AN INVESTIGATION OF THE NEUROREGULATION AND CIRCADIAN EXPRESSION OF BROWN FAT HEAT PRODUCTION

Lisa Ann Kelly

A thesis submitted to the
School of Graduate Studies of the University of Ottawa
as partial fufillment of the requirements for
the degree of Doctor of Philosophy
May, 1995

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

ISBN 0-612-07801-9

Canada

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

ABSTRACT

The overall aim of this work is to gain insight into the physiological role of brown adipose tissue (BAT) thermogenesis in thermoregulation; to that end, the studies reported here comprise an investigation, in the rat, of both the neural control of interscapular brown fat and the daily pattern of BAT temperature change in the rat. In the first experiment, an acute stimulation paradigm was employed in combination with moveable electrodes, in order to precisely map the ventromedial hypothalamus (VMH) for sites which elicit stimulation-induced increases in BAT thermogenesis. Of roughly 30 stimulated sites in the VMH region, 20% gave rise to a BAT response; temperature rises ranged from 0.6 to 4.6°C, and returned to baseline in approximately an hour. It is speculated that the observed variability in BAT response to VMH signals may be due either to regionalized input from adjoining nuclei, or to differing thresholds of activation for various elements of the nucleus.

In the course of this first study, stimulation of a number of extra-VMH sites was observed to evoke a rapid drop in BAT temperature, a response which has not been well-characterized. Therefore, in the second study, structures outside the VMH were systematically evaluated, using moveable electrodes, for sites associated with a BAT temperature decrease. About 70% of electrode tracks located in the ventral lateral thalamic nucleus (VLT) and the zona incerta (ZI) displayed sites which elicited repeatable drops in BAT temperature. The typical profile showed a temperature drop of approximately 0.5°C, and took about 30 min

to return to baseline; in addition, most VLT sites showed a small drop in core temperature. To investigate mechanisms underlying the BAT temperature drops, effective sites in a subset of animals were restimulated after systemic injection of the β-adrenoceptor blocker propranolol or the α-blocker phentolamine. As expected, application of the β-blocker did not prevent the temperature drop, but the α-blocker appeared to severely diminish the response in the three cases examined. This finding, while not yet definitive, is compatible with the notion that a sympathetic vasoconstrictive mechanism underlies the BAT temperature drops.

The observation of bidirectional changes in BAT baseline temperature, combined with reports that BAT heat production contributes to core temperature (eg. Closa et al, 1993; Foster & Frydman, 1978) led to the hypothesis that brown fat displays a circadian profile of heat production similar to that of core. To investigate the daily pattern of BAT temperature changes, radio-frequency disc transmitters (Mini-Mitter Co., Inc.) were implanted beneath the interscapular BAT deposit, and temperatures were recorded throughout the 24 h cycle of equal light/dark periods, for up to six days. The data were analyzed using spectral analytic methods to examine underlying cycles. The circadian component was then statistically subtracted from the temperature pattern ("deseasoning") and the data reanalyzed in order to determine if any ultradian cycles were present; this second procedure reduces interference from harmonic frequencies.

As expected, all data show a circadian cycle; the analysis confirms the strong visual impression given by the temperature

profiles of an elevated nocturnal baseline. However, the presence of ultradian rhythms is less clear; spectral analysis suggests that the 8h and 4h cycles are most consistent, and the 4h cycle may be the stronger as it persists in the analysis of the deseasoned data.

Three subjects also had a second transmitter placed in the intraperitoneal cavity in order to collect simultaneous core and BAT temperature. The temperature profiles we observe appear to be highly correlated; both the BAT and core patterns are characterized by an elevated nocturnal baseline, peaks and valleys throughout the 24h, and an anticipatory rise in temperature that appears about three hours before light offset. This latter feature, along with the persistence of the BAT nocturnal rise even in the 24h lights-on condition, suggests that BAT temperature rhythm is driven by an endogenous pacemaker, similar to the regulation of core temperature.

The cross correlational analysis indicates first, that the precise relationship between the two profiles is highly variable, both within and between rats; second, the predominant underlying ultradian cycle for both appears to range from 3 to 4 h, as corroborated by the results of spectral analyses; third, it appears that the correlation between BAT and core is generally higher at night than during the day; and fourth, in many cases there is a suggestion that the BAT temperature profile leads that of core by 10 to 30 minutes. Thus, the results of the third experiment provide strong evidence that brown fat heat production contributes to core temperature warming patterns.

Acknowledgements

Without question, my first and strongest debt of gratitude is to my supervisor, Dr. Kate Bielajew. Only in the last year have I realized that she has become my standard: her delight in research, her integrity, her humor, her unfailing courtesy, and her matchless grace in the written word. I hope I can live up to her teaching.

As for Janet and Monika - we have been there for each other in so many ways over the last six years. I am all too aware how lucky I was to discover such good friends in my labmates. But now the GOOD stuff begins, remember?? Collaborations, and meeting at conferences, and sharing many, many more great meals over expensive wine...

And our honourary labmate Thom, whose eloquence of speech and boundless joie de vivre brought much to our days in 104. And then there is Dr. George Fouriezos, whose singular view of the world always got me thinking, and whose casual confidence in me over the years was more probably more important than he realized. There are several people out of the department, who very kindly came to my rescue when I needed direction with some aspect of the thesis: Steve Reid, Dr. Fenwick, and Dr. Dabrowski. And I cannot forget my good friend Warren Harris, who gave countless hours of his time, somehow with good humor, whenever I needed him to design yet another innovative instrument.

My thanks must also go out to my committee members, Dr. Merali, Dr. Messier, and Dr. Himms-Hagen, for the time they have

invested in my thesis, and for simply keeping their doors open to me over the years whenever I had questions.

And at last, my wonderful family. My parents, John and Inge Kelly, and my four brothers and sisters, Ken, Suzanne, Joanne and Christopher who always, always believed in me: I simply could not have done this without all of you there for me. And a very warm thank you to my other parents, Hilda and Ed Bond - I cannot tell them what their support has meant to me. And my sister-in-law Deb, who is definitely more my sister than my in-law. And finally, to my husband, the love of my life, Jeb Bond - you are the person who gives all of this meaning.

TABLE OF CONTENTS

List of Figures

List of Tables

INTRODUCTION

The study of brown fat has a rich, engrossing history. It was first thought to be thymus tissue, and was next billed as the "hibernating gland". The earliest written reference to brown fat is generally attributed to Gessner in 1551 (Smith, 1963), although detailed attempts to describe the microscopic anatomy began much later, at the end of the 19th century. Researchers speculated on numerous possible functions for the gland, from a reserve supply of energy to an internal source of water and endocrine hormones (from Johansson, 1958). In 1961, R. E. Smith published an abstract stating that brown fat excised from cold-acclimatized rats displays an elevated in vitro respiration rate compared to that of tissue from control animals; this metabolic change allows five times the heat production of normal tissue. It is this unique facultative thermogenic response, in combination with the fundamental regulatory role of the sympathetic nervous system in BAT metabolism, that places brown fat research squarely at the crossroads of two major investigative fields: thermoregulation and energy regulation. The work described here is intended to contribute to our current understanding of both the neural control of BAT thermogenesis and the physiological role of brown fat heat production.

General Background: Brown fat differs from white fat in distribution, biochemistry, and function, and has thus far been positively identified only in mammals (Trayhurn, 1993). BAT is found in discrete axillary, intercostal, subscapular, and

interscapular regions in the rat, as well as along major abdominal blood vessels (see Himms-Hagen, 1985). The interscapular brown fat deposit is the most accessible, and is therefore most often used in studies of BAT functioning. Mammals vary in their distribution and percentage of BAT, but the tissue is generally most active in neonates, and the amount declines with maturity; BAT thermogenic activity in rat pups, for example, appears to peak at 3 to 4 days post-natal (Sundin & Cannon, 1980). Brown adipose tissue comprises 1 to 5% of total body fat in rats, depending on feeding conditions and ambient temperature (from Arbuthnott, 1989). Active intra-abdominal deposits have been found imbedded within the white adipose tissue of human adults (see Lean, 1989), although the role of BAT in human energy regulation remains unclear.

The characteristic colour of brown fat comes from both the heavy vascularization and the density of mitochondria within individual cells. The level of blood flow through BAT shows a much wider range that of white fat and is correlated with the thermogenic state of the tissue (Foster and Frydman, 1979; Glick et al, 1984; Benzi & Girardier, 1986; Laury et al, 1987). The inner mitochondrial membranes of BAT contain a unique protein known as thermogenin or uncoupling protein (UCP). The UCP is almost solely responsible for the BAT heat production elicited by sympathetic stimulation of the tissue; this is accomplished by allowing protons pumped out of the mitochondria via the electron transport system to leak back through the UCP, instead of returning through the usual ATP-generating channels that define

the final step of oxidative phosphorylation. This mechanism will be described in more detail in section 3.

