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Symposium on 'Seasonality'

Control of seasonality by melatonin

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Many mammals display profound changes in their physiology and behaviour coincident with their seasonal environment. These changes in reproductive activity, food intake, body weight and coat condition are physiological adaptations which restrict the birth and rearing of offspring to an optimum time of the year, reduce metabolic demand in the face of restricted food supply and alter body insulation to match the seasonal fluctuations in temperature. Due to the long-term nature of these physiological changes (e.g. re-growth of testes, change in body mass) such adaptations cannot be reactive, but instead must be initiated before the anticipated changes in climatic conditions. Thus, the mechanisms which regulate these seasonal changes demand a temporal component which ensures that physiological changes remain synchronized with the appropriate time of the year. Photoperiod provides a highly predictable environmental time cue which is used by many mammals to co-ordinate their seasonal cycles in physiology (Lincoln & Short, 1980). It is now well documented that within the animal the photoperiodic message is conveyed by the pineal hormone, melatonin (Bartness & Goldman, 1989). The intention of the present paper is to review what is currently known about seasonal physiology, and to discuss this in relation to recent findings about the mode of action of melatonin.

MELATONIN SIGNAL AND BIOLOGICAL RESPONSES

Melatonin is synthesized and secreted by the pineal gland in a precisely regulated temporal pattern. Low levels of melatonin synthesis and secretion occur during the day, but this increases at night to result in elevated levels of melatonin in the blood. The levels of melatonin remain high for a duration that is directly related to the length of the dark period. Thus, winter and summer photoperiods are reflected in long- and short-duration melatonin signals respectively. Studies from many laboratories using both sheep and hamsters (Syrian (*Mesocricetus auratus*) and Siberian (*Phodopus sungorus*)) have established that it is the duration of elevated melatonin that is the essential characteristic which conveys the photoperiodic message (Bartness & Goldman, 1989).

Given that the duration of the melatonin signal varies in the same manner through the year in all species, a central problem is to resolve how different species interpret the

melatonin message to co-ordinate divergent physiological processes. Seasonal cycles fall into three main categories: (1) those related to altered reproductive state, involving changes in luteinizing hormone (LH) pulse frequency, follicle-stimulating hormone (FSH) secretion, and steroid secretion; (2) altered coat condition, occurring through changes in prolactin (PRL) secretion; and (3) altered food intake and body weight, possibly involving β-endorphin and neuropeptide Y (NPY). The timing of the changes in these physiological processes is individual to each species. Thus, while short photoperiod or melatonin infusions are followed by a rapid decline in prolactin secretion in all species, changes in reproductive activity or body weight are species dependent. Transfer of anoestrus sheep, held under long photoperiod, to short photoperiod or simulation of these photoperiodic changes by timed infusions of melatonin promotes an early onset of oestrus (Karsch et al. 1984). In contrast the same treatments induce the contrary reproductive response, namely gonadal atrophy, in the hamster (Bartness & Goldman, 1989). Differences in response occur even between species of hamster, as the Siberian hamster shows reduction in its body weight following exposure to short days or melatonin (Wade & Bartness, 1984; J. G. Mercer, unpublished results), as opposed to the Syrian which gains weight (Bartness & Wade, 1984). Therefore, the function of photoperiod and, hence, melatonin is to provide temporal information which modulates the activities of different physiological pathways depending on the specific physiological requirements of the animal. Clearly formulating a model of how melatonin regulates seasonality is complicated by these apparently diverse ways in which different species use the signal.

