Effects of placing micro-implants of melatonin in the pars tuberalis, pars distalis and the lateral septum of the forebrain on the secretion of FSH and prolactin, and testicular size in rams

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Abstract

Previous studies involving the placement of micro-implants of melatonin in the brain in sheep exposed to long days have provided evidence that melatonin acts within or close to the mediobasal hypothalamus (MBH) to mediate the effects of daylength on cycles in reproduction, moulting and other seasonal characteristics. To extend these observations, groups of Soay rams have now been treated with micro-implants of melatonin placed in the pars tuberalis (PT) and pars distalis (PD) of the pituitary gland, and in the lateral septum of the forebrain (septum). The PT and septum are potential target sites for the action of melatonin based on the localized binding of iodomelatonin assessed by in situ autoradiography. The animals were initially exposed to alternating 16-week periods of long days (16 h light:8 h darkness; 16L:8D) and short days (8L:16D) to entrain the seasonal cycles. The treatments were started at 10 weeks into a period of long days when the animals had a physiology normally observed in summer (low blood plasma concentrations of FSH and high concentrations of prolactin), and they remained under long days throughout the experiments. In experiment 1, animals received micro-implants of melatonin placed in the PT (n=6) or PD (n=4), or received empty implants in similar sites (n=4) or no surgery (n=4; total control, n=8). In experiment 2, groups of animals received micro-implants of melatonin placed in the lateral septum (septum, n=7) or received corresponding control treatments (total control, n=8). The micro-implants consisted of 22 gauge stainless-steel needles with melatonin fused inside the tip. They were inserted bilaterally in the selected sites and left in place for 14 weeks. The biological effects of the treatments were assessed by measuring the changes in the blood plasma concentrations of FSH and prolactin, growth of the testes and moulting of the pelage over a period of 28 weeks (14 weeks treatment and 14 weeks post-treatment).

The administration of melatonin in the PT, but not in the PD or septum, affected the photoperiodically induced cycle in the secretion of FSH and prolactin. In the PT group there was no significant change in the plasma concentrations of FSH during the treatment with melatonin, but there was a significant (P<0.001, ANOVA) decrease in the levels of FSH after the treatment associated with premature regression of the testes. The plasma concentrations of prolactin were significantly (P<0.001, ANOVA) decreased during the treatment with melatonin in the PT group and increased after the treatment with associated changes in the growth and moulting of the pelage in the most responsive animals. The effects of melatonin in the PT were qualitatively similar but less consistent than those previously observed following placement of micro-implants in the MBH (data included for comparison). The results support the conclusion that melatonin acts, at least in part, in the PT to mediate the inductive effects of photoperiod on the timing of seasonal cycles of reproduction and moulting in rams.

Journal of Endocrinology (1994) 142, 267–276

Introduction

Recent studies in sheep have provided evidence that the pineal hormone, melatonin, acts within or close to the mediobasal hypothalamus (MBH) to mediate the effects of changes in daylength on cycles in reproduction, moulting and other characteristics (Lincoln & Maeda 1989, 1992a,b, Malpaux et al. 1993a). In the first set of studies, adult Soay rams were treated chronically for 12–14 weeks with micro-implants of melatonin placed bilaterally in the MBH or the preoptic area (POA) while the animals remained under long days (16 h light:8 h darkness; 16L:8D) (Lincoln & Maeda 1992a,b). The treatments in the MBH, but not in the POA, induced a full spectrum of responses normally observed following a change to short days (8L:16D) in all animals. This involved an increase in the circulating blood concentrations of FSH and β-endorphin, and a decrease in the concentrations of prolactin, with predictable changes in the size and activity of the testes, total body weight, and moulting and growth of the pelage. All these changes were reversed after the removal of the micro-implants, demonstrating the
effectiveness of the local treatments. In a complimentary study, Ile-de-France ewes (oestradiol-implanted, ovariec-
tomized) were treated with micro-implants of melatonin
placed in the MBH, POA, anterior hypothalamus and
lateral hypothalamus using a similar experimental protocol
(Malpaux et al. 1993a). Again, the treatments in the MBH,
but not in the other areas of the hypothalamus, induced
effects on the secretion of pituitary hormones (increase in
luteinizing hormone (LH) and decrease in prolactin)
similar to those observed following a switch to short days.
Since these endocrine changes are normally induced by an
increase in the daily period of endogenous melatonin
secretion (long duration signal associated with the longer
nights), the results support the view that melatonin acts
within or close to the MBH. This effect is then relayed
within the hypothalamo-pituitary system to influence the
secretion of several different protein hormones from the
anterior pituitary gland to produce the multiple responses
to a change in daylength (Lincoln 1992).