Role of the SNS: Reports of altered metabolism in denervated brown fat pads for both normal and food deprived animals (eg. Hausberger, 1934 as cited in Smith and Roberts, 1964; Sidman et al, 1962) provided the earliest evidence of BAT autonomic innervation, along with the results of histological and biochemical examinations of the tissue for noradrenaline (NE) content (e.g. Wirsen, 1964). Correll (1963) suggested that the SNS exerts an influence on adipose tissue, based on his observation that in-vitro electrical stimulation of BAT causes the release of free fatty acids into the medium. A major finding was reported by Hull and Segall (1965) who demonstrated that a biphasic temperature response was elicited from rabbit brown fat following direct in vivo cervical nerve stimulation; they also observed that denervation caused depletion of fat content in the tissue. Finally, Seydoux and coworkers (1977), in another important advance, recorded increased NAD(P)H [nicotinamide adenine dinucleotide (phosphate)] levels in BAT when the intercostal nerves were electrically stimulated in vitro, indicating an increased capacity to oxidize substrates. They further noted elevated levels of 0_2 consumption in the tissue.

Pagé and Babineau (1950, as cited in Johansson, 1958) were among the first to suggest that the function of brown fat is realized through cold exposure. While an increase in lipid turnover was known to be a pivotal metabolic change underlying

the general cold-induced sympathetic response (Steiner & Cahill, 1964), the direct link between cold stimulation, sympathetic response, and brown fat thermogenesis was only fully elaborated when Smith and Roberts (1964) discussed how the relatively small amount of heat generated by BAT might make a significant contribution to total body thermoregulation.

They argued that the heat from BAT must somehow be directed to a small isolated heat sink. Latex-injected rats were dissected in order to study details of the regional vascular supply, and the authors subsequently described the large venous drainage from the interscapular BAT deposits, known as Sulzers vein. This efferent venous flow from BAT supplies warmed blood directly to the heart, but also acts to heat proximal afferent arterial blood by the method of counter current heat exchange, therefore in turn bathing the thoracic and cervical spinal cord with the warmed arterial blood. Later research demonstrated that brown fat is able to respond to direct, graded stimulation of the nerves with corresponding changes in the magnitude of temperature change, which suggests that increasing degrees of cold exposure may elicit greater heat production in brown fat tissue (Flaim et al, 1976). This facultative heat production induced in BAT by cold-exposure is termed non-shivering thermogenesis.

Characteristics of BAT Innervation: The innervation of the interscapular BAT deposit has been studied using light, electron, and fluorescence microscopy. Each of the two symmetric fat pads are supplied by five intercostal nerves that originate from the

thoracic and cervical ganglia; an additional single bundle enters along the thoracodorsal artery. Flaim et al (1976) observed that the intercostal nerve bundles vary in size and myelination, and Foster et al (1982b) further demonstrated that the exact innervation pattern for each bundle differs across individual rats. The functional cross innervation between the two BAT lobes appears to be negligible (Foster et al, 1982a).

These five nerves supply both the fat cells and the vascular system of the deposit, and terminate in the skin and white adipose tissue surrounding the interscapular BAT area (Foster et al, 1982b). Therefore, the bundles likely contain afferent as well as efferent fibres. Indeed, capsaicin denervation, and immunohistochemical studies indicate that nerves on the vessels contain the typical sensory neurotransmitters substance P and calcitonin gene-related peptide (CGRP) (Nnodim et al, 1988; Himms-Hagen et al, 1990). These sensory nerves may serve to regulate blood flow and trophic processes, through neuropeptide release and possible communication between the sensory fibres and the sympathetic ganglia (Himms-Hagen, 1990).

There are also distinct nerve types within the class of efferent fibres from the intercostal bundles. The fibres that terminate on vessels contain neuropeptide Y co-localized with NE, while those terminating on fat cells express only NE (Cannon et al, 1986; Nnodim et al, 1988); it is assumed that this distinction means that the two fibre types have different origins, but thus far, little is known. In addition, Engel et al (1992) showed that there are functional differences between the

fibres terminating on the vessels and those terminating in the skin.

The sympathetic control of BAT non-shivering thermogenesis was confirmed by studies that described altered levels of NE after starvation and cold exposure (i.e. Schönbaum et al, 1966; Cottle et al, 1967; Steiner et al, 1970). Cold exposure induces a specific sequence of events in brown fat tissue now attributable to chronic NE stimulation. The acute thermogenic response, that is, the events surrounding the activation of the UCP, occurs within minutes of stimulation, as does the increase in blood flow and mRNA for UCP (Desautels and Himms-Hagen, 1979; Rial and Nicholls, 1984; Ricquier et al, 1986); the latter reaches a maximum in 6 to 24 h, followed by elevated rates of UCP synthesis (Peachey et al, 1988). There is also an increase in lipoprotein lipase at the outer cellular membrane, as well as in cellular thyroxine 5'deiodinase (Carneheim et al, 1984; Kopeckey et al, 1986). Finally, the tissue displays both hypertrophy and hyperplasia, reflecting an increase in fuel availability and in the growth of preadipocytes and endothelial cells for capillary beds, respectively.

Adrenoceptors Subtypes: The use of a wide range of adrenoceptor antagonists and agonists in vitro stimulation studies has demonstrated that NE interacts with β_1, β_2 and also α_1 receptors during stimulation of brown fat cells (Fain et al, 1984; see Fain and García-Sáinz, 1983; Horowitz & Hamilton, 1984; Mohell et al, 1987; Rothwell, 1990). The α_1-adrenoceptor

stimulation is shown to have a potentiating effect on β-induced thermogenesis (Ma & Foster, 1984; Thurlby, 1985), but the α_1 stimulation acts through a different mechanism than the β receptors do; instead of stimulating the adenylate-cyclase mediated uncoupling system, the α_1 stimulation alters some aspect of the ATP-ase controlled electron flow of the BAT cell (see Himms-Hagen, 1990). This cooperativity between α and β receptor subtypes contrasts with the agonistic interaction observed in white adipose cells (for review of subtypes, see Lafontan & Berlan, 1993).

There is now evidence that BAT receptors in fact show the strongest affinity for atypical, or β_3, agonists (Arch et al, 1984); the β_3 receptors may mediate both the cellular events of the thermogenic response, and the blood flow increases (Takahashi et al, 1992). It also appears that this receptor subtype does not elicit the increased blood pressure, or tachycardia seen with other sympathomimetics (Holloway et al, 1991; Jecquier et al, 1992). Hence, β_3 agonists represent the newest generation of thermogenic anti-obesity drugs.

Effects of Surgical Denervation: Steiner et al (1970) investigated the role of the SNS in vivo by denervating one of the two interscapular BAT lobes; they found that denervation impaired but did not prevent cold responses. A similar delayed but not abolished elevation of protein and GDP-binding was reported by Park and Himms-Hagen (1988b) and Desautels et al (1986), while Rothwell and Stock (1984) found that denervation

completely prevented hypertrophy due to cold. The results of denervation studies with animals maintained at room temperature or higher generally show no loss of protein, but increased triglyceride content and decreased GDP-binding (Rothwell & Stock, 1984; Desautels et al, 1986; Minokoshi et al, 1986a).

The effects of denervation of interscapular BAT can be summarized as follows (see Himms-Hagen, 1990 or 1991, for further elaboration): approximately 90% reduction in NE content, abolition of the cold-induced increase in blood flow, and reduction of cold-induced increases in protein, UCP, GDP binding, various lipogenic/lipolytic enzymes, and thyroxine 5'-deiodinase activity. It appears that in order for these reductions to occur, denervation must be performed on animals that are cold-exposed prior to surgery, although even room temperature may be low enough to enhance BAT activity to the necessary level. Thyroxine 5'deiodinase activity, believed to amplify the gene expression of UCP, is the event most critically dependent on direct sympathetic input. It decreases within hours of denervation and, unlike the drop in UCP and protein, the decrease is not prevented by chronic NE infusion. Finally, the denervated deposit typically appears pale, due to the loss of mitochondria, and the wet weight may increase as lipolytic stimulation to the tissue is diminished.

Surgical denervation studies report a contradictory range of effects on blood flow. The cold-induced increase in blood flow is abolished, but the effect on basal blood flow appears to depend very much on the method used, unlike the effect on other

aspects of BAT metabolism. Whether the rats are conscious or unconscious is important, as is the type of anesthetic, and the temperature at which core is maintained (Himms-Hagen, 1990). Thornhill and Halvorson (1993) found that denervation prevented VMH-stimulated blood flow as well as BAT temperature increases, consistent with the view that tissue blood volume and adipocyte activity are jointly controlled by the CNS.

Sympathectomy does not prevent the lipolytic, nor GDP-binding response of the BAT deposit to NE infusion (Rothwell & Stock, 1984; Laury et al, 1987) which indicates the retention of brown fat cellular characteristics; it is possible that humoral factors such as epinephrine or glucagon contribute to the partial maintenance of the cold response after denervation. In fact, Steiner and coworkers (1970) postulated that the primary neural influence is exerted on blood flow through the tissue, rather than on cellular metabolism. While this idea is now known to be inaccurate (Foster & Frydman, 1979; Nicholls and Locke, 1984), neurally mediated blood volume redistribution does play a critical role in the BAT thermogenic response.

