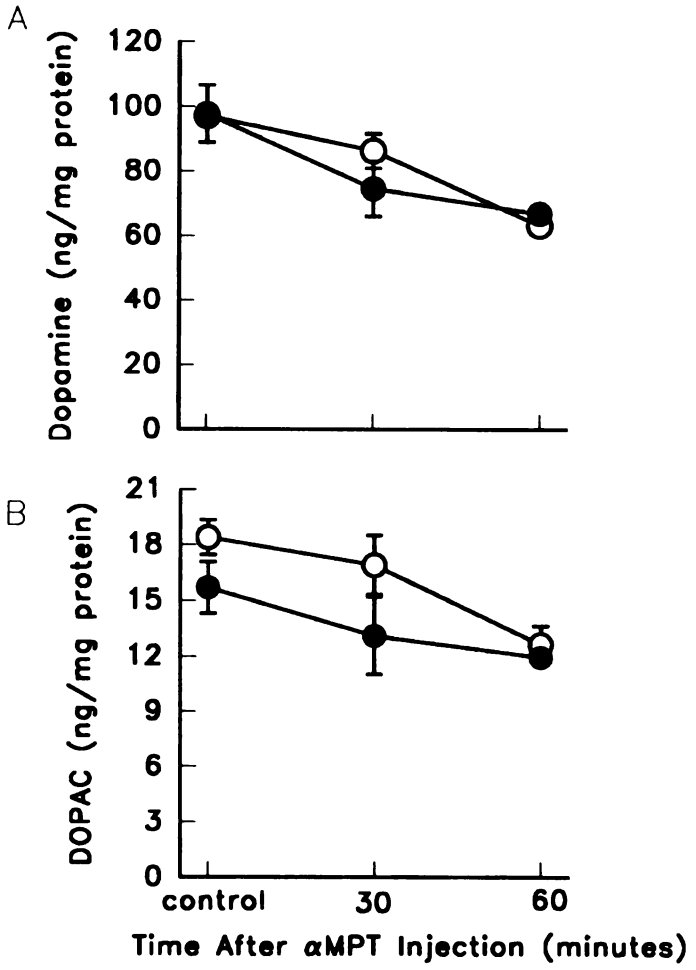

Figure 11. Mean (± sem) concentrations of dopamine (A) and DOPAC (B) in the caudate-putamen (CP) of long- (LD; solid dots) and short-photoperiod (SD; hollow dots) housed killed after an injection of saline or 30 or 60 minutes after an injection of a tyrosine hydroxylase inhibitor, αMPT. Although the data from the 60 minute time point are presented here for reference, they were not included in the analyses (see Analysis section above). Photoperiod did not affect CP dopamine or DOPAC concentrations in any of the animals. Although both dopamine and DOPAC were slightly lower in animals killed 30 minutes after an injection of αMPT, these values were not significantly different from those of controls.

minutes after the injection. However, this decline in dopamine was most prominent in the long-photoperiod housed animals. In fact, the pairwise comparisons revealed that ME dopamine concentrations did not significantly decline in short-photoperiod housed animals. These findings suggest that during the 30 minutes between injection and sacrifice, dopamine was being released from the terminals of animals housed in long but not short photoperiod. The rebound in ME dopamine concentrations in long-photoperiod animals seen 60 minutes after the injection of αMPT was unexpected. Previous work in both the rat (Rance, et al., 1981) and hamster (Steger, et al., 1984) suggest that the decline in ME dopamine concentrations is linear up to 120 minutes after an injection of αMPT. In the present experiment, there was a linear decline in CP dopamine concentrations in long-photoperiod housed animals, but not in ME dopamine concentrations. These findings suggest that there may have been some problem with the dissection or estimation of ME dopamine concentrations in long-photoperiod animals killed 60 minutes after an injection of αMPT.

The failure to see an effect of αMPT on ME dopamine concentrations in the short photoperiod group was unexpected and could have been due to a number of factors; 1) the drug was not reaching its target site within the brain or 2) all releasable stores of dopamine were depleted at the time of injection, and therefore, blocking dopamine synthesis did not result in a further reduction in dopamine

concentrations. The data showing that there is a decrease in CP dopamine concentrations up to 30 minutes after an injection of αMPT indicate that the drug was reaching the brain. More specifically data on ME DOPAC concentrations indicate that the αMPT was reaching the ME and that dopamine synthesis was blocked in animals housed in both photoperiods. Furthermore, ME DOPAC concentrations declined at equal rates up to 30 minutes after an injection of αMPT in both long- and short-photoperiod housed animals. Thus, clearly, the drug was reaching its site of action and having the intended effect (i.e., to block dopamine synthesis). Therefore, it appears as if all releasable stores of ME dopamine were previously depleted in the short-photoperiod housed animals, and that when dopamine synthesis was blocked there was no dopamine left to be released. Results by Steger et al. (1985) also suggest that there is a depletion of dopamine stores from the ME of males housed in a short photoperiod.

These results suggest that after 12 weeks of short-photoperiod exposure the releasable stores of dopamine were gone and almost all newly synthesized dopamine was being released. Since dopamine synthesis is unaffected by short-photoperiod exposure, the fact that dopamine stores are depleted suggests that at some point, dopamine release must have been enhanced in the short-day group. In rats, chronic stimulation of TIDA neurons by prolactin via estrogen treatment (Demarest, et al., 1984; Morehead, et al., 1990),

suckling (Demarest, et al., 1983), and morphine treatment (Alper, et al., 1980) results in a disruption of the coupling between dopamine synthesis and release in TIDA neurons. Therefore, it is possible that exposure to a short photoperiod also disrupts the coupling between dopamine synthesis and release, resulting in a depletion of dopamine stores. Experiments 5-7 will examine the time course of the effects of photoperiod on TIDA neuronal activity to determine when the decrease in dopamine takes place, and if this decrease in dopamine is associated with a change in TIDA neuronal activity.

In this experiment, exposure to a short photoperiod resulted in a decrease in plasma prolactin levels. However, blocking dopamine synthesis with αMPT, which in turn inhibited dopamine release, resulted in an increase in circulating prolactin concentrations in both long and short-photoperiod housed animals. This indicates that dopamine is at least in part responsible for inhibiting prolactin release during short photoperiods. Although prolactin concentrations were increased in short-photoperiod housed animals injected with αMPT, their prolactin concentrations were still lower than those of long-photoperiod animals injected with αMPT. This is probably due to the fact that short-photoperiod exposure has an effect of the ability of the anterior pituitary to synthesize prolactin. Experiments examining the effects of long- and short-photoperiod exposure on anterior pituitary anatomy have found that

pituitaries from short day animals have fewer lactotropes with fewer numbers of granules within each lactotrope (Wang, Liu, & Lin, 1991; Wang, Wu, Lue, Tsa, Chen, & Lin, 1992). These short-photoperiod induced changes may make the pituitary unable to respond fully when inhibition is lifted. Therefore, it is not surprising to see that prolactin levels in short photoperiod animals did not reach long photoperiod levels when dopamine inhibition was removed.

The effects of photoperiod and αMPT injections on CP dopamine and DOPAC concentrations were also measured in this experiment. Photoperiod did not affect dopamine concentrations within the CP of male hamsters. These results are consistent with the findings of Experiment 1 and indicate short-photoperiod exposure has differential effects on distinct groups of dopaminergic neurons. Injections of αMPT resulted in a decrease in CP dopamine concentrations in both long- and short-photoperiod housed animals, however, this decrease was not as robust as the decrease seen in the ME of long photoperiod housed males. The smaller decline in CP dopamine stores 30 minutes after an injection of αMPT may be due to differences in dopaminergic activity between the CP and ME. In the rat, dopaminergic activity is higher in the ME than in the CP (Lookingland & Moore, 1984). Therefore, the effect of αMPT within the CP was not expected to be as pronounced 30 minutes after an injection as it would in the ME. The failure to see a significant effect of αMPT injections on DOPAC concentrations within the CP is

also probably due to the fact that dopaminergic activity is lower in the CP.

In summary, the results of this experiment suggest that the short-photoperiod induced decrease in ME dopamine is the result of a depletion of the releasable pool of dopamine stores. This depletion of dopamine occurs in the ME, and not in other dopaminergic systems. The results of this experiment also suggests that short-photoperiod exposure disrupts the tight coupling between dopamine synthesis and release, such that synthesis cannot keep up with release. Therefore after 12 weeks of short-photoperiod exposure, the releasable stores of dopamine in the ME are depleted, but dopamine synthesis is again equal to release. The postulated increase in the release of dopamine from TIDA neurons may not only be responsible for the short-photoperiod induced decrease in ME dopamine, but it may also be responsible for both the initial decrease and maintenance of low prolactin in short-photoperiod housed animals. The following experiments examine the time course of the effects of photoperiod on ME dopamine and prolactin concentrations and TIDA neuronal activity to determine when these changes take place, and the temporal relationship among them.

EXPERIMENT 5

The Effects of Short Term Exposure to Short Photoperiod on Prolactin and ME DOPAC and Dopamine.

The previous experiments have shown that exposure to a short photoperiod (less than 12.5 hours of light/day) for approximately 12 weeks results in a significant decrease in ME dopamine concentrations and prolactin in male Syrian hamsters (Experiments 1 and 4). According to a published report (Steger & Bartke, 1991) this decrease in ME dopamine may be the result of a reduction in TIDA neuronal activity seen as early as 7 days after transferring the animals from long to short photoperiod. Steger and Bartke (1991) have shown that ME dopamine turnover decreases in animals housed in short photoperiod for only one week. In that experiment, this decrease in turnover persisted for the 10 weeks of short-photoperiod exposure. Because dopamine concentrations and rate constants were not reported separately in the paper by Steger and Bartke (1991), it not possible to determine if the decrease in turnover reported by them was due to a decrease in the rate constant or to a decrease in dopamine concentrations. Therefore, a more detailed description of this early effect of exposure to short photoperiod seems important for two reasons. First, the information may

provide insights into the initial responses that eventually result in the reduction in ME dopamine seen after longer exposures to short photoperiod (i.e., greater than one week). Second, if found to be reliable, the results of Steger and Bartke (1991) would suggest that changes in photoperiod have very rapid effects on the CNS, while effects at the level of the pituitary and gonads depend upon sustained exposure to short days (e.g. 4-6 weeks; Goldman, et al., 1981). Such a model would be valuable for understanding the mechanisms that may be involved in the short-term (e.g. 7 days or less) effects of photoperiod on TIDA neurons versus the long-term (e.g. 6-12 weeks) effects of short photoperiod on the pituitary-gonadal system . ME DOPAC concentrations were used as an index of TIDA neuronal activity (as they were in Experiment 1). However, instead of measuring ME DOPAC and dopamine after 12 weeks of short photoperiod exposure, these measures were taken after a briefer exposure (i.e., 3, 7, 14, 28 or 42 days). Preliminary work (Appendix D) ruled out the possibility that an early effect of short photoperiod exposure on prolactin or TIDA neuronal activity was due to a phase shift in a circadian rhythm of these measures.

