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**STUDIES ON THE INNERVATION OF THE OVINE PINEAL AND THE
REGULATION OF MELATONIN SECRETION**

A thesis presented in partial fulfilment of the requirements for the
Degree of Doctor of Philosophy in Physiology
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Thesis Abstract

The studies described in this thesis were designed to investigate the neural regulation of melatonin secretion from the ovine pineal gland.

Initial studies sought to establish the effects of a range of anaesthetics on the nocturnal rise in plasma melatonin levels in Romney rams and to identify the anaesthetic treatment most suitable for use in future acute studies; this was found to be halothane induction and maintenance.

To investigate the neural mediation of melatonin secretion from the ram pineal, a series of experiments was performed in which the pre-ganglionic sympathetic innervation was stimulated electrically. Acute stimulation during the night or day resulted in an immediate, sustained rise in plasma melatonin levels, with nighttime responses being significantly greater than daytime responses ($P < 0.05$).

Similar studies in conscious rams were subsequently made possible by the development of a cuff electrode which could be implanted around the CST's and remain functional for at least six weeks. In these studies it was demonstrated that photoperiod (16L:8D or 8L:16D) did not influence melatonin output, while responsiveness to stimulation was highest during the middle, rather than at the beginning or end, of the photoperiod.

The second study employing chronically implanted electrodes was designed to evaluate the influence of various parameters of continuously applied CST stimulation on the pineal's melatonin secretory response. Unexpectedly, it was found that only small rises in plasma melatonin levels resulted from stimulation with any of the combinations of stimulus parameters tested and that no one combination was significantly more effective than any other in promoting increases in plasma melatonin levels. These findings appear to have resulted from inadequate performance of the electrodes and/or nerve damage.

The final study undertaken in this thesis was designed to identify the innervation of ovine pineal by immunocytochemically localizing the neuropeptides NPY & VIP, and the enzymes NSE & PNMT. All four antigens were observed in intrapineal nerve fibres, while NSE was also present in pinealocytes. SCGX reduced, but did not eliminate, this immunoreactivity suggesting that both central and peripheral regions innervate the pineal and that partial denervation initiates a neural compensatory mechanism.

Preface

Some material from Chapters 3 & 5 is contained in the following papers:

- (1) B.G. Mockett, K.R. Lapwood, R.J. Pack & D.H. Carr (1991) Induction of pineal melatonin secretion in anaesthetized rams by bilateral electrical stimulation of the cervical sympathetic trunks. *Proceedings of the Physiological Society of New Zealand*, pp 32-33

- (2) B.G. Mockett, K.R. Lapwood, R.J. Pack & D.H. Carr (1991) Innervation of the ovine pineal gland and the regulation of melatonin secretion: An electrophysiological and immunocytochemical study. *Advances in Pineal Research*, Vol 6, 13-16

- (3) B.G. Mockett, K.R. Lapwood, R.J. Pack & D.H. Carr (1991) Regulation of ovine pineal gland function: An electrophysiological and immunocytochemical study. *Proceedings of the International Symposium on Pineal Hormones, Bowral, Australia*. pp 51.

Copies of these are contained in an envelope inside the back cover of this thesis.