Characteristics of Vascularization: The presence of blood flow is one of the reasons that the in vivo cold stimulated activity of BAT is greater than in vitro NE-induced response (Kikuchi et al, 1992). Foster and Frydman (1979) are responsible for the sophisticated study that affirmed the role of BAT heat production in cold-exposed rats. The maximum possible contribution of a tissue to heat production is set by both its

blood flow and the individual O_2 consumption of the tissue. The researchers used radioactive microspheres to measure changes in blood flow distribution in conscious animals, and demonstrated that BAT is by far the dominant anatomical site of increased heat production in cold-exposed, warm-acclimatized rats. The cold-exposed animals showed 25% higher VO_2 and 24-fold increase in blood flow to BAT than did control subjects, compared to a threefold increase in the heart and no change to the hepatosplanchnic region.

This enormous increase in blood flow can be related to the vascular characteristics of the interscapular BAT deposit. Numerous arteriovenous anastomoses (AVA) appear as direct shunts between arterioles and venules, and thus can act to short circuit the blood flow through the BAT capillaries. Assuming that the SNS fibres can regulate the vasomotor tone of the anastomoses seperately from that of the vessels in the BAT deposits, then constriction of the AVA would result in increased blood flow through the BAT capillary bed, while dilation of the AVA would allow a large percentage of blood flow to bypass the capillary bed and thus lessen the oxygen and fuel supply to the thermogenic fat cells.

Sympathetic control of BAT blood flow is not completely understood. While activation of BAT metabolism, whether by NE infusion, CNS stimulation, or direct nerve stimulation, clearly causes an increase in blood flow, this is believed to be secondary to the effect of NE. In fact, adrenergic stimulation induces vasoconstriction initially, as demonstrated by Flaim et

al (1977) who sought to evaluate the precise mechanisms underlying the observed biphasic temperature response of BAT to VMH stimulation. They applied the non-specific α-receptor blockers phentolomine and phenoxybenzamine, as well as the α-adrenergic agonist phenylephrine, and concluded that activation of α-receptors causes vasoconstriction of the arteries and capillaries, thereby giving rise to the initial dip in BAT temperature.

This reduction in BAT blood flow may be counteracted by the tissue in several ways. First, the vasoconstrictive effect itself may function to increase blood flow by constriction of the potential blood reservoir in the AVAs; second, the thermogenic response of brown fat to NE may induce the release of a separate vasodilator, possibly from mast cells found in BAT deposits (Desautels et al, 1994). Adenosine, histamine, the sensory peptide CGRP, and nitric oxide have all been suggested as candidates (Szillat & Bukowiecki, 1983; Desautels et al, 1994; Uchida et al, 1994), while proposed triggering mechanisms include changes in the level of intracellular PO_2, and the buildup of byproducts of thermogenic metabolism (Foster & Depocas, 1980; Himms-Hagen, 1991). This process is an example of the acute control of local blood flow that can occur in any tissue when metabolic rate increases (Guyton, 1991).

Finally, Engel et al (1992) suggest that the SNS fibres terminating in the skin are regulated separately from the fibres terminating at the vessels, despite the fact that there is no clear pattern of origin from the five intercostal nerves entering

the interscapular BAT deposit. The authors dissected branches at each location, and recorded firing rate as a strong cold stimulus was applied to the abdomen of the anaesthetized rats; they found that stimulation increased the activity of the nerve branches to the skin, while it suppressed firing rate in branches to BAT itself. This arrangement could facilitate increased blood flow during thermogenesis both by preventing flow to surrounding skin, and dilating brown fat vessels.

In conclusion, the regulation of brown fat by direct sympathetic innervation contrasts the mainly humoral regulation of white adipose tissue. White fat is regulated by humoral catecholamines released from the adrenal medulla and also by insulin and glucocorticoids, with direct sympathetic stimulation thought to play a lesser role as the adrenergic innervation of white fat is thought to be limited to blood vessels (see Lafontan & Berlan, 1993). While brown fat contains specific insulin and thyroxine receptors, these humoral agents are considered to be permissive or modulating factors, in contrast to the primary regulatory role of sympathetic innervation.

Brown fat and Hypothalamic control: The SNS has long been implicated in the mechanisms of glucose metabolism (Cannon et al, 1924), but evidence of a specific role for the VMH in the control of metabolism appeared more recently. It stemmed from the results of lesion studies (Marshall et al, 1955) and reports of changes in VMH neuronal activity in response to different blood glucose levels (Anand et al, 1964). Direct stimulation of the

VMH is linked to both accelerated hepatic glycogenolysis (Shimazu & Amakawa, 1968) and a rapid rise in plasma glucose (Frohman and Bernardis, 1971)

This work led Shimazu and coworkers to examine hypothalamic control of lipid metabolism. They demonstrated increased plasma glycerol in rabbits after electrical stimulation of the VMH (Kumon et al, 1976); the lack of a parallel increase in plasma free fatty acids was attributed to accelerated oxidation in the fat cell. This finding was contrasted with the lack of lipolytic response after lateral hypothalamic stimulation.

The first study directly linking BAT metabolism to VMH stimulation also came from Shimazu's laboratory (Shimazu and Takahashi, 1980). The researchers reported the rate of fatty acid synthesis by measuring incorporation of labelled water into fatty acids in vivo. Acute electrical stimulation of the VMH was shown to enhance fatty acid synthesis preferentially in interscapular BAT over white adipose tissue; this change occurred in diabetic rats as well, demonstrating that the mechanism does not require insulin mediation. When taken with the previous paper, the results suggest that the rates of both lipolysis and lipogenesis are accelerated by VMH stimulation, which agrees with previous observations of a cold-induced increase in BAT lipid turnover (Steiner & Cahill, 1964; Himms-Hagen, 1965).

Subsequent stimulation studies elaborated the nature of VMH regulation of brown fat. Stimulation of the VMH significantly increases regional blood flow to BAT, less so to the adrenal glands and diaphragm, with no change in blood flow to the liver

(Iwai et al, 1987). Increased NE turnover is also noted (Saito et al, 1989a), which is impaired by denervation (Minokoshi et al, 1986b). Perkins et al (1981) observed an increase in interscapular BAT temperature with VMH stimulation that could be blocked by administration of a β-antagonist. Intrahypothalamic administration of glucose also increases BAT activity (Sakaguchi & Bray, 1987) while intrahypothalamic 2-deoxy-glucose administration, which creates a condition of cellular glucose deprivation, has been shown to have the opposite effect, that of a decrease in BAT activity (Arase et al, 1987).

Evidence of a specific link between the VMH and BAT was further provided by VMH-lesion studies. Seydoux et al (1981) found a decrease in brown fat DNA concentration three days after lesioning the nucleus, and they also used direct nerve stimulation techniques in lesioned animals to demonstrate a diminished capacity for the production of reduced equivalents from fatty acid oxidation. These effects were attributed to a chronic lack of neural activation. The researchers also showed that this defective response can be reversed with cold-exposure or food restriction (Seydoux et al, 1982). Niijima et al (1984) reported a reduction in the spontaneous activity level of BAT sympathetic fibres after VMH lesions, supporting the notion of tonic CNS input in brown fat.

There is a wide range in reported BAT response to VMH destruction; while many studies observed reduced basal metabolic levels (eg. Hogan et al, 1982; Luboshitsky et al, 1983; Saito et al, 1985; Fukushima et al, 1987), others found normal thermogenic

activity (Hogan et al, 1982; Vander Tuig et al, 1985). Variability in the size of the VMH lesions may contribute to the conflicting results (Preston et al, 1989). However, reports of both an unaltered cold-induced response and impaired diet-induced activity are consistent (Hogan et al, 1982; Rohner-Jeanrenaud et al, 1983; Vander Tuig et al, 1985).

This pattern suggests that the VMH-BAT pathway is responsible for diet-induced BAT activity but not for the maintenance of cold-induced thermogenesis. It has therefore been hypothesised that attenuation of the diet-induced BAT response underlies the obesity associated with VMH lesions, as well as the obesity of other conditions (Rothwell and Stock, 1981; Himms-Hagen, 1989b). But despite the number of studies that show a correlation between impaired BAT activity and obesity, or between BAT activity and enhanced energy expenditure, the extent of brown fat contribution to the development of obesity remains equivocal.

Thus emerges a role for brown fat as a discrete region of excess energy expenditure, in which the facultative thermogenic response can be driven either through energy regulation or thermoregulation (Himms-Hagen, 1989a). The dual contributions of BAT, to temperature regulation and to weight gain, will both be addressed in this introduction, but the methods of evaluating BAT thermogenic activity must first be described to facilitate interpretation of the central papers.