Methods

Animals. Sixty intact male Syrian hamsters were obtained from Charles Rivers (Kings, NY). All animals were maintained as described in Experiment 1. Animals were

housed in a long photoperiod (lights on 0400, lights off 2000) for one week. Groups of ten animals were then transferred to a short photoperiod (lights on 0800, lights off 1400) at various time points. There were 6 groups (n = 10 animals/group) spending the following number of days in a short-photoperiod: 0, 3, 7, 14, 28, 42. Animals were transferred to a different colony room with a short photoperiod on a schedule which allowed all animals to be killed on 2 consecutive days.

Procedure: Animals were killed by decapitation. Brains were removed, rapidly frozen on dry ice, and stored at -70°C. Trunk blood and brains were processed as described in Experiment 1.

Neurochemical Estimation and Prolactin Assay: A detailed description of the methods used for dissecting the ME, and measuring DOPAC, dopamine and prolactin can be found in Experiment 1. Prolactin in plasma samples from this experiment and Experiments 6 and 7 was measured in a single assay. The intra-assay CVs for plasma pools containing prolactin which displaced iodinated hormone to 23% and 40% were 8% and 11% respectively.

Analysis Dunnett's tests were used to compare prolactin, and ME dopamine and DOPAC concentrations across groups using the animals kept in long photoperiod (i.e., 0 days) as a common control group for all dependent variables. Significant differences were those with $p < .05$.

DOPAC concentrations were undetectable in three males

(42 day group). The DOPAC in these samples was not detected because the sensitivity of the HPLC system was not set correctly. The setting was corrected for the measurement of the rest of the samples. The data from 10 males (1-42 days, 3-28 days, 1-14 days, 2-7 days, 2-3 days, and 1-0 days) were not included in the analyses because poor sectioning made it impossible to accurately dissect the ME.

Results

As shown in Figure 12, exposure to short photoperiod resulted in an unexpected reduction in plasma prolactin concentrations that was significant at day 3 (i.e., the first sampling time after the transfer to short photoperiod). However, by day 7, prolactin levels of short-photoperiod animals were not different from those of controls, and a sustained suppression of prolactin was not evident until the animals had been in short photoperiod for 28 days (see Figure 12A).

Dopamine concentrations in the ME were not affected by short photoperiod until the animals had been in that photoperiod for 28 days, with that sampling time being the only one significantly different from the control group (see Figure 12C). On the other hand, concentrations of DOPAC in the ME showed a significant reduction after 7 and 14 days in short photoperiod, but returned to control levels after 28 days (see Figure 12B). Testis weights were not significantly reduced after 3-42 days of short-photoperiod

exposure (Figure 12D).

Discussion

Steger and Bartke (1991) reported that ME dopamine turnover decreases as early as one week after the initial exposure to short photoperiod, and that this decrease is maintained in short-photoperiod housed males for at least 10 weeks. The results from this experiment are inconsistent with those findings. Although transfer to a short photoperiod resulted in a decrease in dopamine synthesis (as measured by a decrease in DOPAC in animals housed in short-photoperiod for 7 and 14 days), this decrease was not maintained beyond day 14, and ME DOPAC concentrations were back to control levels after 28 or 42 days of short-photoperiod exposure. It is difficult to interpret this decrease in ME DOPAC concentrations, however, it may have been secondary to the transient (day 3) decrease in circulating prolactin levels, and thus due to a reduction in the tonic stimulatory effects on TIDA neuronal activity by that hormone. The drop in ME DOPAC concentrations, however, was not immediate (i.e., not seen on day 3). This delay may reflect the relatively low sensitivity of TIDA neurons to prolactin feedback in males. For example, treating rats with drugs that increase prolactin results in an increase in TIDA neuronal activity in females, but does not produce a concurrent change in TIDA neuronal activity in males (Demarest & Moore, 1981). Alternatively, the decrease in ME

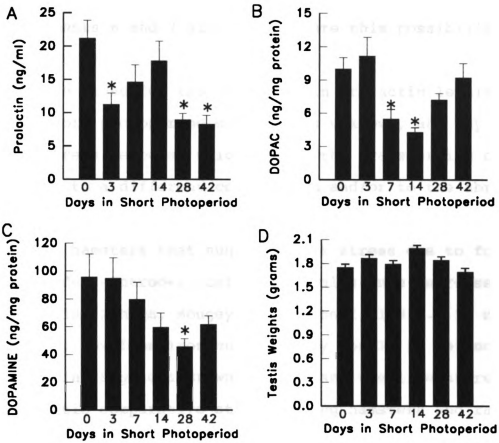

Figure 12. Mean (± sem) concentrations of plasma prolactin (A) and median eminence (ME) DOPAC (B) and dopamine (C) in male hamsters housed in a short photoperiod (SD) for varying amounts of time (i.e., 0, 3, 7, 14, 28 or 42 days). Mean (± sem) testis weights are also presented (D). Animals that were not housed in SD (0 days) were used as controls and asterisks (*) denote means that are different from those of the control animals ($p < .05$). Plasma prolactin levels were decreased in animals housed in SD for 3, 28 and 42 days. ME DOPAC concentrations were decreased in animals housed in SD for 7 and 14 days, however, ME dopamine concentrations were only decrease in animals housed in SD for 28 days. SD exposure did not have a significant effect on testis weights in these animals.

DOPAC concentrations might have been unrelated to the change in circulating prolactin, and thus due to a rapid, direct effect of photoperiod on dopamine synthesis in TIDA neurons. Experiments 6 and 7 will investigate this possibility further.

The cause for the reduction in prolactin levels on day 3 of short photoperiod exposure is unknown, and may be due to a stress response triggered by the transferring of animals to a different colony room and/or to the abrupt change in the light-dark cycle. In fact, there is evidence in male hamsters that suggests that stress due to footshock or resident-intruder conflicts results in a decrease in prolactin (Huhman, Mougey, & Meyerhoff, 1991). In male rats however, novel environments usually result in an increase in prolactin (Seggie & Brown, 1975), and the literature lacks systematic comparative studies using hamsters and rats. Experiment 6 examines the immediate effects of a change in daylength on prolactin and ME DOPAC and dopamine concentrations without introducing the animal to a novel environment.

The anticipated short-photoperiod suppression of prolactin and ME dopamine was evident 28 days after the change in photoperiod. At this time, DOPAC concentrations were similar to those of the long-photoperiod housed animals that displayed high levels of prolactin. Prolactin levels were also decreased in animals housed in short photoperiod for 42 days, however dopamine levels, although low, were not

significantly different from control levels at this time. It should be noted that part of the variability inherent in these time-course studies stems from the fact that individual animals display different rates of regression of the reproductive system after a transfer from long- to short-photoperiod. For example, in this experiment, although the mean testis weights for the groups were not significantly different, some animals housed in short photoperiod for 28 and 42 days did have regressed testes (less than 1 gram in weight versus between 1.5 - 2 grams in control animals). Therefore, the failure to see an effect of photoperiod on ME dopamine after 42 days of short-photoperiod exposure may be due to individual differences in the rate of response. Experiments with more inbred strains may be necessary to fully describe the time course of these effects.

In summary, the results of this experiment suggest that the short photoperiod induced decrease in prolactin and ME dopamine concentrations occur after 28-42 days of short photoperiod exposure. This decrease in ME dopamine concentrations and prolactin seen after 28 days of short-photoperiod exposure is not associated with a change in dopamine synthesis. Although they occur at about the same time, the present data do not provide any insights as to the causal relationship that may exist between reductions in prolactin and ME dopamine concentrations.

EXPERIMENT 6

The Early Effects (less than 7 days) of Transferring Animals from a Long to Short Photoperiod on Circulating Prolactin Levels and TIDA Neuronal Activity.

The results from Experiment 5 suggest that there are early changes seen in ME DOPAC concentrations after transferring male hamsters from a long to short photoperiod. These changes could result from fluctuations in prolactin instead of being the direct result of the change in photoperiod. The cause for the decrease in circulating prolactin levels after only 3 days of short-photoperiod exposure is unknown. Therefore, the purpose of this experiment was to determine if the fluctuations seen in prolactin after a brief exposure to short photoperiod (3 days) could be replicated in a group of animals that were kept in the same colony room for the duration of the study. Animals were killed after 0, 1, 3 or 7 days in a short photoperiod.

If exposure to short photoperiod is responsible for the early fluctuations in prolactin release and ME DOPAC, then the results of this experiment should be consistent with those of Experiment 5. However, it is possible that in Experiment 5, the animals were stressed by the change in

colony rooms as well as by the change in the light-dark cycle. In male and female rats, exposure to a novel environment is stressful, and results in an increase in circulating prolactin concentrations (Demarest, Moore, & Riegle, 1985; Seggie & Brown, 1975). However, in hamsters some stressors result in a decrease in prolactin rather than an increase (Huhman, et al., 1991). Therefore, in hamsters, exposure to a novel environment or change in photoperiod could result in a decrease in circulating prolactin concentrations. In this experiment, animals were kept in the same colony room throughout the experiment, and the change in photoperiod was accomplished by changing the light/dark cycle within the colony room. This was done to eliminate one potential source of stress to the animals.

Methods

Animals. Forty adult male hamsters were obtained from Charles Rivers (Kings, NY). Animals were maintained as previously described in Experiment 1. All animals were maintained in long photoperiod with lights on at 0400 and lights off at 2000 for one week. During this period animals were housed in two separate rooms (10 animals in one room and 30 animals in another room). After the first week, the light-dark cycle was changed in one room so that lights on was at 0800 and lights off was at 1400 (short photoperiod). Animals were killed 1, 3 or 7 days after the photoperiod change. A few animals housed in long photoperiod (controls)

were killed each day along with the short-photoperiod housed animals.

Procedure. Animals were killed by decapitation. The brains were rapidly removed and frozen on aluminum foil over dry ice. The brains were stored at -70°C until used for DOPAC and dopamine measurements. Trunk blood was collected into heparinized tubes. Treatment of the blood and plasma for the prolactin assay is described in Experiment 1.

Neurochemical Estimation and Prolactin Assay: A detailed description of the methods used for dissecting the brain tissue and measuring prolactin can be found in Experiment 1. Coefficients of variation for this assay can be found in the Methods section of Experiment 5.

Analysis Dunnett's tests were used to compare prolactin, ME dopamine and DOPAC concentrations across groups using the animals kept in long photoperiod (i.e., 0 days) as a common control group for all dependent variables. Significant differences were those with $p < .05$. Eight animals (2-7 days, 3-3 days, 1-1 day and 2-0 days) were not included in the analysis because poor sectioning made it impossible to accurately dissect the ME.