ANNUAL CYCLES AND CIRCANNUAL RHYTHMS

For some species one hypothesis suggests that seasonal cycles may be driven by endogenous circannual rhythms. In the ground squirrel (*Citellus lateralis*) it has been shown that in the absence of the pineal gland or in conditions of constant darkness rhythms of body weight, food intake and hibernation free-run for several cycles with an approximate period of 10·5 months (Zucker et al. 1991). Similarly, in the sheep it has been shown that Suffolk ewes, ovariectomized and implanted with oestradiol and maintained under fixed short photoperiod, will continue to display annual cycles of LH and PRL levels in the blood, but with a period usually less than 1 year (Karsch et al. 1989). On this basis it has been argued that seasonal reproductive physiology is regulated through a circannual rhythm and, thus, the function of photoperiod and, hence, melatonin is to synchronize this endogenous rhythm with the seasonal environmental cycle (Karsch et al. 1989). Likewise, gonad-intact Soay rams maintained outdoors display robust annual cycles in a number of physiological factors (Fig. 1(a)), which continue even after removal of the melatonin signal by superior cervical ganglionectomy (SCGx; Lincoln et al. 1989). When maintained indoors under conditions of more constant nutrition and temperature, and on an artificial photoperiod of alternating blocks of 16 weeks of long and short days, the period of the cycles is decreased and clearly entrained by photoperiod (Fig. 1(b)). However, for SCGx rams on the same regime the cycles soon degenerate (Fig. 1(c); Lincoln et al. 1989), which would seem to argue against an endogenous rhythm of reproduction. On the other hand, whilst short photoperiod and, hence, long-duration melatonin seem to activate the gonadal axis (Fig. 1(b)), under natural conditions the initiation of gonadal growth occurs during the spring (i.e. under
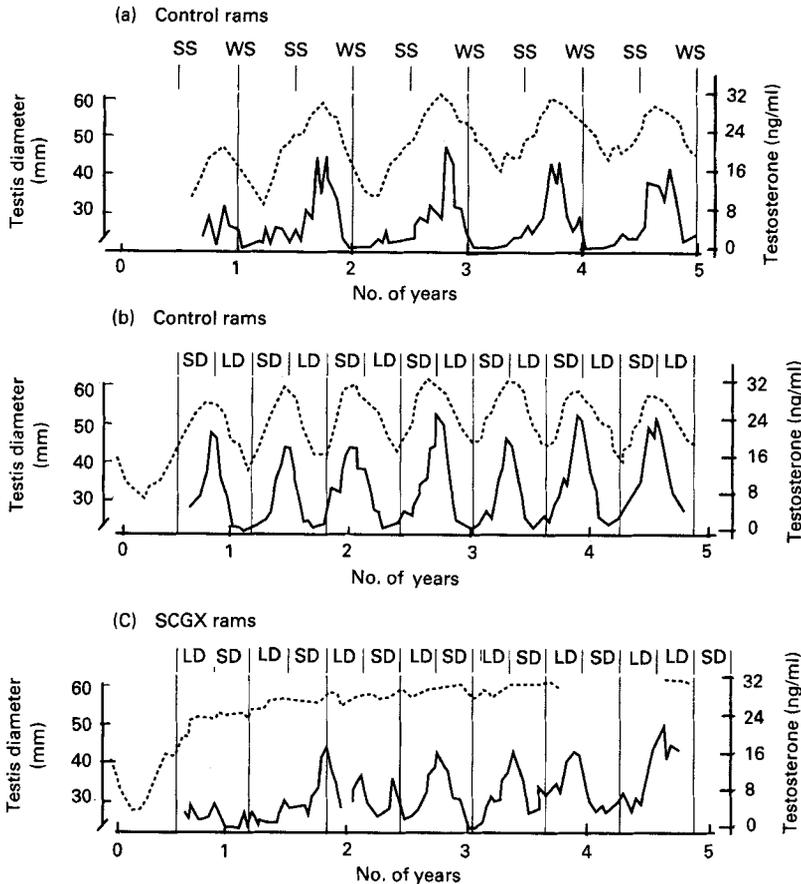
Fig. 1. Cycles in testosterone secretion (—) and testes diameter (– – –) of Soay rams held (a) outside over 5 years under natural photoperiod, (b) under artificial photoperiod of alternating 16 week blocks of long (16 h light (L):8 h dark (D); 16L:8D) and short (8L:16D) days (LD and SD respectively), and (c) as for (b) but following superior ganglionectomy (SCGx). Modified from Lincoln *et al.* (1989). SS, summer solstice; WS, winter solstice.

long days, short melatonin; see Fig. 1(a)), suggesting that melatonin is not the primary factor causing the activation of the reproductive axis in the ram under natural conditions. Although this could be interpreted in favour of a circannual rhythm which is fine-tuned by melatonin, other factors such as nutrition and temperature may also influence the occurrence of annual cycles (Lincoln *et al.* 1989). Thus, seasonal responses in sheep may involve endogenous circannual rhythms, but as a number of modulatory inputs, including photoperiod, nutrition, and temperature, can each influence annual cycles, inevitably this complicates further the analysis of the seasonal process.