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Table of Contents

CHAPTER 1	Introduction	1
1.1	Seasonality and survival	1
1.1.1	Seasonal adaptative changes mediated by the pineal.	1
1.1.2	Short and long day breeders.	2
1.1.3	History of pineal research	2
1.2	Reproductive seasonality in sheep.	3
1.2.1	Behavioural and physical characteristics of seasonality.	4
	(I) Behaviourial	4
	(II) Physical and physiological changes associated with reproductive capacity	5
1.2.2	Ovine seasonal hormonal changes	6
	(I) Ram	6
	(II) Ewe	7
1.2.3	Factors influencing reproductive seasonality.	9
	(I) Photoperiod.	9
	(II) Nutrition	10
	(III) Temperature.	11
	(IV) Pheromones.	12
1.2.4	Seasonal reproduction in New Zealand sheep.	12
1.3	Hypothalamo-Pituitary mechanisms mediating seasonal reproduction	14
1.3.1	Control of GnRH pulse generator activity by photoperiod.	14
	(I) Direct action of photoperiod (Steroid independent effects)	14
	(II) Steroid negative feedback.	15
1.3.2	Neural mechanisms regulating seasonal reproduction.	16
	(I) GnRH release during anoestrus	17
	(II) GnRH release during the breeding season.	17
1.4	Methods used to evaluate the role of the pineal gland and melatonin in mediating seasonal reproduction	18
1.4.1	Pinelectomy	18
1.4.2	Superior Cervical Ganglionectomy.	19
1.4.3	Melatonin Administration.	20
1.4.4	Photoperiod Manipulation.	21
1.5	Physiology and regulation of pineal function.	21
1.5.1	Circadian rhythms, photoperiodism and the pineal gland	21
	(I) General	21
	(II) Formal properties of circadian rhythms	22
	(III) Suprachiasmatic nuclei as circadian pacemakers.	22
	(IV) Pineal-SCN interrelationship	22
1.5.2	Indoles and Peptides - metabolism and function	23
	(I) Indoles	23
	(II) Peptides	24
1.5.3	Environmental factors affecting pineal function.	25
	(I) Light.	25
	(II) Nutrition	26
	(III) Other influences	26
1.5.4	Melatonin's sites and mechanisms of action.	27
1.5.5	Melatonin and the measurement of daylength.	28
1.5.6	Hormonal modulation of pineal function.	29
	(I). Gonadal steroids.	29
	(II) Corticosteroids.	30

1.6	Innervation and electrophysiology of the mammalian pineal gland. . . .	30
1.6.1	Neurological investigative techniques applicable to pineal research.	31
	(I) Lesion studies.	31
	(II) Stimulation studies.	31
	(III) Recording of neuronal/pinealocyte electrical activity.	32
	(IV) Retrograde and Anterograde nerve fibre tracing	33
	(V) Immunological techniques	33
	(VI) Pharmacological methods/studies.	34
1.6.2	Innervation.	34
	(I) Sympathetic (peripheral)	34
	(II) Central.	36
1.6.3	Electrophysiology of the pineal gland.	37
	(I) Patterns of electrical activity.	37
	(II) Characteristics of stimulation affecting the pineal's response.	39
	(III) Effects of sympathetic nerve stimulation.	40
	(IV) Inhibitory regulation of sympathetic nerve activity.	42
1.7	Purpose of this study	43
CHAPTER 2 Materials and Methods		44
2.1	Animals and management.	44
2.2	Surgical techniques.	44
2.2.1	General surgical procedures.	44
2.2.2	Surgical preparation for acute stimulation and recording	45
2.2.3	Chronic electrode implantation	46
2.2.4	Superior cervical ganglionectomy	46
2.3	Equipment for electrophysiological studies.	47
2.3.1	Stimulating electrodes.	47
	(I) Acute stimulation.	47
	(II) Chronic stimulation	48
2.3.2	Recording electrodes.	50
2.4	Stimulus generation and delivery.	50
2.5	Compound action potential recordings.	52
2.6	Blood collection.	52
2.7	Melatonin radioimmunoassay.	53
2.7.1	Reagents.	53
2.7.2	Preparation of standards, tracer & antibody	54
2.7.3	Assay protocol	55
2.7.4	Performance and validation.	55
2.8	Pineal tissue collection and processing.	57
2.8.1	Collection	57
2.8.2	Processing prior to ICC	57
	(I) Tissue fixation	57
	(II) Paraffin wax embedding	57
2.8.3	Immunocytochemistry	58
2.8.4	Evaluation of antigenic immunoreactivity	62
2.9	Experimental design and analysis.	62
2.9.1	Missing data	62
2.9.2	Total melatonin secretion estimates	62