Results

Exposure to short photoperiod did not have an effect on any of the variables measured in this experiment at any time point (see Figures 13A-C). However, because the variance in prolactin levels was so large in the animals housed in short

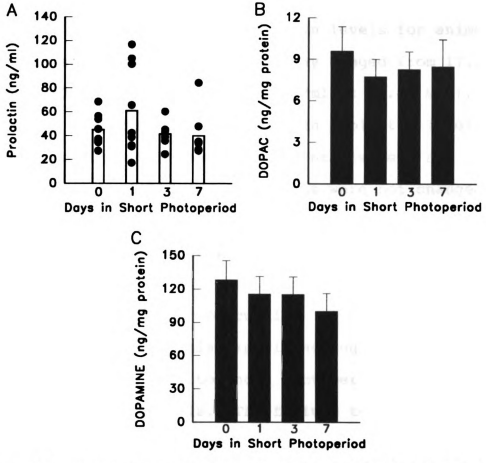

Figure 13. Mean (± sem) concentrations of plasma prolactin (A) and median eminence DOPAC (B) and dopamine (C) in male hamsters housed in a short photoperiod (SD) for varying amounts of time (i.e., 0, 1, 3 or 7 days). Animals that were not housed in SD (0 days) were used as controls. There was no effect of SD exposure on plasma prolactin levels. However, the variance in the 1 day group is significantly greater than the variance in the control animals suggesting that 1 day of SD exposure may have affected prolactin levels in some animals. SD did exposure did not affect ME DOPAC or dopamine concentrations in this experiment.

photoperiod for 1 day, a F-max test was done between this group and the controls to test for homogeneity of variance. A significant difference was found between the variances (F-max (7,8)= 7.31, $p < .05$). Prolactin levels for animals housed in short photoperiod for 1 day ranged from 17.2 to 116.8 ng/ml with a mean of 61.02 ng/ml (± 12.42 SEM). F-max tests done comparing the variances in prolactin levels of the other groups with that of the controls were not significant. ME DOPAC concentrations were not changed in the subset of animals with high prolactin after one day of short-photoperiod exposure.

Discussion

The results from this experiment suggest that the abrupt change from long to short photoperiod may be stressful to some animals. The failure to find a significant effect of photoperiod on prolactin levels in animals housed in short photoperiod for 1 day was probably the result of the high variance in prolactin levels in this group. This increased variance may reflect individual differences in the animals responses to an abrupt change in the light-dark cycle. Although it appears as if a change in photoperiod by itself may stress some animals enough to affect circulating prolactin levels 24 hours after the challenge, no residual effects were evident after 3 days in short photoperiod. It is also possible that there was no effect of stress after 3 days of short-photoperiod exposure

because the change in photoperiod was not accompanied by a change in the environment as it was in Experiment 5.

No changes were seen in ME dopamine or DOPAC concentrations in animals exposed to short photoperiod for 1 to 7 days. These finding suggest that 1) the increase in prolactin seen in the 1 day group may not have been sufficient to significantly affect TIDA neuronal activity at the sampling times used here or 2) that the stress induced by a change in photoperiod does not produce a change in TIDA neuronal activity. Stressors that increase prolactin do not always result in a change in TIDA neuronal activity. For example, in male rats exposed to restraint stress, circulating prolactin concentrations are increased, but TIDA neuronal activity is unaffected (Demarest, et al., 1985).

The results of this experiment and Experiment 5 suggest that there are no reliable effects of short photoperiod on TIDA neuronal activity. In Experiment 5, there was a reduction in ME DOPAC concentrations after only 7 days of short-photoperiod exposure, however, these changes may have been secondary to the decline in prolactin, and could not be replicated in the present experiment. In fact, in this experiment, neither prolactin nor DOPAC changed significantly in animals housed in short photoperiod for one week or less. Although prolactin levels may be affected by some of the manipulations commonly used in experiments on photoperiodic responses (i.e., abrupt changes in the illumination cycle; room changes), such effects are not

robust and may not be seen in all animals. Because the results of this experiment and Experiment 5 are not completely consistent, one more study was done to examine the effects of brief exposures to short photoperiod on TIDA neuronal activity using the method employed in Experiment 2 (DOPA accumulation).

EXPERIMENT 7

The Effects of Short Term Exposure to Short Photoperiod on TH Activity in Male Hamsters.

The results from Experiment 5 suggest that there may be direct effects of brief exposures to short photoperiod on circulating prolactin concentrations and dopamine synthesis within the ME of male hamsters, however this was not replicated in Experiment 6. The purpose of this experiment was to complement those observations by assessing TH activity (or dopamine synthesis) in the ME of animals housed in short photoperiod for various lengths of time (0, 3, 7, 28, 42 days). The method used here was identical to the one employed in Experiment 2 (i.e., measures of DOPA accumulation in the ME following injections of NSD 1015). Because the injection of NSD 1015 blocks the synthesis of new dopamine, inhibition of prolactin release by dopamine is lessened, resulting in an increase in circulating prolactin levels. Therefore, in this experiment prolactin was measured only to evaluate the response of the pituitary to the reduction in dopamine produced by the drug.

Methods

Animals. Sixty male Syrian hamsters were obtained from Charles Rivers (Kings, NY). The housing and treatment of animals are described in detail in Experiment 5.

Procedure. On the day of the experiment, animals were injected with NSD 1015 (100 mg/kg body weight) 30 minutes prior to decapitation. The methods used for storing the brains and for collecting and storing plasma for prolactin assay are described in detail in Experiment 1.

Neurochemical Estimations and Prolactin Assay: A detailed description of the methods used for dissecting the brain tissue and measuring prolactin can be found in Experiment 1. The intra-assay coefficients of variation for the prolactin assay can be found in the Methods section of Experiment 5.

Analysis. Dunnett's tests were used to compare prolactin, and ME DOPA accumulation across groups using the animals kept in long photoperiod (i.e., 0 days) as a common control group for all dependent variables. Significant differences were those with $p < .05$. Nine animals (2-42 days, 2-28 days, 1-14 days, 2-3 days and 2-0 days) were not included in the analysis because poor sectioning made it impossible to accurately dissect the ME.

Results

Exposure to short photoperiod did not have an effect on the accumulation of DOPA in the ME of hamsters (Figure

14A). Prolactin levels in this experiment were high in comparison to those seen in Experiment 5, indicating the effectiveness of the NSD 1015 injections in preventing dopamine synthesis and release, thereby disinhibiting prolactin release. However, in this experiment, animals housed in short photoperiod for 42 days had significantly lower prolactin levels than those of controls (Figure 14B). Testicular weights were also significantly reduced in animals housed in short photoperiod for 42 days (Figure 14C).

Discussion

In this experiment, there was no early effect of short-photoperiod exposure on the accumulation of DOPA in the ME after NSD 1015 injection. Although the fluctuations in the pattern of DOPA accumulation were similar to the pattern of fluctuations in DOPAC concentrations seen in Experiment 5, these changes in DOPA did not reach statistical significance. Therefore, the results of this experiment are consistent with those of Experiment 6 and suggest that there is not an early effect of short-photoperiod exposure on TIDA neuronal activity. These results do not support the findings of Steger and Bartke (1991) that exposure to short photoperiod induces a decrease in ME dopamine turnover within one week of the photoperiod change, with no concurrent effects on circulating prolactin levels. The discrepancies between the results presented here and those

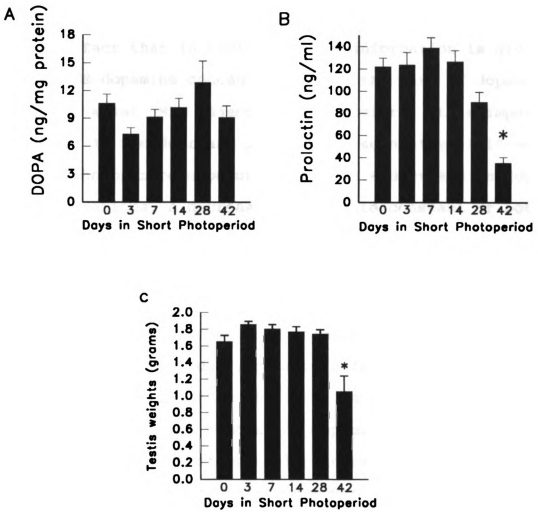

Figure 14. Mean (± sem) levels of DOPA accumulation (A) in the median eminence (ME) and plasma prolactin concentrations (B) 30 minutes after an injection of NSD 1015 in animals housed in a short photoperiod for varying amounts of time (i.e., 0, 3, 7, 14, 28 or 42 days). Testis weights (C; means ± sem) are also presented. Animals that were not housed in SD (0 days) were used as controls, and asterisks (*) denote those means that are different from those of the controls ($p < .05$). Photoperiod did not affect DOPA accumulation in the ME at any time point. Injections of NSD 1015 increased plasma prolactin levels in all animals. However, animals housed in SD for 42 days had lower plasma prolactin levels than control animals. Testis weights were also reduced in animals housed in SD for 42 days.

of Steger and Bartke (1991) may be due to methodological differences. However, a direct comparison of the present data and those from Steger and Bartke (1991) is complicated by the fact that in their paper no information is given about ME dopamine concentrations or the rate of dopamine decline after αMPT injections. Therefore, it is impossible to tell if the decrease in turnover seen after 1-10 weeks of short-photoperiod exposure is due to a decrease in dopamine concentrations, a decrease in the rate constant or both.

The most interesting finding of the present experiment was that the response of the pituitary to a decrease in dopamine changed after animals had been housed in short photoperiod for 42 days. In animals housed in short photoperiod for 42 days, prolactin levels were lower after NSD 1015 injection than they were in control animals, suggesting that the removal of dopaminergic inhibition may not significantly affect prolactin release in short-photoperiod housed animals. However, the results from Experiment 4 indicate that dopamine clearly inhibits prolactin release in males housed in a short photoperiod. Therefore, it is likely that the weak prolactin response seen after dopamine inhibition in this experiment was due to the fact that the pituitary is incapable of greatly increasing the release of prolactin in animals housed in short photoperiod for an extended period of time (at least 6 weeks) even after the removal of dopamine inhibition. Published studies examining the effects of short-photoperiod

exposure on the anterior pituitary lactotropes (prolactin secreting cells) indicate that after 8 weeks of exposure to short photoperiod, lactotropes atrophy and decrease in number (Wang, et al., 1991; Wang, et al., 1992). Therefore, it is possible that the effects of short photoperiod on the ability of the anterior pituitary to secrete prolactin are present after 6 weeks of exposure, and that the relatively low level of circulating prolactin after NSD 1015 injection in those animals was due to the inability of the pituitary to respond to the decrease in dopamine. The effect of short-photoperiod exposure on lactotropes could be the result of an increase in dopamine release (Experiment 4). When lactotropes are exposed to higher concentrations of dopamine, both prolactin release and synthesis are decreased (for review see; Ben-Jonathan, 1985). Therefore, if dopamine release from TIDA neurons was increased in animals housed in short photoperiod for 42 days, both prolactin release and synthesis may have been inhibited, thereby leaving the pituitary unable to respond to a decrease in dopamine inhibition as strongly as it would in a long-photoperiod housed animal. If this is the case, an increased release of dopamine from TIDA neurons may be responsible for both the initial and maintained decrease in prolactin release seen during this time.