Melatonin is presumed to act through specific high-
affinity, membrane-bound receptors, and the tissues in the
brain and pituitary gland in sheep which bind melatonin
indicative of the presence of such receptors have been
mapped by in situ autoradiography using 125I-labelled
melatonin (Morgan et al. 1989, Bittman & Weaver 1990,
de Reviers et al. 1991, Hellwell & Williams 1992). The
results show that the pars tuberalis (PT) of the pituitary
gland is the tissue with by far the highest concentration of
high-affinity binding sites for melatonin. There are also
high levels of binding of melatonin in specific areas of the
brain, particularly in the hippocampus, and in the septum
of the basal forebrain. Notably, there is only a low
concentration of binding sites for melatonin in the MBH.
This raises the possibility that the endocrine effects ob-
served following implantation of melatonin in the MBH
may be due to the action of melatonin in the adjacent PT,
the tissue with the remarkably high concentration of
binding sites. The PT is strategically placed to modulate
the hypothalamic regulation of the anterior pituitary gland,
and shows seasonal changes in morphology and functional
activity in some species (for review see Wittkowski et al.

The principle aim of the current study was to test the
involvement of the PT in the photoperiodic response by
monitoring the effects of placing micro-implants of
melatonin in the PT in Soay rams using a standardized
experimental protocol (Lincoln & Maeda 1992a). The
prediction was that if melatonin acts in the PT to relay the
effects of photoperiod, the placement of micro-implants of
melatonin in the PT in rams exposed to long days would
have the effect of blocking the influence of the prevailing
photoperiod. The result would be an increase in the
secretion of follicle-stimulating hormone (FSH) and a
decrease in the secretion of prolactin during the treatment,
and a reversal of the changes after the treatment, a
sequence similar to that induced by exposure to a corre-
sponding period of short days. The secondary aim of the
study was to document the effects of administering melat-
onein the pars distalis (PD), to assess whether melatonin
affects the secretion of FSH and prolactin by a direct action
on the PD. Also, a further objective was to document the
effects of melatonin in the lateral septum of the forebrain
(septum), a region characterized by a high concentration of
high-affinity binding sites for melatonin.

Materials and Methods

Animals and routine monitoring
The animals used in these studies were adult rams of the
Soay breed of feral sheep which show marked seasonal
cycles in reproduction, moulting and fattening similar to
the wild mouflon (Lincoln & Short 1980, Lincoln 1989).
Groups of seven to eight rams (mean body weight 39·5 kg)
were housed permanently in light-controlled rooms at the
Marshall Building near Edinburgh, UK, and a diet of grass
nits with hay and water was available ad libitum. The
animals were initially exposed to an artificial lighting regimen
of alternating 16-week periods of long days (16L:8D) and short
days (8L:16D) for 34–50 weeks to induce and entrain the long-term cycles in pituitary
hormone secretion. The treatments with the micro-
implants began at 10 weeks into a period of long days
when the animals were sexually inactive with regressed
testes, and were moulting into the summer coat.

To monitor the long-term changes in the secretion of
FSH and prolactin, blood samples were collected by vacu-
tainer from the jugular vein of each animal three times weekly. The blood samples were heparinized and the plasma separated within 30 min and stored at −20 °C until the hormone concentrations were measured by radio-
imnoassay. Every 2 weeks, the diameter of the testes was measured and the intensity of the sexual skin coloration
androgen-dependent coloration of the skin in the inguinal region was recorded for each animal (Lincoln & Davidson
1977). In addition, the growth of the pelage on the scrotum
was visually scored and the moult assessed by plucking hair
from the scrotum (Lincoln & Maeda 1992b).