2.9.3	Transformations.	62
2.9.4	Statistical analyses	63
	(I) Plasma melatonin profiles	63
	(II) Immunoreactivity	63
	(III) Levels of significance	63
CHAPTER 3.	Induction of pineal melatonin secretion in anaesthetized rams by darkness or bilateral electrical stimulation of the cervical sympathetic trunks.	64
3.1	Introduction	64
3.2	Materials and methods.	65
	3.2.1 Experimental procedures.	65
	3.2.2 Stimulus Parameters.	67
	3.2.3 Statistical analysis	68
3.3	Results.	70
	3.3.1 Experiment 1 (The effects of anaesthetic agents on nocturnal melatonin secretion)	70
	3.3.2 Experiment 2. (The effect of CST stimulation on melatonin secretion)	73
3.4	Discussion	81
	3.4.1 Anaesthetic action on nocturnal melatonin secretion.	81
	3.4.2 Neural regulation of pineal secretory activity	83
3.5	Conclusions	88
CHAPTER 4	Bilateral electrical stimulation of the CST's of conscious rams: Effects of photoperiod, time-of-day and stimulus parameters on pineal melatonin secretory responses.	90
4.1	Introduction.	90
4.2	Materials and methods.	91
	4.2.1 Preliminary studies to establish appropriate stimulus parameters.	91
	4.2.2 Experiment 3: Animals, lighting regimes and treatment times.	92
	4.2.3 Experiment 4	96
	(I) Animals and lighting regimes.	96
	(II) Stimulus parameters and treatment times.	96
	4.2.4 Statistical analysis	98
4.3	Results.	100
	4.3.1 Experiment 3. (Effects of photoperiod and time-of-day)	100
	(I) Pre-stimulation values	100
	(II) Stimulation responses	101
	(III) Individual animal stimulation responses	105
	(IV) Post-stimulation values	106
	4.3.2 Experiment 4. (The effect of stimulus parameters)	118
	(I) Pre-stimulation values	118
	(II) Stimulated responses	119
	(III) Post-stimulation values	120
	(IV) CST Morphology	120
4.4	Discussion.	128
	4.4.1 Photoperiod and time-of-day	128
	4.4.2 Stimulus parameters	132

4.5	Conclusions	136
CHAPTER 5 Immunocytochemical evaluation of the peripheral and central innervation of the ovine pineal gland and the effects of unilateral or bilateral superior cervical ganglionectomy.		
5.1	Introduction	137
5.2	Materials and Methods.	138
5.2.1	Experimental procedure	138
5.2.2	Evaluation of antigenic immunoreactivity	139
	(I) NSE	139
	(II) PNMT	140
	(III) NPY	140
	(IV) VIP	140
5.2.3	Statistical analyses	141
	(I) NSE	141
	(II) PNMT, NPY & VIP	141
5.3	Results.	142
5.3.1	NSE	142
5.3.2	PNMT	150
5.3.3	NPY	153
5.3.4	VIP	156
5.4	Discussion.	158
5.4.1	NSE	158
5.4.2	PNMT	163
5.4.3	NPY	166
5.4.4	VIP	168
5.5	Conclusions	171
CHAPTER 6 General Discussion and Conclusions		
6.1	Introduction	172
6.2	Sympathetic regulation of ovine pineal gland function	173
6.3	Central influences on pineal function.	177
6.4	Integrated neural control of ovine pineal function	179
6.5	Future directions for ovine pineal research	179
6.5.1	Melatonin secretion during CST stimulation	180
6.5.2	Electrical activity, central innervation and secretory activity of the ovine pineal gland	181
6.5.3	Immunocytochemistry and its role in ovine pineal studies	183
6.5.4	<u>In vivo</u> pharmacological manipulation of neural activity.	184
6.5.5	Anaesthesia and its role in pineal studies	185
6.6	Conclusion	185
REFERENCES		187