GENERAL DISCUSSION

Because many of the effects of photoperiod on behavior and endocrinology are the result of changes in hypothalamic function, it is important to understand how photoperiod affects hypothalamic neurochemistry. One system that is sensitive to changes in photoperiod is the TIDA neuronal system (Steger & Bartke, 1991; Steger, et al., 1985; Steger, et al., 1984). These neurons play an important role in the regulation of hormone synthesis and release by the anterior pituitary. Previous work examining the effects of short-photoperiod exposure on ME dopamine concentrations and TIDA neuronal activity has produced conflicting results (Steger, et al., 1985; Steger, et al., 1984). The experiments presented here examined the effects of photoperiod on ME dopamine concentrations and TIDA neuronal activity using a number of different indices in an effort to resolve the conflicts found in the literature.

The main finding of the experiments presented here is that there is a decrease in ME dopamine concentrations in male hamsters housed in a short photoperiod (Experiments 1, 4 and 5). This short-photoperiod induced decrease in ME dopamine concentrations does not appear to be dependent upon the decrease in testosterone that occurs at this time (Desjardins, et al., 1971) because castrated males also show

the photoperiod-induced decline in ME dopamine and the effect is not seen in castrates kept in long photoperiods. However, in Experiment 1, the decline in ME dopamine was not as pronounced in castrated animals as it was in intact animals, even though prolactin levels were suppressed in both groups. In male rats, testosterone inhibits dopamine synthesis in the ME of gonadally intact males and castration removes this inhibition (Toney, et al., 1991). A similar relationship between the testes and TIDA neuronal activity seems to be present in the hamster, with dopamine synthesis (as measured by DOPAC) being higher in castrated animals than in intact animals (Experiment 1). Therefore, this increase in dopamine synthesis after castration may help maintain ME dopamine concentrations in short-photoperiod housed males.

In order to understand the functional significance of the short-photoperiod induced reduction in ME dopamine concentrations in males, the mechanisms responsible for the decrease (e.g., changes in synthesis or release) need to be identified. Steger et al., (1985) suggested that the decrease in TIDA neuronal activity (dopamine turnover) seen in short-photoperiod males was due to a reduction in positive feedback by prolactin, and in turn responsible for the lower ME dopamine concentrations. However, data from Experiments 1 and 2 indicate that after 12 weeks of short-photoperiod exposure, the photoperiod-induced decrease in prolactin and ME dopamine are not associated with a decrease

in TIDA neuronal activity. Neither ME DOPAC concentrations (Experiment 1) nor ME DOPA accumulation (Experiment 2) were affected by photoperiod, indicating that dopamine synthesis was the same in long- and short-photoperiod animals. Experiment 3 showed that the number of TH+ neurons in the arcuate nucleus of male hamsters was not affected by photoperiod, suggesting that there is not a decrease in the number of neurons synthesizing dopamine in short-photoperiod animals. Taken together, the results of these experiments indicate that the decrease in prolactin positive feedback in short-photoperiod animals does not result in a decrease in dopamine synthesis, and thus this cannot serve as an explanation for the depletion in ME dopamine.

The data from Experiment 4 however, suggest that the decrease in ME dopamine may be the result of an increase in dopamine release from TIDA neurons without a compensatory increase in dopamine synthesis. If this is the case, then exposure to a short photoperiod disrupts the tight coupling between dopamine synthesis and release. Although dopamine synthesis is regulated by end-product inhibition (Demarest & Moore, 1979a), certain manipulations appear to interfere with this tight coupling in TIDA neurons. For example, in female rats, long-term estrogen treatment (i.e., 12 days) results in an increase in circulating prolactin levels. This increase in prolactin is associated with a decrease in ME dopamine stores, but at this time point ME DOPA accumulation is not changed (Demarest, et al., 1984). Other

manipulations that affect prolactin and ME dopamine concentrations, such as suckling (Demarest, et al., 1983) and morphine treatment (Alper, et al., 1980) also disrupt the coupling between synthesis and release. Thus, decoupling of synthesis and release is not rare, but seems to be produced by manipulations that increase circulating prolactin concentrations. On the other hand, in the experiments presented here, decreased levels of circulating prolactin were associated with the apparent uncoupling of dopamine synthesis and release in short-photoperiod housed males. One could speculate that in short-days, hamsters become hypersensitive to the stimulatory effects of prolactin, and thus, TIDA neurons behave in a fashion similar to what is seen when prolactin is elevated in the other models (i.e., increase in estrogen, suckling etc.). Changes in hypothalamic sensitivity to hormone feedback in short-photoperiod exposed males is a well documented phenomenon (Ellis & Turek, 1979). If in fact, TIDA neurons do become very sensitive to prolactin stimulation during short photoperiods, this change in sensitivity may result in an uncoupling between dopamine synthesis and release, with release at some point exceeding synthesis. An increase in release without a corresponding increase in synthesis would result in a decrease in ME dopamine stores and a sustained inhibition of prolactin, even though circulating prolactin levels are already low.

Therefore, in the proposed model for a long-photoperiod

animal (Figure 15A), dopamine is synthesized and can either be stored within the terminal or released. Both prolactin and the cytoplasmic pools of dopamine are responsible for keeping dopamine stores relatively stable at this time. This tight regulation of dopamine synthesis and release in the long-photoperiod animals helps maintain relatively stable prolactin levels. However, in short photoperiods, TIDA neurons may become more sensitive to prolactin. It is proposed that this enhanced sensitivity has a dual effect on TIDA neurons. One, it causes a uncoupling between synthesis and release and two, it increases release of dopamine. This increase in release then would result in the depletion of dopamine stores. Furthermore, the postulated uncoupling of synthesis and release changes the sensitivity of TH to end-product inhibition such that the significant depletion of stores does not stimulate synthesis. If this is the case, and the short-photoperiod induced decrease in ME dopamine is associated with an uncoupling between synthesis and release, then the decrease in ME dopamine due to enhanced release would not be associated with a change in ME DOPAC concentrations or DOPA accumulation. The results of the experiments presented here are consistent with this idea; The short-photoperiod induced decrease in ME dopamine was not associated with changes in DOPAC or DOPA after 6 or 12 weeks of short-photoperiod exposure. However, if dopamine release had been measured after 4-6 weeks of short-photoperiod exposure, an increase in the rate of release may

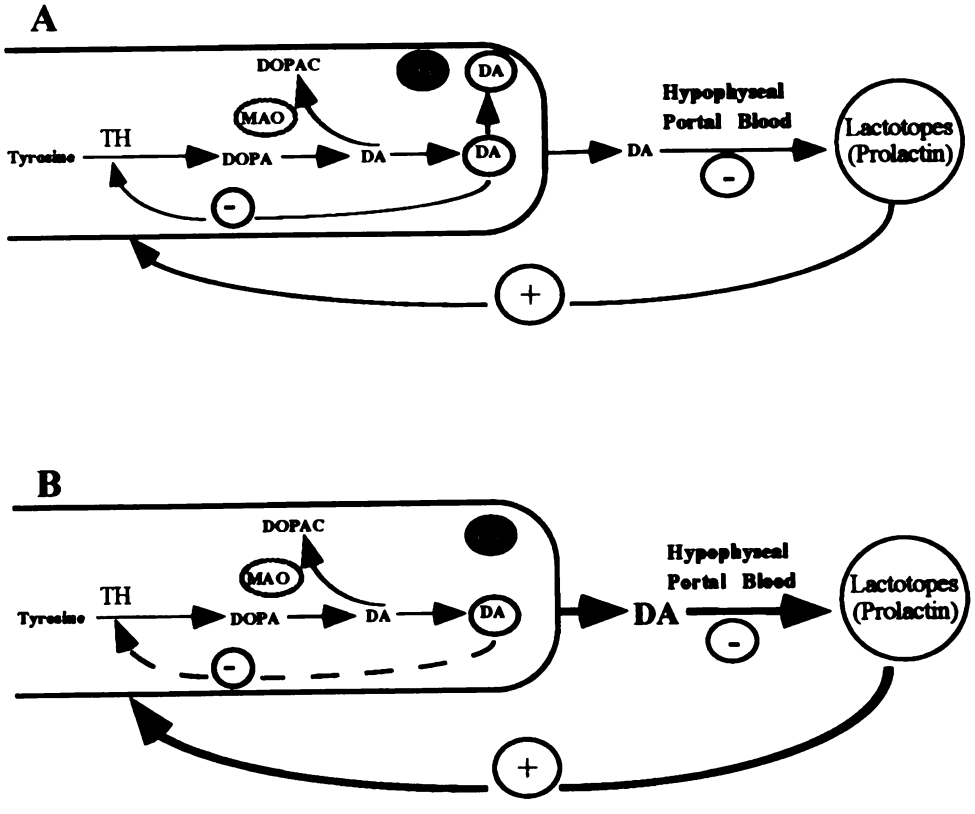
A
DOPAC
DA
MAO
TH
Tyrosine
DOPA
DA
DA
DA
Hypophyseal
Portal Blood
Lactotopes
(Prolactin)
-
+
B
DOPAC
MAO
TH
Tyrosine
DOPA
DA
DA
DA
Hypophyseal
Portal Blood
Lactotopes
(Prolactin)
-
+

Figure 15. Schematic showing the effects of long- (A) and short-photoperiod (B) exposure on dopamine synthesis and metabolism in TIDA neurons of male hamsters. In long photoperiods, dopamine (DA) synthesized by a neuron has one of three fates; 1) It can be released into the hypophyseal portal blood vessels and carried to the anterior pituitary where it inhibits prolactin release, 2) It can be vessicled and stored in either the releasable (white) or nonreleasable (black) pools of DA in the terminal, or 3) it can be metabolized by monoamine oxidase (MAO) to for 3,4-dihydroxyphenylacetic acid (DOPAC). Cytoplasmic DA feeds back onto tyrosine hydroxylase (TH) to keep DA stores within the terminal relatively stable. Prolactin also feeds back onto TIDA neurons to stimulate their activity (synthesis and release of DA). In short photoperiods, DA synthesis is unchanged. However, after extended exposure to a short photoperiod (i.e., maybe 4-6 weeks) the sensitivity of TIDA neurons to prolactin stimulation is enhanced (thick line from prolactin). This increased sensitivity to prolactin disrupts the tight coupling between DA synthesis and release (dashed line from DA), and results in an increase in release without a compensatory increase in synthesis. This increase in release depletes the terminal of all releasable stores of DA, leaving only the nonreleasable pool. This increase in DA release may also be responsible for inhibiting prolactin release from the anterior pituitary. The failure of DA synthesis to increase in response to this increase in release results in newly synthesized DA being either released or metabolized, and not being maintained to replace lost stores.

have been seen in the short-photoperiod housed animals.