Experiments
Two similar experiments were performed to monitor the
effects of placing bilateral micro-implants of melatonin in
the pituitary gland and the brain using a standardized
protocol (Lincoln & Maeda 1992a). In experiment 1, 18
rams were randomly assigned to the following treatments:
(i) micro-implants of melatonin placed in the PT
(n=6), (ii) micro-implants of melatonin placed in the PD
(n=4), (iii) empty micro-implants placed in the PT or
PD (sham-operated control, n=4) and (iv) no surgery
(unoperated control, n=4). In experiment 2, 15 animals
were assigned to the following treatments: (i) micro-implants of melatonin placed in the lateral septum (septum, \( n=7 \)), (ii) empty micro-implants placed in the same area (sham-operated control, \( n=4 \)) and (iii) no surgery (unoperated control group, \( n=4 \)).

In all cases, the implants were left in place for 14 weeks and the biological responses were monitored for a total of 28 weeks (14 weeks treatment and 14 weeks post-treatment) while the animals remained under a lighting regimen of long days.

**Micro-implants of melatonin and surgery**

The micro-implants of melatonin were made from 22 gauge stainless-steel cannulae (outside diameter 0.710 mm; inside diameter 0.415 mm) with \( 8.0 \pm 0.3 \) mg (mean \( \pm \) s.e.m.) melatonin (Genzyme Fine Chemicals, Haverhill, Suffolk, UK) fused inside the tip as described previously (Lincoln & Maeda 1992a). The implants have been shown to release melatonin at a relatively constant rate of \( 3.42 \pm 0.43 \) \( \mu \)g/24 h for up to 14 weeks, after initial higher values in the first week, when incubated in buffer at 37 °C (Lincoln & Maeda 1992a). Also, the implants have been shown to produce a relatively localized concentration of melatonin restricted to within 1 mm of the centre of the implant when placed in the brain in rams, with no detectable increase in the peripheral blood plasma concentrations of melatonin (Lincoln & Maeda 1992a).

The cannulae containing the melatonin, and the empty cannulae for the sham operations, were placed bilaterally in the selected sites for each ram under general anaesthetic using a stereotaxic procedure (Lincoln & Maeda 1992a). Radio-opaque dye (Ultravist 300; Schering Health Care, Burgess Hill, West Sussex, UK) was injected into the lateral ventricles and X-ray photographs using Polaroid instant film (Polaroid Co., Cambridge, MA, USA) were used to define the position of landmarks within the brain, including the infundibular recess, rostral margin of the third ventricle and the ventral surface of the lateral ventricle (Lignereux et al. 1991). Guide cannulae were positioned 2.0 mm from the midline with the tip 3.0–15.0 mm above the target site and fixed permanently to the skull. The micro-implants were introduced through the guides to place the tip in the required position. The implants in the PT were placed 0–0.5 mm anterior to and 1.0–3.0 mm below the lower point of infundibular recess in the pituitary stalk, and the implants in the PD were placed 0.5–2.0 mm posterior to and 2.0–4.0 mm below the infundibular recess in the tissue of the pituitary gland (depth from top of brain to tip of implant: 41–49 mm). For these treatments, implant cannulae with pointed ends were used to allow penetration of the dura mata at the base of the brain. The implants in the septum were placed 3.0–5.0 mm rostral to the margin of the third ventricle, 2.0–6.0 mm from the base of the brain to provide a range of sites in the basal region of the forebrain (depth from top of brain to tip of implant: 29–32 mm).

To complete each operation the upper ends of the implant cannulae were secured to the skull, the skin was sutured over the wound and the animal was returned to its normal pen. The animals received postoperative antibiotics (Cristopen, i.v. day 1; Streptopen, i.m. days 1–3: Pitman Moore, Harefield, Middx, UK) and an analgesic (Finadyne, i.m. day 1; Schering–Plough Animal Health, Mildenhall, Suffolk, UK). The micro-implants remained in place for 14 weeks and were then removed during a brief operation under general anaesthesia. A total of 25 implant operations were performed in this study. There were no fatalities. One animal showed signs of neurological damage following the operation (loss of vision in right eye and head tilted), and was treated on one occasion with dexamethasone and buscopan (Boehringer Ingelheim, Bracknell, Berks, UK) to reduce inflammation. This animal recovered vision and normal behaviour within 4 weeks. All other animals recovered well and showed normal behaviour.