List of Abbreviations

5-HT	Serotonin
ACh	Acetylcholine
APUD	Amine precursor uptake and decarboxylation
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
AVT	Arginine vasotocin
cAMP	cyclic Adenosine monophosphate
cGMP	cyclic Guanylate
CNS	Central nervous system
CSF	Cerebrospinal fluid
CST	Cervical sympathetic trunk
CV	Coefficient of Variation
DAB	Diaminobenzidine
DBH	Dopamine β hydroxylase
d.f.	degrees of freedom
ECN	External carotid nerve
FSH	Follicle stimulating hormone
GABA	Gamma-aminobutyric acid
GnRH	Gonadotrophin releasing hormone
HCl	Hydrochloric acid
H/H	Halothane induction/halothane maintenance
HIOMT	Hydroxyindole O-methyltransferase
HNO ₃	Nitric acid
hr	hour
ICC	Immunocytochemistry
ICN	Internal carotid nerve
ID	Internal diameter
IR	Immunoreactive
IU	International Units
LH	Luteinizing hormone
M	Molar
min	minute
NaCl	Sodium chloride
NAT	N-acetyltransferase
NPY	Neuropeptide-Y
NPY-LI	Neuropeptide-Y-like immunoreactivity
NSE	Neuron specific enolase
NSE-LI	Neuron specific enolase-like immunoreactivity
OD	Outside diameter
OXT	Oxytocin
PNMT	Phenylethanolamine N-methyltransferase
PNMT-LI	Phenylethanolamine N-methyltransferase-like immunoreactivity
PrL	Prolactin
PVN	Paraventricular nuclei
RIA	Radioimmunoassay
SCG	Superior cervical ganglia
SCGX	Superior cervical ganglionectomy
SCN	Suprachiasmatic nuclei
S.E.M	Standard error of the mean
S/H	Saffan induction/halothane maintenance
TH	Tyrosine hydroxylase
VIP	Vasoactive intestinal polypeptide
VIP-LI	Vasoactive intestinal polypeptide-like immunoreactivity

List of Figures

Figure	Page
2.1 Schematic representation of the chronically implanted stimulating electrodes used in Experiments 3 and 4 for bilateral stimulation of the CST's of conscious rams. . .	49
2.2 Schematic representation of the arrangement of Neurolog modules and electrodes for left side CST stimulation.	51
3.1 Effects of various anaesthetics on the nocturnal rise in plasma melatonin concentrations in adult Romney rams.	71
3.2 Effects of various anaesthetics on the average amount of melatonin secreted in adult Romney rams during a 60 min period beginning at lights off.	72
3.3 Daytime plasma melatonin levels before, during and after acute bilateral electrical stimulation of the CST's of anaesthetized rams.	75
3.4 Night-time plasma melatonin levels before, during and after acute bilateral electrical stimulation of the CST's of anaesthetized rams.	76
3.5 Plasma melatonin levels in individual control rams sampled during the period they would otherwise have been stimulated.	78
3.6 Plasma melatonin levels measured in individual rams stimulated during the day. . . .	79
3.7 Plasma melatonin levels measured in individual rams stimulated during the night. . .	80
4.1 Effect of daytime, bilateral, electrical stimulation of the CST's of one conscious ram, with chronically implanted stimulating electrodes on the plasma melatonin profile.	93
4.2 Effect of daytime, bilateral, electrical stimulation of the CST's of one conscious ram, with chronically implanted stimulating electrodes at a range of pulse durations, on the plasma melatonin profile.	94
4.3 Mean plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of conscious rams in a 16L:8D photoperiod.	102
4.4 Mean plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of conscious rams in a 8L:16D photoperiod.	103
4.5 Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #10 in a 8L:16D photoperiod.	107
4.6 Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #18 in a 8L:16D photoperiod.	108
4.7 Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #22 in a 8L:16D photoperiod.	109
4.8 Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #28 in a 8L:16D photoperiod.	110