The problem with this model is that at least in the male rat, prolactin does not appear to have a strong influence on TIDA neuronal activity (Demarest & Moore, 1981). However, because the hamster is photoperiodic, and the short-photoperiod induced change in prolactin may be important for regulating testicular regression (Bartke, Goldman, Bex, Kelch, Smith, Dalterio, et al., 1980; Bartke, Hogan, & Cutty, 1979), the regulation of TIDA neurons by prolactin might be different in these animals. Experiments examining the effects of prolactin feedback onto TIDA neuronal activity are needed to determine how this system is controlled in male hamsters and how photoperiod can affect this system.

Although the results of the experiments in the body of this dissertation suggest that the short-photoperiod induced decrease in ME dopamine is the result of an increase in dopamine release without a compensatory increase in synthesis, a preliminary experiment (Appendix E) suggests that there may also be a decrease in the number of neurons synthesizing dopamine in the arcuate nucleus of short-photoperiod housed males. In that experiment, immunocytochemistry was done for LAAD (the enzyme that converts DOPA to dopamine) in long- and short-photoperiod housed males. The results of that experiment suggest that there is a decrease in the number of LAAD immunopositive cells within the arcuate nucleus of male hamsters housed in

a short photoperiod. If exposure to a short photoperiod results in a decrease in LAAD production in TH producing cells, dopamine synthesis would be decreased. Therefore, it is possible that the short-photoperiod induced decrease in ME dopamine is due to a decrease in dopamine synthesis. This decrease in synthesis would not be evident in measurements of DOPA accumulation, because neurons producing TH would still be producing DOPA, so a decrease in dopamine synthesis due to a decrease in LAAD activity would be consistent with the results of Experiment 2. However, if there is a decrease in dopamine synthesis, ME DOPAC concentrations should have been reduced, and this was not the case in Experiments 1, 4 or 5, thereby suggesting that there is not a decrease in the number of neurons synthesizing dopamine in short-photoperiod housed animals. It is possible, however that in short photoperiods, some neurons stop synthesizing LAAD and dopamine, while other neurons increase their activity in an attempt to make up for this loss of dopamine, and that gross estimates of dopamine synthesis (ME DOPAC) in tissue are not discriminatory enough to detect changes in individual neurons. If some neurons are more active during short photoperiods, not only would they increase their rate of dopamine synthesis, but the rate of release would also be increased. Therefore, it is possible that the decrease in ME dopamine is due to two factors, a decrease in the number of neurons synthesizing dopamine and an increase in dopamine synthesis and release

from TIDA neurons that are still active. Because LAAD is synthesized in a number of neurons (i.e., other catecholaminergic, serotonergic, histaminergic, peptidergic neurons) the photoperiod-induced change in LAAD may be related to changes in one of these other neurotransmitters systems, and not due to change in dopamine producing neurons. To be certain that this change in LAAD immunostaining occurs in dopamine neurons, double-label immunocytochemistry for TH and LAAD should be done to determine if there is a decrease in LAAD staining in TH immunopositive neurons within the arcuate nucleus of males housed in short-photoperiods.

In female hamsters, there was no effect of short-photoperiod exposure on ME dopamine concentrations or TIDA neuronal activity (ME DOPAC), but circulating prolactin concentrations were significantly decreased. The failure to see an effect of photoperiod on TIDA neuronal activity might be due to a number of factors. First, it is possible that females were not killed at the optimal time to detect a photoperiod-induced difference in TIDA neuronal activity. Different from males (Appendix D), female hamsters kept in long photoperiods show robust circadian rhythms in circulating prolactin concentrations (Widmaier & Campbell, 1981). In Experiment 1, long-photoperiod females were killed during a phase of the circadian cycle when prolactin levels are relatively low (Widmaier & Campbell, 1981). Therefore, if this low level of prolactin is associated with

low TIDA neuronal activity, a photoperiod-induced decrease in TIDA neuronal activity may have escaped detection. However, the fact that differences in circulating prolactin levels were detected across photoperiods at this sampling time argues against that interpretation. It is possible that as suggested for males, there is also an increase in the sensitivity to prolactin positive feedback in short-photoperiod housed females, so that even when prolactin is low, TIDA neuronal activity remains unchanged. Further studies investigating the effects of prolactin feedback onto TIDA neuronal activity for both sexes and photoperiods may help us understand the relationship between these two systems in photoperiodic species.

ME dopamine concentrations were also not affected by photoperiod in females. First, it is possible that in this experiment, females began to recrudesce earlier than males. Hypothalamic dopamine concentrations return to long-photoperiod levels in male hamsters before testicular recrudescence is observed (Steger, et al., 1982). Therefore, if females recrudesced earlier than males, ME dopamine concentrations could have already returned to levels seen in long photoperiod. It is also possible that because females have naturally higher levels of TIDA neuronal activity (see Appendix A), dopamine synthesis can replenish lost stores more rapidly. Therefore, a temporary increase in dopamine release may not result in a long-term depletion of dopamine stores in the ME of females. Finally,

it is also possible that there is a sex difference in the effects of photoperiod on TIDA neurons (or in the coupling of release and synthesis).

There are a number of experiments which indicate that photoperiod differentially affects the release of anterior pituitary hormones in males and females. Thus, in retrospect, it is not surprising to find sex differences in how photoperiod affects the hypothalamus. Blask et al. (1986) have shown that the decrease in prolactin seen in blinded hamsters is mediated, in part, by different mechanisms in males and females. Pinealectomy in male hamsters prevents the decrease in prolactin that occurs due to light deprivation. However, in females, pinealectomy only partially blocks this decrease in prolactin, and thus prolactin levels are still lower in blinded-pinealectomized females than in control females (Blask, et al., 1986). The photoperiodic regulation of the gonadotropins is also different in male and female hamsters. In males exposure to a short photoperiod results in a decrease in circulating gonadotropin levels (Turek, et al., 1975). However, in females, exposure to a short photoperiod results in daily afternoon surges of the gonadotropins (Bridges & Goldman, 1975). Therefore only in males is there a short-photoperiod suppression of gonadotropin release. Because TIDA neurons are capable of inhibiting GnRH release (Rasmussen, 1991) they could be involved in the decline in these hormones seen in males exposed to short-photoperiod. In females however,

there is no decrease in the release of gonadotropins, but rather a change in the timing of release, indicating that some other mechanism, perhaps a change in dopaminergic activity in the preoptic area (MacKenzie, et al., 1988) or a change in the suprachiasmatic nuclei (Stetson & Watson-Whitmyre, 1976), may mediate this effect of photoperiod. Therefore, future experiments examining the effect of photoperiod on the hypothalamus of female hamsters should focus on other hypothalamic sites as well as on other neurochemical systems.

Contrary to previous reports (Widmaier & Campbell, 1981), ovariectomy did not affect prolactin concentrations. There also was not an effect of ovariectomy of TIDA neuronal activity, as there is in female rats (Demarest & Moore, 1981; Gudelsky & Porter, 1981). Because the effects of ovariectomy on TIDA neuronal activity are the result of the decrease in prolactin in female rats (Demarest & Moore, 1981), it is not surprising that TIDA neuronal activity was not affected in these hamsters. Also, as mentioned in Experiment 1, in other experiments females were ovariectomized for a brief period of time before sampling (i.e., 1-2 weeks), whereas animals in this experiment were ovariectomized for 12 weeks when these variables were measured. Thus, the failure to see an effect of ovariectomy may have been due to a reorganization of these systems in the prolonged absence of steroid hormones.

Regardless of possible changes in sensitivity to

prolactin stimulation in short-photoperiod animals, there is evidence that indicates that dopamine plays a role in the control of prolactin in short days. For example, when dopaminergic inhibition is removed (as in Experiment 4 and 7) prolactin levels increase in both long- and short-photoperiod housed animals. If the prolactin levels from Experiment 5 are used as baseline measures of prolactin, and the increase in prolactin seen after an injection of NSD 1015 in long- and short-photoperiod housed animals is compared to baseline measures, the control animals in Experiment 7 show a 5-fold increase in prolactin after an injection of NSD 1015 and the animals in short photoperiod for 6 weeks show a 4-fold increase. In Experiment 4, long-photoperiod animals injected with αMPT show a 4-fold increase in prolactin over baseline, but short-photoperiod animals injected with αMPT show an 8-fold increase in prolactin above baseline. Therefore, even though the absolute levels of prolactin are different in long- and short-photoperiod animals injected with NSD 1015, the increase in prolactin from baseline in these animals is comparable. This suggests that dopamine inhibits prolactin release in short-photoperiod animals. *In vitro* work has also shown that dopamine inhibits the release of prolactin from the anterior pituitary of males housed in a short photoperiod for 27 or 77 days (Steger, Bartke, Goldman, Soares, & Talamantes, 1983). In fact, the results of that experiment indicate that the pituitary of those animals is

more sensitive to the inhibitory effects of dopamine than that of long-photoperiod animals. Thus, these studies indicate that prolactin release is inhibited by dopamine in short-photoperiod males. Although the available data indicate that dopamine can inhibit prolactin release from the anterior pituitaries of males, this does not rule out a role for other prolactin inhibiting factors in hamsters. In rats, dopamine from the posterior pituitary is also capable of inhibiting prolactin release (Peters, Hoefer, & Ben-Jonathan, 1981). Therefore, it is possible that short-photoperiod induced decrease in prolactin in male and female hamsters is related to a change in posterior pituitary dopaminergic activity. Photoperiod exposure may also result in a decrease in prolactin releasing factors (e.g., vasoactive intestinal peptide (for review see; Neill & Nagy, 1994)) and thereby influence prolactin release. However, at this time, the effects of photoperiod on these other factors is unknown.

In summary, the results of the experiments presented here indicate that exposure to a short photoperiod results in a decrease in ME dopamine concentrations in male hamsters. This decrease in ME dopamine may be the result of an increase in dopamine release from TIDA neurons without a compensatory increase in synthesis. However, future experiments should focus on examining the effects of short-photoperiod exposure on dopamine release after only 4-6 weeks of short-photoperiod exposure, when the decrease in

dopamine first takes place. More direct methods for estimating release might also be helpful. By cannulating the portal blood vessels or the anterior pituitary, both the amount of dopamine released into the hypophyseal portal blood and the amount of dopamine reaching the anterior pituitary could be measured. These methods could directly test the hypothesis that the short-photoperiod induced decrease in ME dopamine is due to an increase in dopamine release, and that this increase in dopamine release is responsible for the short-photoperiod induced decrease in prolactin.