In contrast to the sheep, there is no evidence that the hamster, a popular alternative model for the study of seasonality, expresses self-sustaining circannual rhythms. Instead the hamster if exposed initially to long days (e.g. 16 h light (L):8 h dark (D); 16L:8D) and then transferred to short days (e.g. 8L:16D) will undergo one cycle of reproductive

quiescence and subsequent spontaneous recrudescence. Re-occurrence of such a cycle is contingent upon the animal seeing another period of long days (Zucker *et al.* 1991). These changes in reproductive physiology induced by shortened photoperiod can be directly mimicked in hamsters which have been pinealectomized and given infusions of melatonin directly related in duration to those experienced under long and short days (Maywood *et al.* 1990). Thus, hamsters interpret a long-duration melatonin signal as a direct inhibitory drive on the reproductive neuroendocrine system.

DECODING OF MELATONIN SIGNAL IN THE BRAIN

It has been argued for many years that melatonin must act within the brain to co-ordinate the annual waxing and waning in reproductive activity. The main evidence to support such a contention stems from a number of different studies. First there is a pronounced interval between the change in the duration of a melatonin signal and a measurable physiological response. This is particularly well exemplified in the ewe where transition from anoestrus to oestrus can be advanced by daily treatment with exogenous melatonin, yet this transition is accompanied by an abrupt rather than gradual change in LH pulse frequency (Karsch *et al.* 1984; Robinson *et al.* 1992); furthermore, down-regulation of the gonadotropin-releasing hormone (GnRH) receptors in the pituitary by prolonged treatment with a GnRH agonist does not interfere with the ability of the ewe to respond to the melatonin treatment (Robinson *et al.* 1993). These results suggest that 'programming' by the melatonin signal must occur at a level at or above the GnRH neurone. A second piece of evidence is the need for a sustained period of treatment with melatonin to elicit a response. Again findings from the ewe have revealed that a 30 d period of daily treatment starting in May is inadequate, whereas a period of 60 d or longer will successfully advance oestrus in Scottish Blackface ewes (Robinson *et al.* 1992). These findings argue that the advance of oestrus induced by melatonin is dependent on a minimum number of signals. The third piece of evidence in favour of a brain site of action is the demonstration of photoperiodic memory. The nature of an animal's response to photoperiod (melatonin) is not entirely dependent on the absolute duration of the melatonin signal, but also on its direction of change. For example, Hastings *et al.* (1989) showed that Syrian hamsters transferred from a 16L:8D to a 12L:12D cycle showed gonadal regression and associated changes in FSH and testosterone levels. In contrast, hamsters transferred from an 8L:16D to a 12L:12D cycle showed an increase in testes size and associated changes in FSH and testosterone, despite being at the same final photoperiod as the first group of animals. Similar results have been reported for sheep (Robinson & Karsch, 1987). The main conclusion from these studies is that the animals must maintain a memory of their photoperiodic history so that they can measure the duration and the direction of change of the melatonin signal. In contrast to the effect on the reproductive indices the photoperiodic history had no effect on the PRL response. Compatible with this is evidence from the sheep which shows that the reproductive and PRL responses can be dissociated (Worthy & Haresign, 1983; Karsch *et al.* 1986), and, thus, it has been suggested that the PRL–coat-growth axis is regulated by an independent photoperiodic system to the reproductive axis (Hastings *et al.* 1989). There have been few studies addressing food intake and body weight control, so it is not yet known whether this response can be dissociated from others.
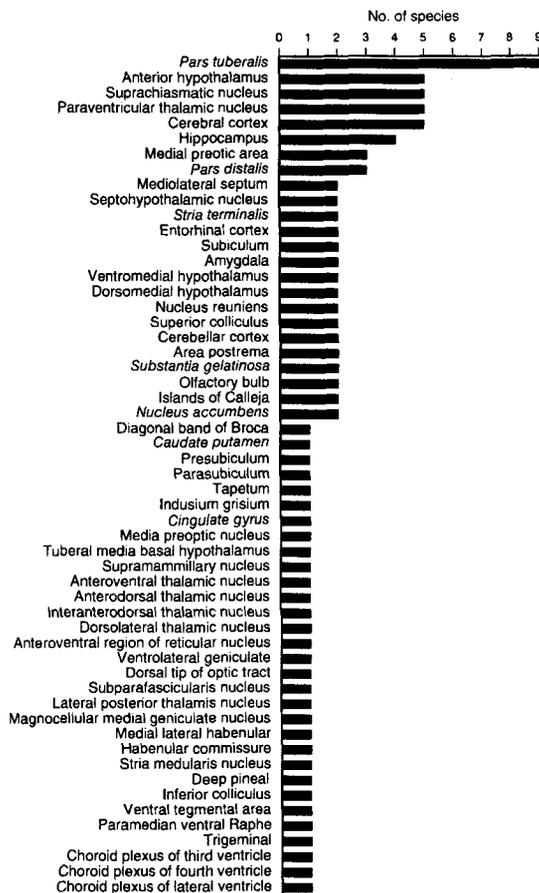
Fig. 2. Pseudo-frequency distribution chart of occurrence of [2-^{125}I]iodomelatonin-binding sites in nine mammalian species, including the sheep, goat, deer (*Cervus elaphus*), rabbit, mouse, rat, ferret (*Mustela furo*), Syrian (*Mesocricetus auratus*) and Siberian (*Phodopus sungorus*) hamsters (see Morgan *et al.* 1994*b* for details).