Assessment of BAT activity: The basis of heat production in NE-stimulated BAT cells lies at the mitochondrial membrane level,

in the uncoupling of the oxidative-phosphorylation chain by the uncoupling protein. Heat is routinely released in conjunction with complete substrate oxidation in all cells, but the overall rate of oxidation, and thus heat release, is normally limited by the rate at which ADP becomes available for the final step of proton-translocating ATP-synthetase. However, when the UCP of brown fat mitochondria is turned on, the proton flow through the ATP-synthetase channel is prevented by a short-circuit, eliminating the role of ADP as an intracellular control of fuel oxidation. The activity of the electron transport chain then accelerates, in turn increasing heat release. The following is a summary of the five sequential events leading to BAT facultative thermogenesis (from Himms-Hagen, 1990):

1) Sympathetic nerve endings release NE which acts on β-receptors found in the BAT cell membrane.
2) Adenylate cyclase is activated, causing intracellular levels of cAMP and other second messengers to rise.
3) Hormone-sensitive triacylglycerol lipase is activated, resulting in an increase in intracellular fatty acid levels.
4) Fatty acids serve two roles: first, as the primary fuel for the electron transport system and second, as a signal to turn on the UCP in a manner not yet understood.
5) UCP acts to dissipate the proton gradient created by the electron transport chain through translocation of protons back into the mitochondria, thereby uncoupling the respiratory chain from ATP production. This removes the limiting factor on the rate of the electron transport system,

in turn sending more of the abundant fatty acids through the oxidation process to supply fuel and therefore increasing heat release.

The BAT uncoupling mechanism depends on the presence of UCP, which is unique to this tissue (Cannon et al, 1982). The protein is a recently evolved member of the family of mitochondrial carrier proteins; it consists of a single polypeptide chain with six hydrophobic transmembrane α-helices (see Trayhurn, 1993). Among the earliest papers documenting its existence are Nicholls, 1976 and Ricquier & Kader, 1976 (for review of background studies see Flatmark and Pedersen, 1975). UCP was first isolated completely by Lin & Klingenberg (1980), then cloned in 1985 (eg. Bouillaud et al, 1985). The complete sequence of both hamster and rat UCP is known; the proteins are similar but not identical (Himms-Hagen, 1990).

Uncoupling protein is activated by fatty acids and inhibited by purine nucleotide binding; however, very little is known about the mechanisms behind fatty acid activation, as the in-vitro changes in secondary structure can thus far only be induced by nucleotide binding, specifically GDP. The C-terminal region of the UCP, which is located on the cytosolic side, is known to contain a nucleotide binding site and possibly also the regulatory site. It appears that proton translocation involves a distinct site, perhaps close to the N-terminal end (see Klaus et al, 1990)

Additional effector mechanisms, beyond acute activation of UCP, can contribute to the BAT response. For example, short-term

heat stress provokes a decrease in the content of cytochrome b, in turn reducing the oxidation rates to required levels (Gaikwad et al, 1990). Brown fat contains a lysosomal proteolytic pathway that regulates the rapid protein loss and reduced thermogenesis resulting from a 24h fast (Desautels et al, 1990). The regulation of several physiologically distinct stores of UCP also influences the amount of the protein present; the UCP incorporated in the inner mitochondrial wall is the most stable form (Puigserver et al, 1992). Finally, within hours of cold exposure or NE injection, there is a strong increase in UCP mRNA levels that is critical to the chronic cold response (Trayhurn & Nichols, 1986).

GDP-binding is currently considered the most accurate indicator of the activity of the proton conductance pathway, and thus of the current thermogenic activity level of the tissue (Trayhurn & Milner, 1989), although recently it has been suggested that GDP-binding may not be a true quantitative measure since there is no evidence of close stoichiometry between mitochondrial proton conductance and GDP-binding (Goubern et al, 1991). Levels can increase as quickly as 20 minutes after cold exposure in rats (Swick & Swick, 1986) and are also affected by NE infusions, warm exposure after cold-acclimation, and arousal from hibernation (eg. Rothwell and Stock, 1981; Nedergaard and Cannon, 1984; Peachey et al, 1988). Binding levels appear to be correlated with the rapid unmasking of high-affinity binding sites in the UCP (termed B_{max} in Scatchard analysis) rather than changes in binding affinity (K_d in Scatchard analysis) or

increases in absolute concentration of UCP (Trayhurn et al, 1987; Peachey et al, 1988; Park and Himms-Hagen, 1988b).

Total UCP concentration, in contrast, indicates the thermogenic capacity of the tissue, while the presence of UCP is the definitive criterion of functional brown adipose tissue (Trayhurn, 1989); a very rapid, and sensitive immunoassay was developed from antisera to the isolated protein (eg. Lean et al, 1983). Because several hours are required to elicit an increase in UCP mRNA, the measurement of this substrate is most useful in identifying the factors regulating the activity of the UCP, while the total DNA or protein content can provide an index of major change in the state of the tissue (Trayhurn, 1989).

Other measures of BAT activity include BAT sympathetic nerve firing rates, NE turnover, NADPH production, and lipid turnover. Under different conditions, these acute snapshot indices can be dissociated from each other or from the thermogenic activity that they are believed to measure (eg. Luboshitsky et al, 1983; Henningfield and Swick, 1987; Minokoshi et al, 1988; Ma & Foster, 1989; Sakaguchi et al, 1989). Therefore, they may not be able to reveal actual heat production patterns as well as does the more time-consuming chronic method of direct measurement of brown fat O_2 consumption (Foster & Frydman, 1979).

Measurement of in vivo BAT temperature change is also direct (eg. Perkins et al, 1981), but anesthetic effects may act as a confound (Shimizu & Saito, 1991). Whole body O_2 consumption levels have been used to indicate BAT activity (Rothwell & Stock, 1984; Morimoto et al, 1986), but the method is indirect. Wet

weight is perhaps the least desirable measure, as there is no evidence of a consistent relationship to BAT activity level (from Himms-Hagen, 1990; Trayhurn, 1989).

BAT and Mammalian Energetics:

i) Diet-induced Thermogenesis

Rothwell and Stock followed up an earlier suggestion that brown fat could be largely responsible for the phenomenon of diet-induced thermogenesis (Himms-Hagen & Desautels, 1978; Himms-Hagen, 1979) with a study on the metabolic changes to BAT in cafeteria-fed rats (Rothwell & Stock, 1979). They speculated that the disproportionately low weight gain per gram of food eaten in the voluntarily hyperphagic rats was due to an increase in the facultative thermogenesis of BAT, as the animals maintained on this diet presented evidence of both increased brown fat activity (interscapular skin temperature and NE-stimulated lipolysis) and increased NE-induced O_2 consumption.

The term diet-induced thermogenesis refers to the facultative portion of meal-related energy expenditure (also known as the specific dynamic action of food). This contrasts with the thermic effect of food, which describes the obligatory heat production due to the digestion, absorption, and metabolic processing of fuel. These two food-related components represent one portion of total daily thermogenesis; the obligatory metabolic heat released as an inefficient by-product of all cellular processes makes up a second portion; and finally, the facultative heat production of both shivering and non-shivering

thermogenesis, and of voluntary movement, represents the remainder. Obligatory heat production and heat loss are regulated by thermal demands, but the facultative components appear to be subject to additional regulation by food intake and subsequent energy balance (see Himms-Hagen, 1989a and Gordon, 1990 for reviews).

Rothwell and Stock outlined the similarities between cold- and diet-induced thermogenesis (1980) by comparing metabolic indices of animals that had been either cafeteria fed or exposed to cold. Resting VO_2, was elevated in both conditions, and during cold exposure, cafeteria animals maintained higher rectal temperatures with less shivering than did control animals. Propranolol abolished these changes, which suggests that the facultative increase in heat production in both the overfeeding and cold conditions is sympathetically mediated. Subsequent research has examined the effect of several feeding conditions on BAT activity, from single meals (eg. Glick et al, 1981, 1983, 1984) to fasting (eg. Desautels, 1985; Champigny et al, 1990). Almost all studies report evidence of various propranolol-inhibitable increases in BAT metabolism associated with food intake, including BAT protein content and activity of respiratory enzymes (Brooks et al, 1980; Tulp et al, 1982), GDP-binding (Nedergaard & Cannon, 1984; Yoshida et al 1987), NE-turnover levels, (Young et al, 1982), activity of BAT sympathetic nerves (Niijima, 1986) and the thermogenic response to NE infusion (Rothwell & Stock, 1979).

Research on two other animal models of obesity, experimental

and genetic, has confirmed the occurrence of obesity-related defects in basal BAT metabolism (eg. Seydoux et al, 1981; Triandafillou & Himms-Hagen, 1983; Rohner-Jeanrenaud et al, 1983; Holt & York, 1989). A number of studies have since attempted to determine, by careful design or manipulation, the contribution of BAT to the development of obesity. For example, brown fat activity, as assessed by GDP-binding, was found to be defective in 2 day old genetically obese (fa/fa) rats, well before the onset of visible obesity (Bazin et al, 1984), but Blouquit et al (1992) observed that defective BAT and obesity appeared concurrently.