APPENDICES

APPENDIX A

APPENDIX A

Dopaminergic Activity in the Central Nervous System of the Syrian Hamster.

A number of studies have shown that gender and gonadal steroids affect the activity of dopaminergic neurons in the rat. For example, TIDA neuronal activity is higher in female rats on day one of diestrus than in male rats (Demarest et al., 1981). This difference in TIDA neuronal activity is in part due to a sex difference in the circulating levels of prolactin, with female rats having higher levels of circulating prolactin than male rats (Neill, 1972). In hamsters, there is also a sex difference in circulating prolactin levels (Borer, Kelch, & Corley, 1982), however, it is not known if higher prolactin levels in female hamsters are associated with higher levels of TIDA neuronal activity. The goal of this experiment was to see if as in the rat, differences in circulating prolactin levels were associated with differences in TIDA neuronal activity (as measured by DOPAC concentrations in the ME).

Methods

Animals. Eight adult male and eight adult female hamsters were obtained from Charles Rivers (Kingston, NY). All animals were housed in hanging cages and supplied with Rodent Laboratory Chow 5001 (Purina) and water ad libitum. Animals were maintained on a long photoperiod with lights on at 0400 and lights off at 2000 (16L:8D). Females were checked daily for the presence of proestrus discharge (Orsini, 1961).

Procedure. On day one of diestrus, females and an equal number of males were killed by decapitation between 0900 and 1000. Brains were rapidly removed and frozen on aluminum foil over dry ice. Trunk blood was collected into heparinized tubes and centrifuged at 4000 rpm for 30 minutes. The plasma was removed and frozen at -20°C until assayed for prolactin.

Neurochemical Estimation and Prolactin Assay. A detailed description of the methods used to dissect the ME and measure concentrations of ME DOPAC and dopamine and prolactin can be found in Experiment 1. The coefficients of variation for the prolactin assay can be found in Experiment 5.

Analyses. Concentrations of DOPAC and dopamine in the ME, and plasma prolactin levels of males and females were compared using a Students t-test. Differences were considered significant if $p \leq .05$.

Results and Discussion

Plasma prolactin levels were higher in female hamsters than in male hamsters [Figure 16A; ($t(14)= 2.12$, $p \leq .05$)]. This finding is consistent with previous work showing a sex difference in circulating prolactin levels in hamsters (Borer, et al., 1982). There was also a sex difference in ME DOPAC concentrations, with females have higher concentrations of DOPAC than males [Figure 16B; ($t(14) = 3.32$. $p \leq .05$)]. Gender did not affect ME dopamine concentrations (Figure 16C).

In female hamsters, high levels of circulating prolactin were associated with increased TIDA neuronal activity (higher DOPAC concentrations) and in male hamsters, lower levels of circulating prolactin were associated with decreased TIDA neuronal activity. These data are similar to data from the rat showing that TIDA neuronal activity is higher in females than in males (Demarest, et al., 1981). The results of this experiment are important because they suggest that prolactin is feeding back onto TIDA neurons to regulate their activity in hamsters, as it does in rats. This suggests that the regulation of TIDA neuronal activity in the rat and hamster are similar, and therefore, the model used to describe the relationship between TIDA neuronal activity and prolactin in the rat may also be useful for studying this relationship in the hamster.

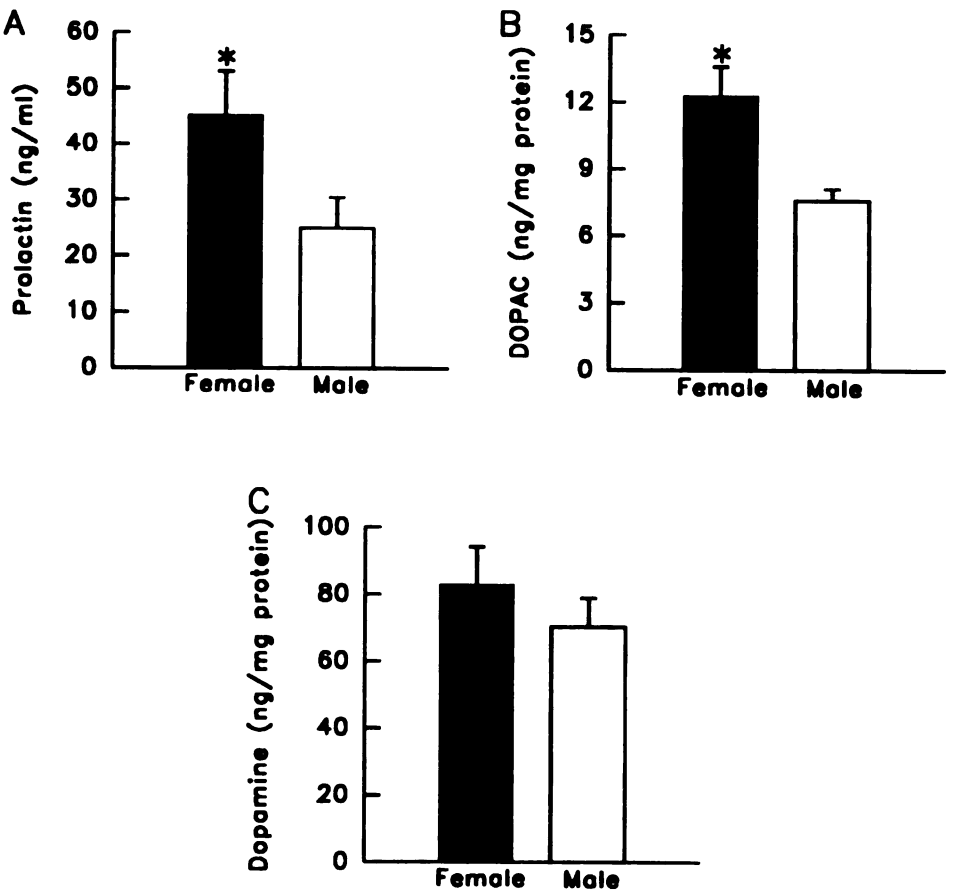

Figure 16. Mean (± sem) concentrations of plasma prolactin (A) and median eminence (ME) DOPAC (B) and dopamine (C) concentrations in male and female hamsters housed in a long photoperiod. The asterisk (*) denotes that mean levels in one sex are greater than those in the other sex ($p < .05$). Plasma prolactin levels were higher in female hamsters than in male hamsters. These increased levels of prolactin in females were associated with higher ME DOPAC concentrations. ME dopamine concentrations were not different between males and females.

APPENDIX B

APPENDIX B

DOPA Accumulation in the ME of the Male Syrian Hamster After an Injection of an l-Aromatic Amino Acid Decarboxylase Inhibitor.

One method that has been used to estimate dopaminergic activity is to measure the accumulation of DOPA after the injection of a L-aromatic amino acid decarboxylase inhibitor such as NSD 1015 (Carlsson, et al., 1972). DOPA accumulation has been used as an estimate of TH activity (Carlsson, et al., 1972) and dopamine synthesis (Demarest & Moore, 1980). In the ME, DOPA accumulates in a linear fashion for at least 30 minutes after NSD 1015 injection in rats, after which point the increase in accumulation levels off because of end-product inhibition (Carlsson, et al., 1972). The purpose of this experiment was to determine the time course for DOPA accumulation in the hamster.

Methods

Animals. 50 adult male Syrian hamsters were obtained from Charles Rivers (Kingston, NY). The animals were maintained as previously described. All animals were housed in a long photoperiod for one week, with lights on at 0400 and lights off at 2000 for one week.

Procedure. On the day of the experiment, groups of 10 animals were injected with NSD 1015 (i.p. 100 mg/kg) 15, 30, 45 or 60 minutes before decapitation. One group of animals (controls) was injected with saline vehicle 60 minutes prior to decapitation. After decapitation, the brains were rapidly removed and frozen on aluminum foil over dry ice.

Neurochemical estimates. DOPA accumulation in the ME was measured using HPLC-ED. A detailed description of the methods can be found in Experiment 1.

Analysis. DOPA accumulation in the ME over time was analyzed using a oneway ANOVA. Pairwise comparisons were done using a Tukey-HSD. Trend analysis was also done to determine if there was a linear component to the increase in DOPA after an injection of NSD 1015. Differences with $p < .05$ were considered significant.

Results and Discussion

Figure 17 demonstrates the accumulation of DOPA in the ME at various time points after NSD 1015 injection. There was a significant effect of time after injection on ME DOPA accumulation ($F(4,36)= 13.59$, $p < .05$). Pairwise comparisons revealed that DOPA accumulation increased significantly over time ($p < .05$) except between 45 and 60 minutes. ME DOPA accumulation 60 minutes after an injection of NSD 1015 was not different from DOPA accumulation 30 or 45 minutes after injection. These results suggest that DOPA accumulation leveled off between 45 and 60 minutes after NSD

1015 treatment. These results are consistent with the findings in rats (Carlsson, et al., 1972). Trend analysis revealed that there was a significant linear component to the data ($F(1,36)= 13.59$, $p < .05$). This linear component occurs between 0 and 45 minutes after injection. Therefore, measurement of TH activity in other experiments will be accomplished by measuring DOPA accumulation in the ME 30 minutes after an injection of NSD 1015.

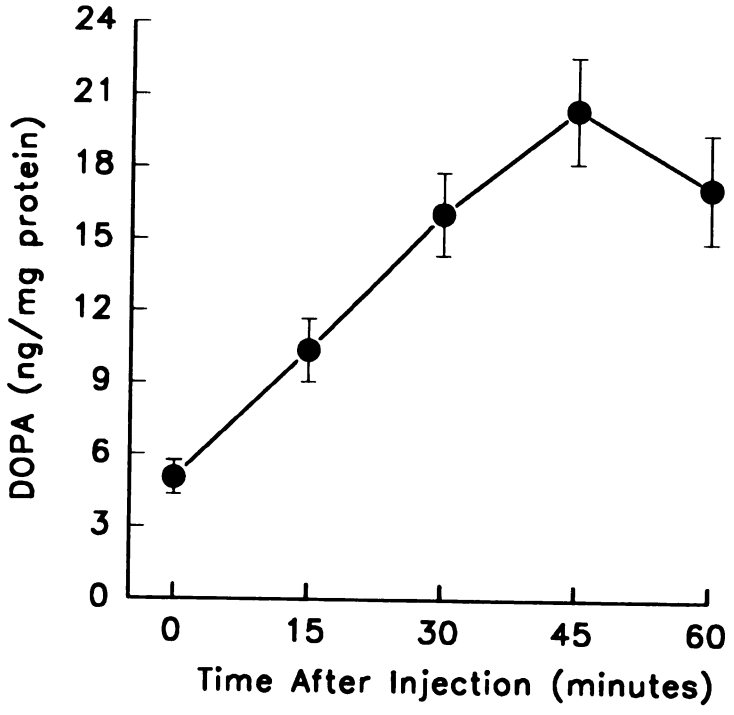

Figure 17. The time course of DOPA accumulation in the ME of male hamsters injected with an LAAD inhibitor, NSD 1015. Values represent means ± sems. The accumulation of DOPA in the ME was linear between 0 and 45 minutes after an injection of NSD 1015.