4.9	Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #32 in a 8L:16D photoperiod.	111
4.10	Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #10 in a 16L:8D photoperiod.	112
4.11	Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #11 in a 16L:8D photoperiod.	113
4.12	Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #18 in a 16L:8D photoperiod.	114
4.13	Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #22 in a 16L:8D photoperiod.	115
4.14	Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #28 in a 16L:8D photoperiod.	116
4.15	Plasma melatonin levels before, during and after bilateral, electrical stimulation of the CST's of ram #32 in a 16L:8D photoperiod.	117
4.16	Effect of 30 min continuous electrical stimulation of CST's of conscious rams, with stimuli of varying pulse duration, on mean plasma melatonin levels.	121
4.17	An example of the effect of 30 min continuous electrical stimulation of CST's of sheep D, with stimuli of varying pulse duration, on plasma melatonin levels.	122
4.18	Effect of 30 min continuous electrical stimulation of CST's of conscious rams, with stimuli of varying current strength, on mean plasma melatonin levels.	123
4.19	An example of the effect of 30 min continuous electrical stimulation of CST's of sheep F, with stimuli of varying current strength, on plasma melatonin levels.	124
4.20	Effect of 30 min continuous electrical stimulation of CST's of conscious rams, with stimuli of varying pulse frequency, on mean plasma melatonin levels.	125
4.21	An example of the effect of 30 min continuous electrical stimulation of CST's of sheep D, with stimuli of varying pulse frequency, on plasma melatonin levels.	126
4.22	Transversely sectioned CST's stained with Masson's Green Trichrome to show cell nuclei (blue/black), collagen (green) and myelin (red).	127
5.1	Paramedian section through the epithalamic region of the sheep brain showing the position of the pineal gland and associated structures.	143
5.2	Outline diagram to show the anatomical position of the pineal gland and associated structures in the epithalamic region of the sheep brain.	144
5.3	NSE-like immunoreactivity in the apex of a pineal gland with intact sympathetic innervation.	145
5.4	NSE-like immunoreactivity in the apex of sympathetically denervated pineal glands.	145
5.5	NSE-LI cells and nerve fibres in the habenular region of the pineal stalk.	148

5.6	NSE-LI cells and nerve fibres in the caudal commissure (CC) and pineal stalk (PS).	148
5.7	NSE-LI cells and nerve fibres (arrows) in the habenular region of the stalk of a pineal (PS) with intact sympathetic innervation.	148
5.8	Bundles of PNMT-LI fibres (arrows) travelling through the interstitial tissue of the body of the pineal.	151
5.9	Transversely sectioned PNMT-LI fibres (arrows) emerging from the habenular nucleus (HN) and travelling into the stalk of the pineal gland (P).	151
5.10	PNMT-LI fibres (arrows) in the caudal commissure (CC).	152
5.11	Parallel tracts of PNMT-LI fibres in the apex of the pineal gland.	152
5.12	NPY-LI fibres shown in close association with blood vessels in the apex of the pineal gland.	154
5.13	NPY-LI fibres (arrows) in the interstitial tissue of the mid-pineal region.	154
5.14	NPY-LI fibres (arrows), without vascular association, travelling through the habenular nucleus (HN) and projecting into the stalk of the pineal gland (PS).	154
5.15	NPY-LI fibres (arrows), without vascular association, travelling through the caudal commissure (CC) and projecting into the stalk of the pineal gland (PS).	155
5.16	NPY-LI associated with blood vessels in the pineals of unilaterally SCGX rams.	155
5.17	NPY-LI in the pineal gland of a bilaterally ganglionectomized ram.	155
5.18	NPY-LI fibres (arrows), without vascular association, travelling through the habenular nucleus (HN) and projecting into the stalk of the pineal gland (PS).	157
5.19	VIP-LI nerve fibres (arrows) associated with blood vessels in the pineal of a control ram.	157
5.20	VIP-LI nerve fibres (arrows) in close association with a blood vessel in the pineal of a control ram.	157
5.21	Low magnification photomicrograph to show the regionalized distribution of VIP-LI nerve fibres which was observed in some pineals.	159
5.22	VIP-LI nerve fibres in the pineal of a unilaterally ganglionectomized ram.	159
5.23	VIP-LI nerve fibres in the pineal of bilaterally ganglionectomized ram.	159
5.24	Caudal commissure (CC) of the pineal stalk of a control ram. Note that this region is totally devoid of VIP-LI.	160
5.25	Habenular region (HN) of ganglionectomized rams.	160