Kaul et al (1990) raised fa/fa rats in thermoneutral conditions to eliminate a possible role for defective BAT thermogenesis, and found the onset of obesity delayed but not ultimately prevented. Puigserver et al (1992) reported evidence that BAT is functionally altered by the obese condition; however, Levin and coworkers conducted a series of studies on susceptible and resistant rats (e.g. Levin at al, 1983, 1984, 1989), and concluded that altered energy expenditure appears to be a result rather than a cause of the obesity. Cheng et al (1990) found that obesity prone and resistant animals displayed similar VO_2 levels, but the resistant group had a significantly lower respiratory quotient that indicated more fat oxidation; thus, it was concluded that resistant animals compensated for increased fat intake by adjusting the profile of substrate types being oxidized, rather than adjusting energy expenditure through BAT.

Foster's laboratory (1988) performed a critical experimental

step by quantifying BAT blood flow and arterio-venous O_2 differences in response to cafeteria feeding. Their obese rats displayed the typical characteristics of diet-induced thermogenesis, but did not exhibit greater BAT O_2 consumption than control rats. These results contradict the theory that BAT is the main source of diet-induced thermogenesis, and the researchers suggest that the liver makes the major contribution. However, our current understanding of BAT blood flow regulation is poor, as the reports of dissimilar effects of denervation testify. Since it is possible that a cold-induced axon reflex through the sensory nerves is necessary to invoke increased blood flow, Himms-Hagen (1990) has suggested that this mechanism might lie behind the failure to observe either blood flow or O_2 consumption differences with cafeteria-fed rats.

Other researchers have reported a failure to find a change in O_2 consumption with cafeteria diets (Tulp et al, 1982; Armitage et al, 1983); measurement issues related to energy expenditure (Hervey and Tobin (1983) and confounds due to variability in diet composition (Moore, 1987) have been cited as explanations for the lack of findings. Finally, as Himms-Hagen (1990) has stated, the differing effects of standard room temperature and a thermoneutral environment on BAT activity are a critical and often neglected factor.

The effects of complete removal of BAT on energy expenditure and obesity have been difficult to test, whether surgical or chemical methods are employed (eg. Desautels and Dulos, 1991; Rothwell and Stock, 1992). However, a recent paper (Lowell et

al, 1993) reports the creation of novel lines of transgenic mice with markedly decreased UCP levels; the mice consistently develop obesity in the absence of hyperphagia, which fits with the idea of a critical role for brown fat in the development of obesity.

In summary, the phenomenon of sympathetically mediated diet-induced thermogenesis, which manifests as a greater than expected energy expenditure during overfeeding, has been repeatedly confirmed. The weight of research findings strongly suggests a role for BAT in the phenomena of diet-induced thermogenesis, but conclusive evidence is still lacking.

ii) BAT and Thermoregulation

Non-shivering thermoregulation was initially considered to play a quantitatively important role only in hibernating animals, neonates, and cold-exposed animals. However, it now appears that BAT heat production contributes to daily thermoregulation in animals living below thermoneutrality (eg. Brown et al, 1991; Closa et al, 1992), in addition to its facultative role in diet-induced thermogenesis. The circadian regulation of core temperature represents a pivotal issue in the thermoregulatory field, and current research is seeking to define the role of BAT in this daily pattern.

The following sections provide a background on both circadian rhythms in general and the specific regulation of core temperature. Circadian rhythms are defined and CNS control areas are discussed, as are the primary entrainment cues: light and food intake. A brief description of the thermoregulatory aspects

of core temperature and the relationship between core temperature and activity patterns is provided. Finally, there is a discussion of what is known about the mechanisms underlying the circadian rhythm of core temperature, particularly as it relates to brown fat.

Definition of Circadian Cycles: Core temperature and activity levels are the two physiological functions most commonly used to study circadian rhythms. Both oscillate from generally high values in the awake phase, to low values during sleep, and both persist despite isolation from external time cues. Any process that repeats once every 24 h is referred to as daily or nyctohemeral, while a 24 h rhythm that is endogenously generated, such as body temperature or activity level, is further termed circadian (Refinetti & Menaker, 1991). Rats are a nocturnal species, and therefore the active phase occurs during the dark period.

Circadian rhythms are defined by their 24 h period, as well as by amplitude and mean value (termed "mesor"). In rats, core temperature ranges from approximate values of 37.3°C during the day to 38.1°C at night (Gordon, 1990). When the rhythm is free-running, that is in the absence of any lighting schedule or other time cues, the core temperature period is slightly greater than 24 h. However, the period will entrain precisely to any light:dark schedule that cycles at approximately 24h, plus or minus several hours; this moulding of the shape of the rhythm by an external cue is known as entrainment, and will be addressed

later (see page 27).

The free-running period length of core temperature, as for other endogenous cycles, is genetically determined by a CNS pacemaker; thus far, the suprachiasmatic nucleus (SCN) of the hypothalamus is the only region confirmed to function as a circadian oscillator. The key identification studies (from Mistlberger, 1994) come out of the ablation and stimulation work, and the results of unit recordings.

CNS Pacemakers: Ablation of the SCN results in the disruption of circadian core temperature cycles in both constant light and normal light:dark cycles (eg. Moore & Eichler, 1972; Rusak, 1977), and SCN transplant studies demonstrate the opposite process, that is the resumption of cycles of specific periodicity. Multiple and single unit recordings from the SCN reveal circadian variation, and finally, there is a clear phase-shifting effect of both chemical and electrical stimulation of the SCN, similar to the effect of a pulse of light in the dark period.

However, there is evidence of a second pacemaker outside of the SCN. It is known that not all physiological rhythms are controlled by the nucleus; heart rate and follicular development, for example, are regulated by pacemakers with distinct periodicities (Refinnetti & Menaker, 1991). Further, it has been reported that the circadian rhythm of core temperature can persist despite SCN ablation (eg. Fuller et al, 1981; Satinoff & Prosser, 1988). However, this finding may be due simply to

incomplete destruction of the SCN (Eastman et al, 1984; Ruis et al, 1987; Honma et al, 1988). Finally, both core temperature and activity can be affected by an apparent second pacemaker that responds to a cyclic regime of restricted food intake, rather than to photic cues. These studies report that rats display anticipatory behaviour or temperature increase when food availability is restricted to the same daily interval (Bolles et al, 1969; Krieger, 1970; Borer & Clover, 1993). The behaviour disappears within a few days of the resumption of ad-lib feeding, but will reappear without further cues of interval feeding if complete food deprivation is later imposed; this has been observed even with delays of several months of ad-lib feeding (Mistlberger, 1994).

Cues for Circadian Cycles: Both light/dark cycles and restricted food intake are entrainers, or "zeitgebers", that act through neural pacemakers to determine circadian cycles. These two entrainers must be distinguished from other events, such as muscular exertion, sleep habits, food intake schedules, and environmental disruptions (eg. animal care procedures), which are termed "masking agents". While both masking agents and zeitgebers can influence endogenous circadian rhythms, masking is distinguished by the acute, irregular changes that it imposes, in contrast to the rhythm-inducing effect of zeitgebers (for review, see Johnson, 1992; Rietveld et al, 1993). For example, locomotor activity acts as a masking agent because it induces a rise in core temperature regardless of the circadian schedule. As well,

changes in various hormone levels follow food intake; lipogenic enzymes, blood glucose, insulin, and cholesterol all display an apparent circadian cycle, but if food intake is distributed evenly throughout the 24 h period, the rhythms disappear and are therefore considered as simple metabolic responses to food (Cohn & Joseph, 1967; Saito et al, 1989).

Human studies use a "constant routine" protocol to reduce the masking effects of both internal and external cues, but it is still difficult to isolate the purely endogenous component of the oscillatory system from the environmental feedback that acts to modify it (Reitveld et al, 1993). This notion is illustrated in the dilemma of whether to accept the masking effects of sleep periods on observed rhythms, or to impose the masking effect of sleep deprivation. In the case of animal research, the aim is to eliminate light entrainment through constant lighting conditions and to reduce external timing cues through white noise and physical isolation.

Core Temperature Regulation: A number of prominent characteristics of core temperature rhythm should be mentioned. First, only those zeitgebers or cues that fall within range of the endogenous period are able to entrain it; thus, light/dark cycles, for example, must range from approximately 21 to 27 hours in order to influence core. Second, despite its endogenous nature, core temperature rhythm has been shown to disintegrate over weeks or months in constant light, as do other circadian processes. It is speculated that in the absence of a zeitgeber

to act as a "primer", the component oscillators of the circadian pacemaker drift apart (Refinetti & Menaker, 1991). Third, additional cycles, termed ultradian, can be seen within the 24 h period. Rats show core temperature ultradian cycles from 2-8 h (Stupfel & Pervely, 1990; Closa et al, 1993) but these may not be regulated by the circadian oscillators, since they persist after SCN lesions (Eastman & Rechtschaffen, 1983). Finally, the absolute temperature values are regulated by the preoptic hypothalamic area and not the SCN. Lesions of the SCN disrupt solely the period, while preoptic lesions cause an increase in the mean and the amplitude size but no change in rhythmicity.