CENTRAL SITES OF MELATONIN ACTION

In view of this evidence it is not surprising that a considerable amount of effort has been spent trying to localize the target sites essential to the photoperiodic mechanisms of action of melatonin within the brain. The relatively recent introduction of *in vitro* autoradiography using the radioligand [2-^{125}I]iodomelatonin (I-MEL) has enabled precise localization of melatonin target sites within the brain in several mammalian species. An extensive review of these binding sites has recently been published (Morgan *et al.* 1994*b*). In synthesis these studies have revealed a wide species-specific distribution of I-MEL-binding sites within the mammalian brain (see Fig. 2).

Potential candidate sites such as the suprachiasmatic nucleus (SCN) have been found to possess I-MEL binding in a number of species, including the Siberian and Syrian hamsters, the white-footed mouse (*Peromyscus leucopus*), the rabbit and the rat, the latter being a non-photoperiodic species (Morgan *et al.* 1994*b*). Notably, however,

I-MEL-binding sites have not been found in the SCN of sheep, deer (*Cervus elaphus*), ferrets (*Mustela furo*), ground squirrels or goats, all highly seasonal animals. This, therefore, raises a question over the general importance of the SCN to the photoperiodic response. Consistent with this view, recent studies have shown that pinealectomized Syrian hamsters, bearing SCN lesions, still show testicular regression in response to timed melatonin infusions (Maywood *et al.* 1990). In marked contrast, similar experiments in the Siberian hamster have shown that SCN lesions abolished not only the reproductive response but also the decline in PRL and body weight in response to melatonin (Bartness *et al.* 1991). This work was supported further by studies where timed infusions of picogram amounts of melatonin into the SCN of juvenile male Siberian hamsters stimulated gonadal regression and lowered PRL levels (Badura & Goldman, 1992). Nevertheless, infusions of melatonin into other sites where I-MEL binding has been observed in this species, including the reuniens nucleus and the paraventricular nucleus of the thalamus (Duncan *et al.* 1989; Weaver *et al.* 1989), also resulted in gonadal collapse, whereas infusions into areas lacking I-MEL binding, notably the hypothalamic ventromedial nucleus (VMN), were without effect (Badura & Goldman, 1992). Lesioning studies on pinealectomized Syrian hamsters, on the other hand, have shown that different brain areas are critical to the melatonin response. Lesions to the paraventricular nucleus of the thalamus, the SCN or the preoptic area (POA) of the hypothalamus do not alter the effect of timed infusions of melatonin on the reproductive response (Ebling *et al.* 1992; Maywood & Hastings, 1994). In contrast, lesions to either the anterior hypothalamus (AHA) or the VMN abolish the reproductive response (Bonnefond *et al.* 1989; Maywood & Hastings, 1994). Interestingly, VMN lesions did not affect the PRL response and, thus, these findings provide supportive evidence that the seasonal PRL and reproductive responses may be regulated through independent sites (Maywood & Hastings, 1994). I-MEL binding has been reported in the VMN of the Syrian hamster (Williams *et al.* 1989) but not the Siberian hamster (Duncan *et al.* 1989; Weaver *et al.* 1989), implying that this may be a species-specific primary site of action. However, it is important to note that the AHA lacks I-MEL binding in the Syrian hamster.

A ROLE FOR THE *PARS TUBERALIS*

Lesioning studies of I-MEL-binding sites in other species have not yet been performed, but other anomalies still have to be resolved. For example, the only I-MEL-binding site that has been observed in all mammals studied to date, with the possible exception of man, is the *pars tuberalis* (PT; Morgan *et al.* 1994*b*). This site, originally incorrectly described as the median eminence of the hypothalamus, is part of the pituitary gland. In the ferret, ground squirrel and western spotted mink (*Mustela vison*), studies of I-MEL binding have each shown an apparent absence of I-MEL binding in any areas of the brain, yet the PT possesses I-MEL-binding sites in each case (Weaver & Reppert, 1990; Stanton *et al.* 1991; Boissin-Agasse *et al.* 1992). Therefore, as these animals are all highly seasonal it is difficult to dismiss a role for the PT.