List of Tables

Table	Page
2.1 Between- and within-assay CV for melatonin radioimmunoassay based on repeated measurement of ovine plasma samples.	56
2.2 Effect of dilution with assay buffer on estimates of melatonin concentration in two quality control plasma samples. All samples were assayed twice.	56
2.3 Paraffin wax processing schedule.	58
2.4 Antibodies used in ovine pineal tissue immunocytochemistry.	59
2.5 Immunocytochemical procedure for the identification of antigens in pineal tissue. . .	60
3.1 Coefficients used in partitioning treatment effects in Experiment 1.	68
3.2 Summary of analyses of variance of data from Experiment 1.	70
3.3 Mean melatonin concentrations S.E.M (pg/ml) prior to stimulation and mean hourly areas under melatonin response curves S.E.M (pg/ml.hr) during and after acute or sham bilateral electrical stimulation of the CST's of anaesthetized rams in Experiment 2.	73
3.4 Summary of analyses of variance of data from Experiment 2.	74
4.1 Times for each photoperiod during which blood sampling occurred in Experiment 3.	95
4.2 Combinations of electrical stimulus parameters applied to each ram in Experiment 4.	97
4.3 Orthogonal coefficients used in partitioning treatment effects in analysis of variance of data from Experiment 3.	99
4.4 Coefficients used in partitioning treatment effects in analyses of variance of data from Experiment 4.	100
4.5 Mean (\pm S.E.M) pre- and post-stimulation plasma melatonin levels (pg/ml) and mean (\pm S.E.M) melatonin secretory responses to CST stimulation (pg/ml.hr) under 16L:8D and 8L:16D photoperiods, when nerve stimulation was performed at the beginning, middle or end of the photophase.	101
4.6 Summary of analysis of variance of pre-stimulation data from Experiment 3.	101
4.7 Plasma melatonin secretory responses (log pg/ml.hr & pg/ml.hr) to bilateral CST stimulation adjusted for the regression of stimulated responses (log pg/ml.hr) on pre-stimulated responses (log pg/ml).	104
4.8 Summary of analysis of residual variance of stimulation and post-stimulation data from Experiment 3.	104
4.9 Plasma melatonin secretory responses (log pg/ml & pg/ml) following bilateral CST stimulation adjusted for the regression of post-stimulation responses (log pg/ml) on pre-stimulated responses (log pg/ml).	106

4.10	Mean (\pm S.E.M) pre- and post-stimulation plasma melatonin levels (pg/ml) and mean (\pm S.E.M) melatonin secretory responses (pg/ml.hr) to 30 min of CST stimulation in Experiment 4.	118
4.11	Summary of analysis of variance of pre-stimulation data from Experiment 4.	119
4.12	Mean plasma melatonin secretory responses (pg/ml.hr) to bilateral CST stimulation and mean post-stimulation responses (pg/ml) adjusted for the regression of stimulated responses (pg/ml.hr) on pre-stimulation responses.	119
4.13	Summary of analyses of residual variance of stimulation (pg/ml.hr) and post-stimulation (pg/ml) data from Experiment 4.	120
5.1	Coefficients used in partitioning treatment effects on NSE-LI for pineal apex and body data from Experiment 5.	141
5.2	Mean count \pm S.E.M of NSE-like immunoreactive cell-, non-immunoreactive cell- and interstitial space-grid point intercepts per 400 grid points in the apex and pineal body regions of rams in Experiment 5.	147
5.3	Summary of analyses of variance of NSE-like immunoreactive cell data from the apex and body of the pineal.	149
5.4	Summary of analyses of variance of Non-NSE immunoreactive cell data from the apex and body of the pineal.	149
5.5	Summary of analyses of variance of interstitial space data from the apex and body of the pineal.	150