Relationship between Core Temperature and Activity: Because locomotor activity and core temperature vary together, it is probable either that one is simply a consequence of the other, or else that both are controlled by the same timing mechanism; evidence points to the latter explanation (Refinetti & Menaker, 1991), for the following reasons. Core temperature usually precedes, or is phase-advanced relative to activity, such that temperature begins to rise before the active phase begins (eg. Smolander et al, 1993). Second, while core temperature always correlates with activity, its baseline values are higher at night regardless of activity level (Honma & Hiroshige, 1978; Refinetti, 1994); also, the correlation between the two rhythms are shown to be higher at night (De Castro, 1978). Third, under constant lighting conditions, the circadian cycle of wake/sleep can be shown to disintegrate earlier than that of temperature (Eastman &

Rechtschaffen, 1983). Perhaps the strongest evidence comes from human studies showing that inactive volunteers still display circadian temperature changes (see Refinetti & Menaker, 1991). Overall, research indicate that while activity level can influence, or mask, circadian temperature rhythm, the core temperature cycle cannot be considered a mere consequence of the activity cycle.

Photic vs. Non-Photic Pacemakers: This conclusion, in concert with the results of SCN ablation studies, has led to several hypotheses concerning the organization of CNS oscillators. There may be one CNS oscillator with separate pathways, or several distinct centres, or a hierarchy of multiple oscillators. It is known that SCN neuronal activity will not entrain to restricted feeding schedules, as it will to light/dark cycles, suggesting the existence of a physically separate pacemaker (see Mistlberger, 1994). The results of SCN lesion studies likewise support this hypothesis, as light/dark entrained cycles disappear with SCN destruction, while those induced by restricted feeding persist (Krieger & Hauser, 1977; Stephan, 1986).

The current thinking is that there is at least one non-SCN pacemaker, and the distinguishing characteristic is the response to non-photic input; this contrasts the earlier view that SCN and non-SCN oscillators were distinguished by virtue of which rhythm they regulated, either temperature or activity. Examples of non-photic input or cues include daily exposure to restricted food

intake, restricted novel wheel running, or methamphetamine administration (Hiroshige et al, 1991; Mrosovsky & Janik, 1993). Thus, most work now focuses on revealing the strength of coupling between different pacemakers, and between each pacemaker and each type of physiological rhythm.

Relationship between Core Temparature and Thermoregulation: The final issue that will be addressed here is the thermoregulatory mechanisms underlying the circadian rhythm of core temperature. Initial rat studies assessed the impact of both heat-generating activities and low ambient temperatures on thermoregulatory responses to dark and light, and the data are consistent with the interpretation that core temperature values are constrained to a lower baseline in the day (De Castro, 1978; Refinnetti & Menaker, 1991); it is sugested that this apparent change in set point is independent of behavioural thermoregulatory mechanisms, in view of the fact that rats sleep more during the light hours, and therefore make less use of the most effective heat loss mechanism of saliva spreading.

It is not clear whether a circadian variation in thermoregulatory set point is necessary to account for the two different temperature baselines seen in light and dark periods. If the circadian changes in the levels of heat loss can occur without a permissive change in thermoregulatory set-point, then it should follow that the thermoregulatory mechanisms will oppose the changes. This logic provides a behavioural tool for investigation, as rats should then prefer the ambient temperature

which will help compensate for the change in heat loss. To date, however, the results of numerous studies strongly conflict with each other, such that there is no clear answer. Perhaps the most concrete summary statement is simply a cautionary note, that the concept of set point in mammalian thermoregulation is more complex than the engineering term from which it was borrowed, as a biological set point is influenced by far more factors, both external and internal (Krauchi & Wirz-Justice, 1994).

Circadian Regulation of Core Temperature and BAT: When ambient temperature is stable, any changes in core temperature are the simple result of differences between total metabolic heat production and subsequent heat loss. Thus, periodic variations in heat production and heat loss are thought to underlie the circadian rhythm of body temperature; it is hypothesised that heat loss plays the major role, and that the daily rhythm of heat production is simply a correlate of the activity/feeding rhythm (see Krauchi & Wirz-Justice; 1994; Refinnetti & Menaker, 1991). This area of investigation is most relevant to the thesis, and will therefore be addressed in detail.

Metabolic heat production is usually measured by indirect calorimetry (O_2 consumption is converted to joules/min). In an equilibrium state, heat loss must be equal to heat production and so the terms are sometimes interchanged; but typically the measurement of heat output through direct heat calorimetry is referred to as heat loss, rather than heat production, since changes in core temperature are observed and thus equilibrium is

disturbed.

As described earlier, heat production in rats occurs through both obligatory processes, such as thermic effects of food and of cellular activity, and through facultative events, such as BAT thermogenesis and movement. Heat loss occurs primarily by convection and radiation through the skin, and thus core temperature changes are due mainly to variation in distal blood flow. In rats, the tail, feet, and distal portions of the limbs are well vascularized with arterial anastomoses (Gordon, 1991).

Fuller et al (1985) examined the circadian rhythms of 0_2 consumption, body temperature, and heat loss. If metabolic heat production were the sole driving force in the nightly increase in CT, then 0_2 consumption should show a strongly circadian pattern. The averaged pattern was weakly circadian, but the pattern between individuals was highly variable and in fact was clearly not circadian in some cases. Rather, it appeared that heat loss, as measured through various distal temperature changes, showed a strong circadian rhythm.

Further work from Shido and coworkers (1986) have shown that the nightly increase in core temperature in rats may be the results of a nightly change in the slope between body temperature and heat loss response; this suggests both that the nightly temperature increase is a regulated change and that a nightly alteration in the heat loss effectors is involved. Shido (1987) also showed that there is a nightly increase in the threshold body temperature required to evoke tail vasodilation. The flatter slope describing heat loss response at night is believed

to reflect primarily a higher underlying vasomotor skin tone; thus, the circadian rhythm of the autonomic nervous system may be a major factor underlying the circadian changes in body temperature.

In fact, a significant circadian rhythm has been demonstrated for distal skin temperature, and thus distal blood flow, in humans (Krauchi & Wirz-Justice, 1994). The pattern is found to be phase advanced by 25-100 min with respect to body temperature, which implies active regulation of core by distal heat loss. Distal sites such as the hands and feet are known to be rich in arteriovenous anastomoses; these can be constricted by the autonomic system to cause passive blood flow elevation to proximal areas, and thus regulate CT.

Direct measurement of distal blood flow by laser-doppler flowmetry confirms the presence of a circadian rhythm in humans, and further demonstrates that the distal temperature pattern precedes the core pattern, in this case by about 240 minutes or 4 h (Smolander et al, 1993); the two patterns thus appear to be roughly the reverse of each other. The authors hypothesize that the distal circulation reflects a circadian rhythm of catecholamines; it is likely that passive vasodilation of arteriovenous anastamoses is responsible for the nightly increase in distal blood flow (when autonomic tone is low in humans) and this would correlate with the nightly decrease in plasma NE and epinephrine.

Circadian Rhythm of BAT and Core Temperatures: A similar role for hemodynamic changes in the circadian rhythm of core temperature has been found in rats (Gomez-Sierra et al, 1993), although ultradian cycles also appear to contribute to the pattern. The patterns of aortic core temperature and of heat output were analyzed, and both showed a circadian pattern, as well as ultradian cycles of 8, 5, 4 and 2 h. However, the different ultradian cycles varied in the strength of their influence on the shape of each of the two patterns. For example, the 2 h rhythm was found to play only a minor role in core temperature, but appears instead as an important component of heat output, and possibly of autonomic tone. There was a 30 min delay in the daily heat output pattern compared to that of aortic core temperature; the authors suggest that aortic temperature reflects core (essentially organ) heat generation, and that this is later released with 30 min delay.

Another study by the same group (Closa at al, 1993) found strong agreement between BAT and core temperature patterns, although this conclusion was based on a simple sign test. Analysis of BAT blood flow specifically during the warming portions of the 2 h cycles in core temperature found a 200% increase in blood flow over basal levels, which suggests a role for BAT in temperature regulation.

In an extension of this idea, Himms-Hagen (1995) has proposed a critical role for BAT in both the initiation, and termination of periodic meal eating; the mechanism is based on the presence of regular, ultradian drops in core temperature which are

postulated to trigger BAT thermogenesis. The consequent enhanced consumption of glucose by the tissue causes an acute drop in blood glucose, which in turn represents the signal for food consumption. The enhanced BAT heat production may also contribute to satiation, by increasing body temperature to the threshold value associated with the termination of eating.

Given these research pursuits, it is clear that the daily pattern of brown fat heat production may have powerful implications for a number of metabolic processes.