Representative animals from each experimental group (mostly half the animals/group) were killed after the end of the study. A block of tissue surrounding the site of implantation was collected from each animal and frozen on dry ice, and later sectioned with a cryostat. The position of the implants, seen as a tract through the tissue, was assessed with reference to the macroscopic features of the pituitary gland and the brain (Lincoln & Maeda 1992a). In addition, tissue sections were prepared and stained with haematoxylin and eosin. These were used to confirm the position of the implants using an atlas of the brain of the sheep and goat (Yoshikawa 1968, Mori et al. 1990). In all cases the implants were located within 1.5 mm of the centre of the target sites in the position predicted from the X-ray photographs.

**Hormone assays**

Concentrations of FSH and prolactin in the blood plasma collected three times weekly were measured by a radioimmunoassay using the method of McNeilly et al. (1986) for FSH, and that of McNeilly & Andrews (1974) for prolactin. The lower limit of detection (10% decrease in binding relative to \( B_0 \)) for the FSH assay was 1.0 \( \mu \)g NIH-FSH-S14/l plasma, and the intra- and interassay coefficients of variation were 6.6% and 12.6% respectively, based on the mean of low, medium and high quality control samples measured in ten assays. The corresponding values for the prolactin assay were 0.5 \( \mu \)g NIH-PRL-S15/l plasma, 5.5% and 7.0% based on eight assays.

**Statistical analysis**

In both experiments, the effects of the treatments on the weekly changes in the blood plasma concentrations of FSH
and prolactin during the 14-week treatment and the 14-week post-treatment periods were assessed for significance by analysis of variance (ANOVA) using a CLR ANOVA program (Clear Lake Research, Houston, TX, USA). The magnitudes of the FSH and prolactin responses within rams during the experiment were each calculated as the ratio of the mean hormonal concentration for the periods 4–9 weeks (period A; middle of treatment period) to that for the period 18–23 weeks (period B; middle of post-treatment period) of the experiment. The two periods were preselected as the times of maximum effect based on a previous study in Soay rams treated with subcutaneous implants containing melatonin (Lincoln & Ebling 1985). The correlation between the magnitude of the responses for the two hormones was calculated (Cricket Graph Software Inc., Philadelphia, PA, USA) and a P value <0.05 was considered significant.

Results

Effects of micro-implants of melatonin in the PT and PD (experiment 1)

The long-term changes in the blood plasma concentrations of FSH and prolactin, and the size of the testes in the rams which received micro-implants of melatonin in the PT and PD are summarized in Figs 1 and 2. At the beginning of the experiment, the animals were sexually inactive with low plasma concentrations of FSH and regressed testes, and high concentrations of prolactin associated with the development of the summer pelage. In the animals receiving no melatonin there was a gradual increase in the plasma concentrations of FSH from 0–20 weeks (10–30 from the beginning of exposure to long days) and slow growth of the testes as normally occurs during prolonged exposure to long days (Lincoln & Maeda 1992a). At the same time there was a progressive decrease in the plasma concentrations of prolactin. There were no significant differences in the endocrine profiles between the two control groups (sham-operated and unoperated controls) and the results were combined for comparison with the melatonin-treatment groups.

The placement of micro-implants of melatonin in the PT, but not the PD, resulted in an alteration in the photoperiodically induced cycle in the plasma concentrations of FSH and prolactin. In the PT group, there was no significant change in plasma levels of FSH during the treatment with melatonin but there was a significant decrease following the treatment, once the micro-implants were removed (post-treatment period, PT vs control, P<0.001, ANOVA). This was associated with a marked regression of the testes. There was a significant decrease in the plasma concentrations of prolactin during the treatment with melatonin (pre treatment period, PT vs control, P<0.001, ANOVA), and an increase after the end of treatment (post-treatment period, PT vs control, P<0.001, ANOVA), with an inverse relationship to the changes in the secretion of FSH.