APPENDIX C

APPENDIX C

The Effects of α-MPT on Dopamine Concentrations in the ME of Male Hamsters.

Turnover is one method that has been commonly used as a measure of dopaminergic activity. To measure turnover, a TH inhibitor, such as α-methylparatyrosine (αMPT), is used to block dopamine synthesis. The rate at which stores decline after injection is then measured so that turnover may be calculated (Brodie, et al., 1966). This decrease in dopamine is due to release, and therefore, the rate of decline of dopamine can be used as an index of dopamine release. Exposure to short- photoperiod may be decreasing ME dopamine concentrations by increasing the release of dopamine from the ME. Therefore, we would expect that the rate of dopamine decline after αMPT injection would be much faster animals exposed to a short photoperiod than in those exposed to a long photoperiod. Because the rate of decline in dopamine in the short-photoperiod housed animals may be very rapid, it is important to determine the earliest time point at which the effects of αMPT on ME dopamine concentrations can be measured. The purpose of this study was to determine if a decrease in dopamine could be measured as early as 15 minutes after αMPT injection. Because αMPT blocks the synthesis of new dopamine, DOPAC concentrations

should also decrease.

Methods

Animals. 16 male hamsters were obtained from Charles Rivers (Kingston, NY) and maintained as described in Experiment 1. All animals were housed in a long-photoperiod with lights on at 0400 and lights off at 2000 for one week.

Procedure. On the day of the experiment, animals were injected with either αMPT (250 mg/kg, i.p.; Steger, et al., 1982) or saline 15 minutes prior to decapitation. After decapitation, the brains were rapidly removed and frozen on aluminum foil over dry ice.

Neurochemical Estimations. A detailed description of the method used to dissect the tissue and measure ME DOPAC and dopamine can be found in Experiment 1.

Analyses. The effects of αMPT on ME DOPAC and dopamine concentrations were analyzed using a Students *t*-test. Differences were significant if $p < .05$.

Results and Discussion

ME DOPAC and dopamine concentrations were not significantly affected 15 minutes after αMPT injection (Figures 18A and B). Previous work in the hamster has shown that there is a linear decline in hypothalamic dopamine concentrations when the following time points after injection are used; 30, 60 and 120 minutes (Steger, et al., 1982). Therefore, in Experiment 4, animals will be killed

30 and 60 minutes after αMPT injection for estimating dopamine turnover.

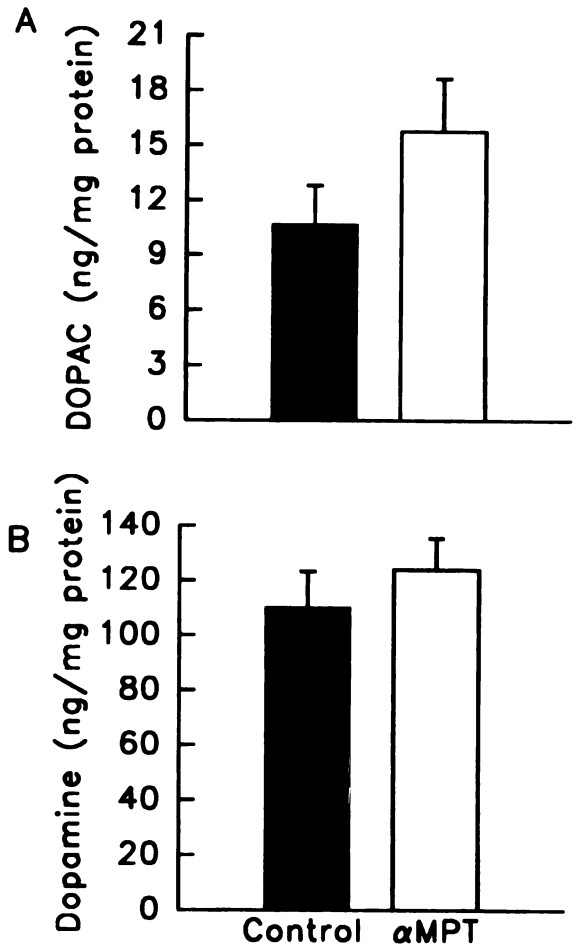

Figure 18. Mean (± sem) concentrations of DOPAC (A) and dopamine (B) in the median eminence (ME) of male hamsters killed after a saline injection or 15 minutes after an injection of a tyrosine hydroxylase inhibitor, αMPT. Neither ME DOPAC nor dopamine concentrations were reduced from control levels 15 minutes after an injection of αMPT.

APPENDIX D

APPENDIX D

The Effect of Time of Day on Circulating Prolactin and TIDA Neuronal Activity in Male Hamsters.

Steger and Bartke (1991) have suggested that a change in photoperiod can have an effect on TIDA neuronal activity as early as one week after animals are transferred from a long to short photoperiod. Dopamine turnover in the ME of male hamsters was significantly decreased in animals that were housed in short photoperiod for one week, and this decrease was maintained for at least 10 weeks (Steger & Bartke, 1991). This rapid decrease in ME dopamine turnover could be the result of short-photoperiod exposure, however, these findings could also be the result of a shift in a circadian rhythm. Although there is no evidence for a circadian rhythm in TIDA neuronal activity, there is a rhythm in prolactin release in female hamsters (Widmaier & Campbell, 1981), and possibly a rhythm in the release of prolactin in male hamsters (Goldman, et al., 1981). Because circulating prolactin levels and TIDA neuronal activity are tightly coupled, it is possible that a rhythm in prolactin could be associated with a rhythm in TIDA neuronal activity. If exposure to a short photoperiod shifts these rhythms, comparing control (i.e., housed in long photoperiod) animals

and animals housed in short photoperiod at a single clock time may result in apparent differences in TIDA neuronal activity that are actually the result of the phase-shifted rhythm. For example, both plasma prolactin and ME DOPAC concentrations might be low 0400 (the time of lights on), but increase significantly by 0800 in long-photoperiod housed animals. In short-photoperiod housed animals, the rhythms in plasma prolactin and TIDA neuronal activity might shift to fit the light/dark cycle, and therefore prolactin and DOPAC concentrations would be low at 0800 (time of lights on) but increase significantly by 1200. If this is the case, sampling at a single clock time (i.e., 0800) would indicate that there is a photoperiod-induced decrease in prolactin and ME DOPAC concentrations. However, this decrease would be an artifact of the testing time rather than a direct result of photoperiod exposure, and testing over the circadian cycle would reveal that there is a shift in the rhythm and not a photoperiod-induced decrease in these measures.

The purpose of this experiment was to determine if there was a circadian rhythm in prolactin and TIDA neuronal activity in male hamsters housed in long photoperiod. Circulating prolactin levels and ME DOPAC and dopamine concentrations were measured in male hamsters that were killed every three hours over a 24 hour period. The presence of a rhythm would suggest that the photoperiod induced decrease in prolactin and ME dopamine may be due to

a change in the rhythm rather than to a direct effect of photoperiod.

Methods

Animals. Sixty-four adult male Syrian hamsters obtained from Charles Rivers (Kingston, NY) were maintained in a long photoperiod as previously described in Experiment 1.

Procedure. Animals were decapitated at various times after lights on (in hrs: 0, 3, 6, 9, 12, 15, 18, 21). A description of the removal and storage of the brains, and the collection of blood for the prolactin assay can be found in Experiment 1. The animals that needed to be killed during the dark phase of the cycle were killed under red light.

Neurochemical Estimation and Prolactin Assay. These procedures are described in detail in Experiment 1. The coefficients of variation for the prolactin assay can be found in Experiment 5.

Analyses. Plasma prolactin, and ME DOPAC and dopamine concentrations were analyzed using a oneway ANOVA. Differences were significant if $p < .05$.

Results

Graphs depicting circulating prolactin levels and ME DOPAC and dopamine concentrations over a 24 hour period can be found in Figure 19 (A-C). Time of day did not have an

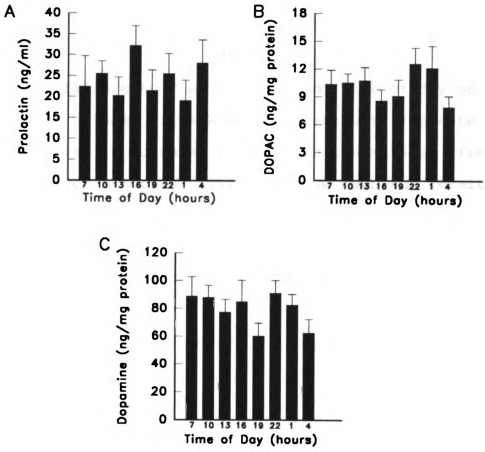

Figure 19. Mean (± sem) concentrations of plasma prolactin (A) and median eminence DOPAC (B) and dopamine (C) measured every 3 hours over a 24 hour period in male hamsters. There was no apparent circadian rhythm in any of these measures.

effect on any of these variables, suggesting that there is not a circadian rhythm in either prolactin release or TIDA neuronal activity in male hamsters.

Discussion

The failure to find an effect of time of day on prolactin release or TIDA neuronal activity suggests that the decrease in dopamine turnover in the ME seen after one week (Steger & Bartke, 1991) may have been the result of the change in photoperiod. Therefore, the effects of photoperiod seen after prolonged exposure may be due to changes happening after relatively brief exposures to short photoperiod.

This possibility was tested in Experiments 5-7 of this dissertation.

APPENDIX E

APPENDIX E

LAAD Immunostaining in the Arcuate Nucleus of Long- and Short-Photoperiod Housed Males

In Experiment 3, immunocytochemistry (ICC) for TH was done to determine if short-photoperiod exposure resulted in a decrease in the number of neurons synthesizing dopamine within the arcuate nucleus of male hamsters. The results of that experiment indicate that there is no effect of photoperiod on the number of TH+ cells in the arcuate nucleus, and thereby suggest that exposure to a short-photoperiod does not affect the number of dopamine synthesizing neurons. Although TH is the rate limiting enzyme in the production of dopamine, LAAD is also necessary for the conversion of DOPA to dopamine within TIDA neurons. If short-photoperiod exposure affects LAAD availability in the arcuate nucleus, this in turn could affect ME dopamine synthesis. The purpose of this experiment was to examine the effects of short photoperiod on the number of LAAD neurons in the arcuate nucleus of male hamsters. ICC was used to visualize LAAD containing neurons within the arcuate nucleus. In rats, there are region specific differences in the co-localization of LAAD and TH within the arcuate

nucleus. In the dorsomedial portion of the arcuate nucleus, most TH producing neurons also produce LAAD, however in the ventrolateral portion of the arcuate nucleus, there are many TH containing neurons that do not produce LAAD (Jaeger, Ruggiero, Albert, Joh, & Reis, 1984) in rats. Therefore, in the present experiment, neurons within these two divisions of the arcuate nucleus were analyzed to determine if photoperiod differentially affected one region or the other.