In the sheep, microimplants of melatonin have been used in an attempt to target specific areas of the brain. In two independent studies it has been shown that implants placed in the medial basal hypothalamus (MBH), but not the POA, could mimic a short-day response, causing the premature activation of the reproductive axis and

suppression of PRL (Lincoln & Maeda, 1992a,b; Malpaux *et al.* 1993). These studies have been interpreted as demonstrating that a region in the MBH or an adjacent region such as the PT may mediate the actions of melatonin on the reproductive axis, yet to date only very weak I-MEL binding has been observed in the sheep MBH (Helliwell & Williams, 1992). In contrast to microimplants placed in the MBH, a more recent study has shown that implants placed in direct apposition to the PT are ineffective at inducing premature activation of the reproductive axis (Malpaux *et al.* 1994). Thus, the role of the PT as a mediator of photoperiodic responses has been questioned. However, at present it would be premature to exclude a role for either the MBH or the PT, and indeed it may be that both sites are important to one or more of the photoperiodic responses mediated by melatonin. In this context it is interesting to note that sheep which have undergone hypothalamic–pituitary disconnection at the level of the median eminence still appear to show seasonal changes in PRL following the transition from anoestrus to oestrus and vice versa (Thomas *et al.* 1986). This result was interpreted as showing evidence of hypothalamic independent control of seasonal PRL secretion (Thomas *et al.* 1986).

Cytologically, the ovine PT (oPT) is composed of two predominant secretory cell types (Morgan *et al.* 1991). The first is similar to that found in the anterior pituitary, and is characterized by the presence of abundant dense-core granules. In contrast, 85–90% of the cells have an agranular appearance at the ultrastructural level, which indicates that they are PT-specific and, thus, unlike other cells in the *pars distalis*. The agranular cells of the oPT appear able to synthesize and secrete proteins as they have abundant rough endoplasmic reticulum and active Golgi bodies, but they lack dense-core granules, indicating that the cells do not accumulate their secretory products as is typical of endocrine cells of the *pars distalis*. Studies, using [^{35}S]methionine labelling to follow synthesis and secretion of protein and peptide products from the oPT cells in culture confirm that the cells do indeed secrete proteins which are synthesized *de novo* (Morgan *et al.* 1992). By modulating the cyclic AMP levels through an inhibitory pathway melatonin influences the synthesis and secretion of these proteins. Two proteins are of interest. The first has a molecular weight of 72 kD (p72), and is sensitive to inhibition by melatonin (Morgan *et al.* 1994a). The second protein is PRL, identified by immuno-precipitation, and it is of interest as it is insensitive to melatonin (Morgan *et al.* 1992). These results demonstrate the presence of both melatonin-sensitive and melatonin-insensitive cells in the oPT gland. In combination with findings on I-MEL binding visualized by *in vitro* autoradiography, these findings are consistent with the interpretation that the majority of cell types responsive to melatonin in the oPT are agranular cells. In addition there must be a small population of lactotrophs which are not sensitive to melatonin. In view of the suggestion that there may be a hypothalamic independent regulation of PRL secretion by melatonin (Thomas *et al.* 1986), the lack of effect of melatonin on PRL synthesis and secretion would seem to suggest a chronic rather than an acute regulatory mechanism, possibly at the level of transcriptional control.

The structure and function of p72 is not known, but its role is clearly not specific to the mode of action of melatonin, as p72 is also synthesized and secreted by the *pars distalis* of the pituitary, although in these cells it is not regulated by melatonin (Morgan *et al.* 1992). Moreover, metabolic labelling of newly synthesized proteins and peptides has not revealed any PT-specific secretions (Morgan *et al.* 1992, 1994a). Therefore, it has been postulated that the oPT may produce a low-molecular-weight secretion through an

enzymic pathway, in a manner similar to the biosynthesis of melatonin by the pineal gland (Morgan *et al.* 1994*b*).