Data for two representative animals treated with melatonin in the PT, and one control receiving empty implants in the same area, are illustrated in Fig. 3. In one of the melatonin-treated animals, classified as a responder (Fig. 3a), there was a clearly defined increase in the plasma

![Graph](http://example.com/graph.png)
The changes in the plasma concentrations of FSH and prolactin following implantation of melatonin in the POA and MBH in Soay rams using the same procedure as in the current study (Lincoln & Maeda 1992a,b) are summarized in Fig. 5 for comparison with the effects of the treatments in the PT, PD and septum. The administration of melatonin in the MBH produced the most marked and consistent effects on both pituitary hormones. All animals treated in the MBH showed a rapid and highly significant increase in the plasma concentrations of FSH and a decrease in the levels of prolactin, with a reversal of the changes once the micro-implants were removed. Overall, melatonin had no effect in the septum, a small effect in the POA, a maximum effect in the MBH, a reduced effect in the PT and no effect in the PD. When the data for all experiments were combined (40 rams received micro-implants of melatonin in the different sites), there was a significant correlation between the magnitude of the FSH response and the magnitude of the prolactin response ($r^2=0.81$, $P<0.001$).
FIGURE 3. Changes in the blood plasma concentrations of FSH and prolactin (●), diameter of the testes (○), and period of the sexual skin flush (SF, an androgen response) and moult of the pelage (solid bars) in three adult Soay rams: (a) treated with micro-implants of melatonin placed in the pars tuberalis (PT; classified as a responder), (b) treated with micro-implants of melatonin placed in the PT (classified as a partial responder), and (c) empty implants placed in the PT (control). The treatments (open box) were initiated at 10 weeks into long days (16L:8D) when the rams were sexually inactive, and the parameters were monitored for 28 weeks (14 weeks treatment and 14 weeks post-treatment) while the animals remained under long days.
Discussion

The current results demonstrate that the local administration of melatonin in the PT, but not the PD or the septum, caused significant changes in the secretion of FSH and prolactin in rams maintained under long days. Since these changes are qualitatively similar to those induced by exposure to short days (Lincoln & Maeda 1992a), the results provide evidence that endogenously secreted melatonin acts within, or close to, the PT to mediate the inductive effects of changes in daylength on cycles in gonadal activity and moulting in sheep. Furthermore, the total lack of an effect of melatonin in the PD indicates that melatonin does not act directly on the gonadotrophs and lactotrophs in the PD to affect the secretion of FSH and prolactin. Also, the lack of an effect of melatonin in the septum shows clearly that the neural tissues in this part of the forebrain, which have a high concentration of melatonin-binding sites (Bittman & Weaver 1990), are not involved in the photoperiodic regulation of pituitary gland.

The prediction for the current study was that if the PT is the principle site for the action of melatonin in relaying the photoperiodic responses, then the local administration of melatonin within the PT would cause particularly pronounced effects on the secretion of FSH and prolactin. This was based on the simplistic assumption that the closer the melatonin is delivered to the active site the greater the biological response. The results show that the treatments in the PT produced less consistent effects than similar treatments in the MBH (Lincoln & Maeda 1992a,b). Only the animals which received micro-implants of melatonin in the PT, immediately adjacent to the MBH, showed the full sequence of endocrine responses, and animals treated with implants lower in the pituitary stalk showed a much reduced response or no response at all.