OBJECTIVES

Brown fat thermogenesis is believed to exert a strong influence on two energy regulation systems, thermal homeostasis and intake/expenditure. Examination of the neural circuitry that regulates BAT activity, and the innervation and vasculature of the tissue forms the backbone of investigation into BAT functioning, in concert with studies on the thermogenic response to cold and diet. Although the significance of brown fat metabolism is at present better understood in terms of thermoregulation than diet, the precise nature of BAT contribution to daily core temperature rhythm is not well researched. Given that the overall aim of the thesis is to enhance understanding of the physiological role of BAT heat production in thermal processes, the objectives of the three individual experiments were twofold: the first two studies addressed the neural control of acute changes in BAT temperature, while the third evaluated the daily pattern of BAT temperature changes.

One of the primary routes of investigation into the neural control of BAT tissue involves the assessment of tissue response after stimulation of the hypothalamus and other CNS regions. The results of such studies have led to general agreement that the VMH is the main site of BAT thermogenic control (Shimazu and Takahashi, 1980; Seydoux et al, 1981; Perkins et al, 1981; Iwai et al, 1987), but the complexity of afferent and efferent VMH connections demands further delineation of the effective areas within the nucleus. As the findings thus far on the nature of

sites within the VMH are conflicting (Perkins et al, 1981; Freeman & Wellman, 1987; Holt et al, 1987; Hugie et al, 1992), the purpose of the first study was to explore the VMH area for brown fat responses with more precision than have previous studies. To achieve that, we used moveable electrodes (Miliaressis & Gratton, 1981) to finely map the location of sites which evoke a BAT temperature increase.

A presentation of the pattern of effective sites represents one step towards the characterization of the neural substrate underlying BAT regulation, along with studies that investigate whether the tissue response involves activation of cell bodies that originate in the VMH or fibres of passage through the nucleus (Amir, 1990), assess the relationship between stimulation parameters and magnitude of response (Flaim et al, 1976; Thornhill & Halvorson, 1992; Woods and Stock, 1994), or provide a neuroanatomical description of hypothalamic pathways (eg. Luiten et al, 1987; Amir et al, 1989; Mitsushimi et al, 1994).

In the course of this study we discovered extra-VMH sites that induced an acute drop in BAT temperature, a finding which has received little attention in the literature. Thus, in the second study, we evaluated structures that evoked a drop in BAT temperature and investigated possible circulatory mechanisms underlying the temperature decrease through the application of adrenoceptor blockers.

A physiological context is necessary in order to learn the significance of sites that induce abrupt drops in BAT temperature. The research suggests that decreased sympathetic

input mediates other reported drops, whether due to signals from baroreceptors (Shibata, 1982), or thermoreceptors (Hinckel et al, 1983), or CNS infusions of neuropeptide Y (Szreder et al, 1994). In one study, the drop in BAT temperature was accompanied by additional thermoregulatory changes, and similarly, we report that a drop in core temperature paralleled the induced brown fat response. This finding, when considered with studies demonstrating BAT thermogenesis in response to cold stimulation (eg. Foster & Frydman, 1978; York et al, 1985; Ricquier et al, 1986), led to the supposition that there are daily oscillations in BAT heat output, presumably driven by cycles in sympathetic input.

In view of the well-documented presence of circadian and ultradian oscillations in core temperature (see Gordon, 1991), and the demonstration in the first study that increases in BAT temperature can influence core temperature, the objective of the third study was to investigate the diurnal pattern of BAT temperature change as a potential contributor to core temperature patterns (Closa et al, 1993). Rats were implanted with radio-frequency transmitters to first, determine if there was a circadian rhythm underlying daily BAT temperature changes and second, to investigate the presence of any consistent ultradian rhythms. In a subset of subjects, intraperitoneal temperatures were simultaneously monitored in order to explore the relationship between BAT and core temperature patterns.

EXPERIMENT 1

Regulation of feeding behaviour, metabolic rates, and body temperature have all been linked to the hypothalamus; the VMH in particular may be involved in each of these areas of energy regulation through its control of BAT thermogenesis. The results of stimulation and lesion studies have confirmed the role of the VMH in the sympathetic control of brown fat metabolism (eg. Shimazu and Takahashi, 1980; Seydoux et al 1981; Hogan et al, 1982; Iwai et al, 1987). In order to further investigate the contribution of this BAT-VMH connection to the altered energy regulation associated with diet-induced thermogenesis, Rothwell & Stock (Perkins et al, 1981) stimulated the VMH in anaesthetized rats, and directly recorded temperature changes in interscapular BAT. This simple design has the advantage of measuring acute, continuous BAT response within the animal; the report was among the first to confirm both a functional connectivity between the VMH and BAT and the inability of the lateral hypothalamus to elicit a BAT response, which were initially demonstrated by Shimazu & Takahashi in 1980.

The design was later employed to investigate the contribution of other CNS regions to BAT regulation; however, there have been conflicting results with regards to the response of BAT to VMH stimulation. For example, Freeman & Wellman (1987) observed little change in BAT temperature following electrical stimulation of the VMH, reporting an average rise of 0.25°C across animals compared to the 0.8°C value reported by Perkins et al (1981). In contrast, Holt et al (1987) and Thornhill and Halvorson (1990)

did observe increases in BAT temperature as a consequence of VMH stimulation, which grew progressively larger with increasing current (Holt et al, 1987).

The discrepancy surrounding the precise role of the VMH in regulating BAT activity is illustrated in the finding of Hogan and coworkers (1982); only VMH lesions which had invaded the paraventricular nucleus were effective in decreasing BAT activity. Thornhill suggests that cold acclimation, age, and strain differences can account for some conflicting results (Thornhill and Halvorson, 1990; Hugie et al, 1992).

Effective sites have also been found in the preoptic anterior and posterior hypothalamic areas, and the paraventricular nucleus, while the dorsomedial hypothalamus has been reported as both effective and ineffective in eliciting BAT response (Perkins et al, 1981; Holt et al, 1987; Freeman & Wellman, 1987; Amir, 1990).

By adapting the stimulation paradigm to include moveable electrodes, we sought to finely map the region within the VMH nucleus which affect brown fat thermogenesis. The small controlled changes in electrode position coupled with low currents permits a degree of anatomical resolution that is not possible with fixed electrodes in different animals.

METHOD

Seventeen male hooded rats (Charles River, Quebec), weighing 305 to 400 g at the time of surgery, were housed individually at 19-23°C and placed on a 12 hour light/12 hour dark cycle with

lights on at 0600 h. Chip bedding was used in the clear plastic cages, and standard lab chow and water were freely available. Surgeries were always begun between 0800 and 1000 h, and conducted at housing temperature range.

The animals were anaesthetized with sodium pentabarbitol (Somnotol, 65 mg/kg, i.p.) and rompun (Xylazine, 3mg/kg, i.m.). They were supplemented roughly every 1.5 h with 20 mg/kg i.p. of Somnotol, or exposed to the inhalant anesthetic methoxyflurane (Metofane), in order to maintain deep anesthesia throughout the stimulation session; if the sessions were longer than about five hours, a second injection of rhompun was given. We have found this regime sufficient to support stable respiration and muscle atonus. Core temperatures were monitored with a rectal thermometer, and ranged from 34.5 to 37°C, values comparable to those reported elsewhere for anaesthetized rats (Refinetti & Carlisle, 1988). A thermocouple thermoprobe (Harvard Scientific Ltd) was placed between the pad of the shoulder muscle and the interscapular BAT deposit to monitor BAT temperature.

Using standard stereotaxic procedures, a single moveable nonrotating electrode (Kinetrods Reg'd) aimed at the VMH was implanted in each rat; the flat-skull coordinates, based on the Paxinos and Watson (1986) atlas, were as follows: 1.2 - 2.8 mm posterior to bregma, 1.5 mm lateral to the midsagittal suture, and 7.5 - 8.5 mm below dura. The electrodes were manufactured from short lengths of stainless steel wire, 250 µ in diameter, and insulated with Formvar to their semi-spherical polished tips. Four to ten sites, spaced 160 µ apart, were stimulated per

animal. A manipulator calibrated in 80 µ steps was used to advance the electrode. We did not begin stimulation sessions until BAT temperature was stable for fifteen minutes; stability was defined as fluctuations no larger than 0.1°C, based on observation of BAT temperatures in non-implanted anaesthetised animals.

The 500 ms stimulation trains comprised 25 x 300 µA square wave cathodal pulses of 100 µs duration, and were delivered for trials of 60 s. The total charge was therefore equal to 1.5 µcoulombs. Stimulation was provided by constant-current stimulators (Mundl, 1980) and in-house manufactured pulse generators; between pulses, the electrode was grounded through a low resistance path to prevent charge accumulation at the tip. The stimulation parameters were constantly monitored on an oscilloscope by reading the voltage drop across a 1 KΩ precision resistor in series with the rat.