The anatomical location of the PT allows it to interface with both the median eminence of the hypothalamus and the anterior pituitary which suggests that the PT may play a role in modulating the output of the neuroendocrine system. Four different mechanisms by which this might occur have been postulated, but each mechanism is contingent on the secretion of a novel product by the oPT (Morgan *et al.* 1994*b*). The simplest mechanisms are for a PT-specific product to influence the output of the median eminence, where all the neurosecretory terminals from the hypothalamus terminate, or for this product to modulate the secretory output of the anterior pituitary. More speculatively the PT-specific product might modulate the activity of neurons in the median eminence which convey neural impulses to a circannual timer, or this connection could occur via a humoral route. Establishing whether any of these hypothetical pathways has credibility depends on the identification of tissue-specific function for the PT. To this end, more recent studies have focused on tissue-specific gene expression in the oPT.

A NUTRITIONAL PERSPECTIVE

Seasonal animals modify their food intake, even in the presence of *ad lib.* food supply. This suggests that fundamental changes occur in response to photoperiod in the hypothalamic appetite control centres where nutrient, endocrine and neuropeptide signals are integrated. Evidence for such alterations in animals with seasonally-modulated appetite is presently limited, although preliminary findings suggest that neuropeptides do contribute to the regulation of circannual cycles of feeding. In laboratory rodents and other mammalian species, NPY and galanin both elicit a powerful feeding response on injection into the paraventricular nucleus (PVN) and other hypothalamic sites (Leibowitz, 1992; White, 1993). Furthermore, both peptide concentration and gene expression of NPY change with nutritional status. Consistent with a role in pathways regulating the seasonal changes in energy metabolism and appetite regulation, both neuropeptides increase food intake when injected into the third ventricle of the photoperiodically-sensitive ground squirrel (Boswell *et al.* 1993). Furthermore, the expression of the NPY gene in the hypothalamic arcuate nucleus and the galanin gene in the dorsomedial nucleus were found to be highest at the times of maximum food intake and fat deposition in this species (Boswell *et al.* 1992).

The effect of photoperiod on the nutritional balance of the Siberian hamster is particularly intriguing as, although food intake is depressed by exposure to short days, weight loss is initiated before the expression of hypophagia (Wade & Bartness, 1984). This suggests that short photoperiod may act as a cue for seasonal increases in energy expenditure and, furthermore, implies involvement of the sympathetic nervous system (SNS). An attractive hypothesis to explain the early catabolism expressed by the Siberian hamster in short photoperiod is of increased brown adipose tissue (BAT) thermogenesis following sympathetic activation. However, although thermogenic activity (estimated by mitochondrial GDP binding) is enhanced after extended periods of maintenance in short photoperiod, enhanced activity cannot be detected during the early stages of photoperiod-induced weight loss (J. G. Mercer, unpublished results). Although the possibility cannot be discounted that very discrete changes in thermogenic activity, extending over periods of several weeks, could bring about weight reductions, it seems

unlikely that increased BAT thermogenesis initiates or contributes to the early stages of photoperiod-induced weight loss. Rather, the late onset increase in thermogenic activity may be an adaptive response to the increased potential to lose heat due to lowered body insulation caused by a reduced level of adiposity, and smaller body size. Nevertheless, although BAT thermogenesis does not increase significantly during the initiation of weight loss, a general activation of the SNS by exposure to short photoperiod in the Siberian hamster has been reported (McElroy *et al.* 1986). Further support for involvement of the SNS in the loss of body-weight response is provided by the critical role of the SNS in normal metabolism, where there is a reciprocity between food intake and sympathetic activity (Bray, 1990). An alternative possibility for the apparent state of energy deficit without a concomitant change in energy intake is altered transit time of digesta, possibly affecting digestibility or the efficiency of absorption.

CONCLUSION

In summary, it is presently not possible to formulate a consensus view on how melatonin might mediate the photoperiodic effects on the neuroendocrine system. In view of the species-specific I-MEL binding in the brain, and the species-specific nature of the physiological responses, also evident in the findings from lesioning and melatonin microimplant studies, it is tempting to conclude that different species have evolved their own distinct mechanisms. However, this seems unsatisfactory, and we must be prudent in our interpretation. Studies using the radioligand I-MEL may not yet have revealed all melatonin-binding sites in each species, and whilst lesioning and microimplant studies can provide powerful evidence they could be equally misleading. However, it seems likely that more than one site is involved in mediating the effects of melatonin on different physiological responses, at least with respect to PRL and the reproductive response. Therefore, convincing demonstration of the site or sites which mediate the specific melatonin responses demands verification by a number of different approaches.

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