There are three possible interpretations of these results. The first is that melatonin acts exclusively in the MBH to mediate the multiple effects of photoperiod, and the observed endocrine changes following the administration of melatonin in the PT are due to the transfer of the hormone from the PT to the MBH. This is consistent with the regional differences in the effectiveness of the treatments in the PT. This is also supported by results from a recent study (Malpoux et al. 1993b) in ewes which demonstrated that the placement of moulded, silicone elastomer implants containing melatonin against the pituitary stalk, which increased the concentration of melatonin in the PT above the normal night-time range, had no effect on the secretion of LH in ovarioectomized oestradiol-implanted ewes exposed to long days. This was in contrast to the effectiveness of micro-implants of melatonin placed in the third ventricle immediately above the MBH, which caused a significant increase in the secretion of LH (Malpoux et al. 1993b). The action of melatonin in the MBH could involve effects on the dopaminergic and/or opioidergic neural pathways which modulate the secretion of gonadotrophin-releasing hormone (GnRH) from the hypothalamus to regulate the release of LH and FSH, and which influence the secretion of neurotransmitters and peptides to regulate the release of prolactin (Rasmussen 1991, Lincoln & Maeda 1992a,b).
The second possible interpretation is that melatonin acts exclusively in the PT to mediate the multiple effects of photoperiod, and the observed endocrine changes following the administration of melatonin in the MBH are due to the transfer of the hormone from the MBH to the PT. This is possible since the PT has a much higher concentration of specific high-affinity binding sites for melatonin than the MBH (Morgan 1991), and the microvasculature of the hypothalamo-pituitary portal system favours the transfer of products from the MBH to the pituitary gland (Page 1982). The greater consistency in the effectiveness of melatonin treatments in the MBH, compared with the PT, may reflect the efficiency of the vascular system at delivering the exogenous melatonin from the MBH to the PT compared with the delivery of melatonin by the physical placement of implants in the PT which may cause local damage. Attempts to measure radiolabelled melatonin in the pituitary gland following implantation in the MBH have failed to detect transfer from the MBH to the pituitary gland (Lincoln & Maeda 1992a, Malpaux et al. 1993a); however, the technique is unlikely to be sufficiently sensitive to monitor the local concentration gradients in the tissues. These will be markedly affected by the complex vasculature in the region of the MBH/PT. Furthermore, since the photoperiodic responses are normally activated by the very low concentrations of melatonin in the peripheral blood (0–500 pmol/l, Lincoln & Maeda 1992a), it is entirely possible that the administration of 3-4 μg/day within the MBH could produce a sufficient increase in the PT to negate the effect of the endogenous long-day melatonin signal and activate the short-day endocrine responses. A number of different mechanisms have been proposed to account for the action of melatonin in the PT, including an action on the follicular stellate cells to influence the expression of a product which affects the hypothalamic regulation of the PD (Morgan 1991, Morgan et al. 1992), and an action on the gonadotrophs in the PT to influence the secretion of LH and FSH through the local negative feedback regulation of GnRH secretion from the hypothalamus (Nakazawa et al. 1991).

The third possible interpretation is that melatonin acts both in the MBH and PT to mediate the multiple effects of photoperiod through a combination of these effects. At the present time it is not possible to discriminate between the three interpretations. However, based on the current observations that melatonin administered in the PT has significant effects on the photoperiodically induced cycle in the secretion of FSH and prolactin, and the knowledge that the PT has a very high concentration of binding sites for melatonin, it is concluded that melatonin acts, at least in part, in the PT to mediate the effects of photoperiod on seasonal cycles in reproduction, moulting and other characteristics in the ram.

Postscript

Since completing this manuscript we have produced definitive evidence, using Soay rams in which the hypothalamus is surgically disconnected from the pituitary gland, that melatonin acts directly in the pituitary gland to mediate effects of photoperiod on the secretion of prolactin (Lincoln & Clarke 1994).

Acknowledgements

I am grateful to N Anderson for collecting blood samples and overseeing the experiments, to the staff at the Marshall Building for the care of the animals, to I Swanston, F Khaleele, V Grant and I Cooper for the expert assistance with the radioimmunoassays, to T McFetters and T Pinner for the art work and to D Tortone for helpful comments on the manuscript. The standard preparations of pituitary hormones were generously provided by NIMDDK.