Methods

Animals. The tissue used for LAAD ICC came from the same animals used in Experiment 3. Details regarding housing and light/dark schedules are also described in the Methods section of Experiment 3. All animals were housed in a long or short photoperiod for 12 weeks.

Immunocytochemistry. The protocol used for tissue preparation and storage are described in detail in Experiment 3. LAAD ICC was performed on every fourth section. The protocol for performing the LAAD immunolabelling was identical to that used for performing the TH labelling in Experiment 3 with the following exceptions; The primary antibody (anti-rabbit LAAD; Eugene Tech International, NJ) was diluted 1:1000 in 0.1 M Tris buffered saline (TBS) plus 0.3% Triton-x and the secondary antibody was goat-anti-rabbit (Vectastain). The chromogen used for the visualization of LAAD immunopositive (LAAD+) cells was 0.1% DAB with 50 µl/ml of 500 mM nickel chloride

and 0.50 µl/ml 30% H_2O_2. To test for the specificity of the secondary antibody, a sections from 2 animals were processed as described above, but the primary antibody was not used. Another control involved deleting the incubation in the secondary antibody to test for non-specific binding of the avidin-biotin complex.

Analysis. Examination of the tissue revealed that there were many LAAD+ cells in the arcuate nucleus of male hamsters. Therefore, a grid was used to outline a specific region in which LAAD+ cells would be counted. The grid was placed over a specific portion on the nucleus at a magnification of 100X. The magnification was then increased to 400X and the number of LAAD+ cells within the 300 µm X 300 µm square grid was counted. Grids were placed bilaterally, in both the dorsomedial or ventrolateral portion of the arcuate nucleus (see Figure 20). The number of LAAD+ neurons/grid was counted throughout the rostral-caudal extent of the arcuate nucleus. Only cells that were obviously stained dark purple were counted (see Figure 21). The effects of photoperiod on the number of LAAD+ cells/grid within the dorsomedial and ventrolateral arcuate nucleus was analyzed using Student's *t*-tests. Differences were significant if $p < .05$.

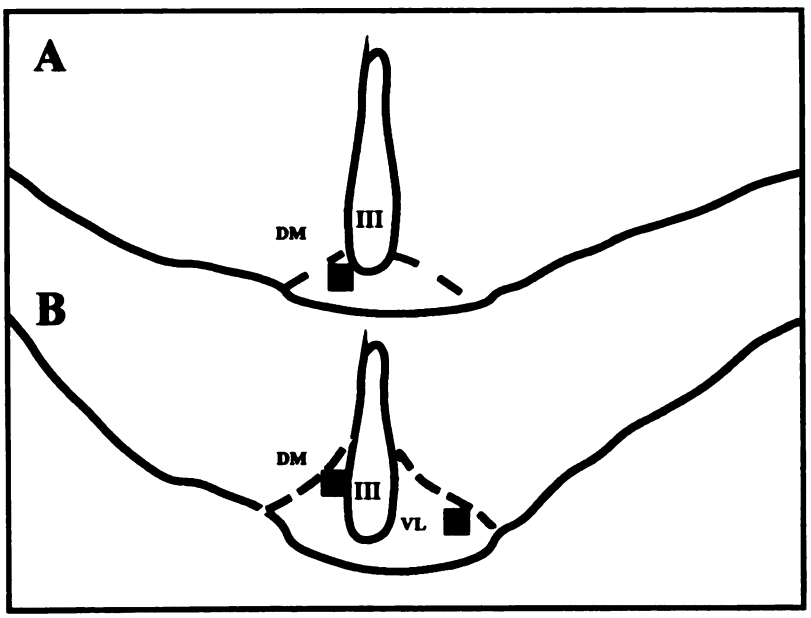

Figure 20. A diagram demonstrating the placement of grids over the dorsomedial (DM) region of the rostral (A) and caudal (B) arcuate nucleus (ARC) and over the ventrolateral (VL) region of the caudal (B) arcuate nucleus. Grids (300 μm X 300 μm squared), which are represented as black boxes were bilaterally placed in the DM or VL portion of the arcuate nucleus at a magnification of 100x. The magnification was then increased to 400x and the number of LAAD immunopositive neurons within each grid was counted. The dashed lines represent the boundaries of the arcuate nucleus and the location of the third ventricle (III) is also noted.

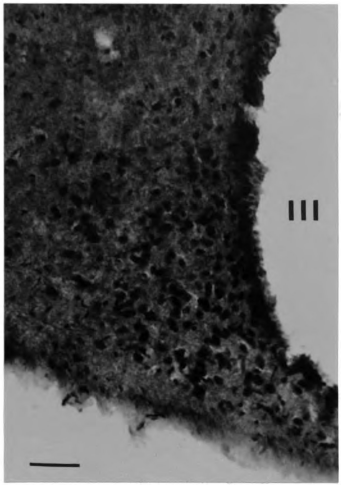

Figure 21. A photomicrograph showing LAAD immunolabelled (LAAD+) cells in the arcuate nucleus of a male hamster housed in long photoperiod. LAAD+ cells were stained dark purple, and easily identified against the red counterstained background. Although there were many LAAD+ cell bodies very few processes were darkly labelled. Abbreviations; third ventricle (III), bar = 50 μm.

Results

Figures 22A and B depict the number of LAAD+ cells/grid in the dorsomedial and ventrolateral arcuate nucleus. Exposure to a short photoperiod resulted in a decrease in the number of LAAD+ cells/grid in both the dorsomedial ($\underline{t}(8)= 4.82$, $p < .05$) and ventrolateral ($\underline{t}(8)= 3.33$, $p < .05$) arcuate nucleus.

Discussion

Exposure to a short photoperiod results in a significant reduction in the number of LAAD+ cells within both the ventrolateral and dorsomedial arcuate nucleus of male hamsters. The results from Experiment 3 suggest that there is not a change in the number of neurons synthesizing TH within the arcuate nucleus of male hamsters. However, if these neurons producing TH lack LAAD, they will not make dopamine, but will instead synthesize DOPA as an end-product. Therefore, this decrease in LAAD production could be responsible for the short-photoperiod induced decrease in ME dopamine concentrations.

A decrease in LAAD activity would not affect DOPA synthesis within the ME, so these findings are consistent with those of Experiment 2 showing that photoperiod does not affect DOPA accumulation in the ME after an injection of an LAAD inhibitor. However, ME DOPAC concentrations also did not change as a result of photoperiod (Experiment 1). Because DOPAC is the metabolite of dopamine, the results

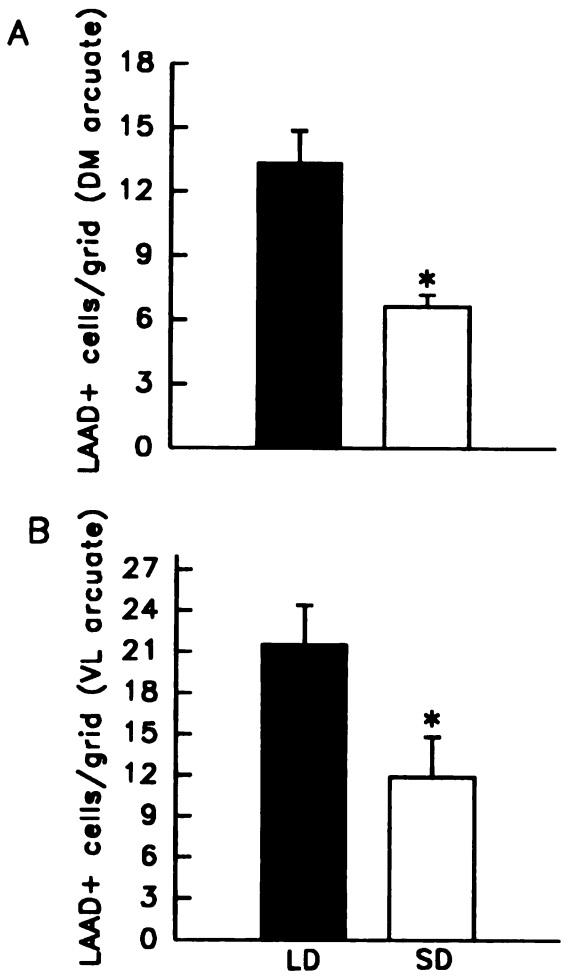

Figure 22. Mean (± sem) LAAD immunopositive (LAAD+) cells/grid in the dorsomedial (A) and ventrolateral (B) regions of the arcuate nucleus in male hamsters housed in either a long (LD) or short (SD) photoperiod. Exposure to a short photoperiod resulted in a decrease in the number of LAAD+ cells/grid in both regions of the arcuate nucleus. The asterisk (*) denotes significantly less than long-photoperiod animals ($p < .05$).

from Experiment 1 suggest that dopamine synthesis was not decreased in short-photoperiod housed animals. However, it is possible that in short photoperiods, some TH producing neurons stop producing LAAD, and that other TH neurons that are still producing LAAD, increase their activity to compensate for the inactive neurons. If this is the case, measuring gross changes in DOPAC at the level of the ME may not be a sensitive enough to detect changes in the activity of individual cells. Therefore, a decrease in dopamine synthesis due to a reduction in LAAD is not necessarily incompatible with the results of Experiment 1. If some neurons do increase their activity in response to the decrease in LAAD production, the active neurons must also be increasing their rate of dopamine release for dopamine concentrations to decrease. Thus, it is possible that the short-photoperiod induced decrease in ME dopamine is the result of a decrease in the number of neurons synthesizing dopamine in the arcuate nucleus, plus an increase in dopamine synthesis and release from neurons that are still active.

The enzyme LAAD is not only present in dopaminergic cells, but it also synthesized in other aminergic and peptidergic cells (for review see; Jaeger, et al., 1984). Therefore it is possible that short-day exposure did not affect LAAD production in TH producing cells, but instead affected LAAD production in one of these other systems. To be certain that the effects of photoperiod on LAAD occur

within dopaminergic cells, double-label ICC for TH and LAAD should be done. If short photoperiod exposure results in a decrease in the number of neurons that are double-labelled for TH and LAAD, then these findings would suggest that there is a decrease in the number of neurons synthesizing dopamine in short days. The next step would then be to determine if this sub-population becomes, in fact, hyperstimulated and to investigate the role of prolactin feedback in that increase in activity.

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