References


FIGURE 5. Summary of the changes in the blood plasma concentrations of FSH and prolactin in groups of adult Soay rams treated with micro-implants of melatonin placed in different regions of the hypothalamus and pituitary gland (A–E, see tissue map for location). The treatments (open boxes) were initiated at 10 weeks into long days (16L:8D) when the rams were sexually inactive, and the parameters were monitored for 28 weeks (14 weeks treatment and 14 weeks post-treatment) while the animals remained under long days. The values are mean±s.e.m. (n=4–12) for melatonin-treated (●) and control groups (○). The data for the treatments in the MBH and POA were obtained from previous publications (Lincoln & Maeda 1992a,b). Abbreviations: AC, anterior commissure; AHA, anterior hypothalamic nucleus; ARC, arcuate hypothalamic nucleus; DMH, dorso-medial hypothalamic nucleus; MM, medial mamillary nucleus; OC, optic chiasm; OVLT, organum vasculosum laminae terminalis; POA, preoptic area; PT, pars tuberalis; PD, pars distalis; PVH, paraventricular hypothalamic nucleus; SCN, suprachiasmatic hypothalamic nucleus; SM, medial septal area; THAL, thalamus; VMH, ventro-medial hypothalamic nucleus; MBH, mediobasal hypothalamic.
Lincoln GA & Clarke IJ 1994 Photoperiodically-induced cycles in the 
scretion of prolactin in hypothalamo-pituitary disconnected rams: 
evidence for translation of the melatonin signal in the pituitary 
Lincoln GA & Davidson W 1977 The relationship between sexual and 
aggressive behaviour, and pituitary and testicular activity during the 
seasonal sexual cycle of rams and the influence of photoperiod. 
Lincoln GA & Ebling FJP 1985 Effect of constant release implants of 
melatonin on seasonal cycles in reproduction, prolactin secretion and 
Lincoln GA & Maeda K-I 1989 Site of action of melatonin in the 
No. 13.
Lincoln GA & Maeda K-I 1992a Reproductive effects of placing 
micro-implants of melatonin in the mediobasal hypothalamus and 
Lincoln GA & Maeda K-I 1992b Effects of placing micro-implants of 
melatonin in the mediobasal hypothalamus and preoptic area on the 
secretion of prolactin and ß-endorphin in rams. *Journal of 
Endocrinology* 134 437–448.
Lincoln GA & Short RV 1980 Seasonal breeding: nature’s 
McNeilly AS & Andrews P 1974 Purification and characterization of 
McNeilly AS, Jonassen JA & Fraser HM 1986 Suppression of follicular 
development after chronic LHRI immunoneutralization in the 
Malpaux B, Daveau A, Maurice F, Gayraud V & Thiery J-C 1993a 
Short-day effects of melatonin on luteinizing hormone secretion in 
the ewe: evidence for central sites of action in the mediobasal 
Malpaux B, Daveau A, Maurice F & Locatelli A 1993b Does the pars 
tuberalis transduce the effects of melatonin on gonadotrophin 
secretion in the ewe? 6th Colloquium of European Pituitary Society, 
Morgan PJ 1991 The pars tuberalis as a target for melatonin action. 
*Advances in Pineal Research* 6 149–158.
Morgan PJ, Barrett P, Davidson G & Lawson W 1992 Melatonin 
regulates the synthesis and secretion of several proteins by pars 
tuberalis cells of the ovine pituitary. *Journal of Neuroendocrinology* 4 
557–563.
Melatonin receptors on the ovine pars tuberalis: characterization 
and autoradiographical localization. *Journal of Neuroendocrinology* 1 
1–4.
Mori Y, Takeuchi Y, Shimada M, Hayashi S & Hoshino K 1990 
Stereotaxic approach to hypothalamic nuclei of the Shiba goat with 
339–349.
Nakazawa K, Marubayashi V & McCann SM 1991 Mediation of the 
short-loop negative feedback of luteinizing hormone (LH) on 
LH-releasing hormone release by melatonin-induced inhibition of 
LH release from the pars tuberalis. *Proceedings of the National 
Academy of Sciences of the USA* 88 7576–7579.
E427–E442.
Rasmussen DD 1991 The interaction between medio-basal 
hypothalamic dopaminergic and endorphinergic neuronal systems as 
a key regulator of reproduction: a hypothesis. *Journal of 
Endocrinological Investigation* 14 323–352.
de Reviers MM, Tillet Y & Pelletier J 1991 Melatonin binding sites 
in the brain of sheep exposed to light or pinealectomized. 
Wirtkowskl WH, Schulze-Bonhage AH & Bockers TM 1992 The 
 pars tuberalis of the hypophysis: a modulator of the pars distalis? 

Revised manuscript received 3 December 1993
Accepted 24 February 1994