Brown fat temperature was monitored continuously from the initial delivery of stimulation through any subsequent rise and return toward baseline levels, until the value had either achieved pre-stimulation status, or remained stable (±0.1°C) for 20 to 30 minutes; this procedure was followed even if the temperature had not returned to baseline values. We defined a temperature rise as a change of at least 0.3°C; this criterion was based on prior observation of BAT temperature changes in anaesthetized animals. Stimulation sessions lasted from 3 to 7 h, and if prolonged, rats were given subcutaneous injections of Ringers solution to prevent dehydration. If no temperature

change was observed for 10 minutes following stimulation, that site was considered ineffective, the electrode lowered 0.16 mm to the next site, and the stimulation trial repeated.

In approximately a third of the animals with electrode placements effective in eliciting an increase in BAT temperature, the stimulation trials were repeated in order to confirm the effectiveness of the site. Following the second trial, the electrode was lowered and the standard procedure described earlier applied. If BAT temperature did not change at any of the sites sampled, that animal was injected i.p. with NE, and BAT and core temperature monitored. This procedure was followed to ensure that BAT was functional and that the probe was properly placed; thus, if BAT temperature failed to rise at this point, the animal was removed from the study.

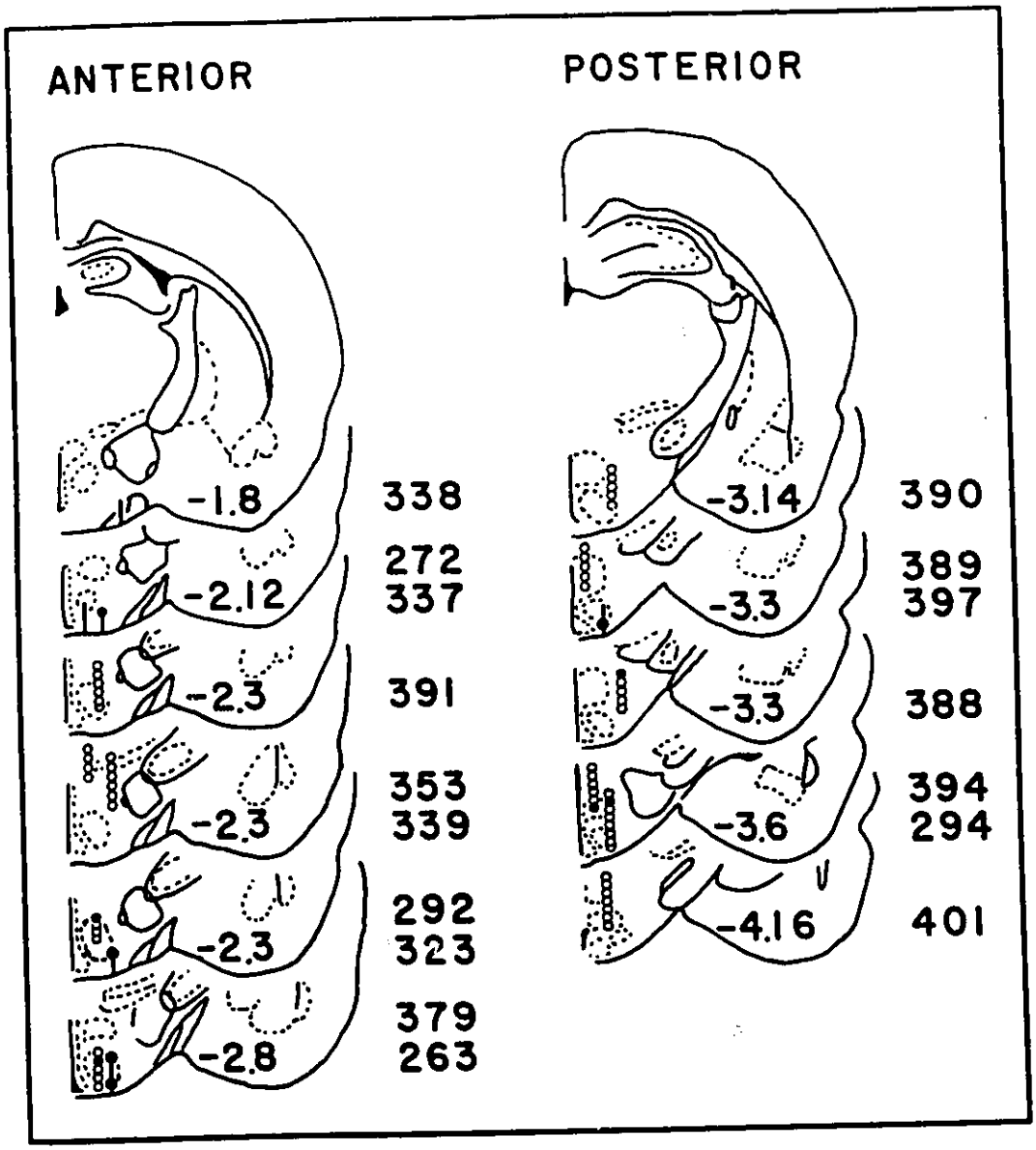
At the end of all stimulation trials, each rat was injected i.p. with a lethal dose of sodium pentobarbitol and perfused with buffered formalin. The brains were frozen, sectioned at 40 µ, and stained with cresyl violet to reveal cell nuclei.

RESULTS

Of the seventeen implanted electrodes, seven penetrated the VMH, three were located immediately adjacent to it (rats 323, 337, 390) and seven were located within 2 mm of the VMH, in surrounding nuclei. Figure 1 details the histologically verified locations of all electrode placements; filled circles represent those sites which were effective in stimulating a brown fat temperature rise ("active" sites), while either blank circles or

Figure Caption

Figure 1: Tracings from the atlas plates (Paxinos and Watson, 1986) that best correspond to sections judged to contain the electrode tips. The seventeen electrode placements are indicated by either a series of circles, or a straight line. The former refers to those electrode tracks for which the tip was clearly identified, while the lines are used in the case of tracks where the tip appeared to penetrate the base of the brain and therefore the position had to be estimated. The active sites, those for which a BAT response was observed after stimulation, are illustrated with a filled circle; these represent both confirmed and estimated positions. Inactive sites are indicated either by the unfilled circles, or straight lines. The numbers on the sections refer to the anteroposterior level (mm distance behind bregma); subjects are identified on the right of each section, those with medial placements listed first.


ANTERIOR
POSTERIOR
-1.8
338
-2.12
272
337
-2.3
391
-2.3
353
339
-2.3
292
323
-2.8
379
263
-3.14
390
-3.3
389
397
-3.3
388
-3.6
394
294
-4.16
401

straight lines represent sites where no BAT response was recorded after stimulation. Note that in the case of the electrode track associated with rat 294 the dorsal sites were located distal to the VMH, while more ventral ones penetrated the nucleus.

Of the ten series of sites that either penetrated or were proximal to the VMH, six yielded at least one site each that induced an increase in BAT temperature; in one series (rat 263), there were two adjacent active sites. In contrast, only three of the seven series of sites located distal to the nucleus yielded an active site, and in fact two of these were found in one region, the dorsal medial hypothalamus. Exact sites could not be determined in the case of six electrode tracks, shown as a line in Figure 1, because the tips had penetrated the base of the brain. The position of any active sites from these electrode penetrations was estimated from the number of site stimulated, and the relative position of the active site in the series (eg. first of six sites). It is also important to note that in no instance could the unvarnished portion of the electrode tip have completely emerged from the brain tissue into fluid, because the large drop in resistance would have shown up on the oscilloscope as a striking change in the shape of the stimulating pulse.

Effective Sites in the VMH: Five of the six individual effective sites found in the VMH (rats 292, 323, 263, 379) were clustered at the mid-anterior level. According to the Paxinos & Watson atlas (1986), these sites are roughly 2.3 and 2.8 mm behind bregma, at a level just caudal to the initial appearance

of the zona incerta.

In one of the these tracks (rat 263) there were two non-consecutive effective sites, the second and sixth of eight. Although the electrode tip of this track penetrated the base of the brain, the responses were presumably due to activation of the VMH and not cerebrospinal fluid, otherwise sites subsequent to the sixth one would have shown a temperature response as well. Furthermore, resistance levels appeared to be stable for all eight stimulation trials.

The active site of track 292 was verified to be at the dorsal border of the mid-anterior region; however, stimulation of what appeared to be the same area in another rat (391) did not alter BAT temperatures. Finally, one active VMH site (397) was posterior to the others, 3.3 mm behind bregma (Paxinos & Watson, 1986).

Non-VMH sites: Outside the VMH, three of seven tracks showed sites that gave rise to a BAT temperature increase; two tracks were found in the dorsomedial nucleus (394, 294), and the third a little more lateral in the perifornical nucleus (388). Note that two other tracks, 389 and 390, also penetrated the dorsomedial hypothalamus, but were not effective in producing stimulation-induced change in BAT temperature. The remaining two tracks, which were located dorsal to the VMH (339, 353), showed no active sites, but several of the sites in 353 gave rise to striking increases in core temperature with no corresponding change in BAT temperature.

Temperature Profiles: The typical stimulation-induced pattern showed BAT temperature rising from 0.6 to 4.6°C above baseline, and taking from 1 to 2 hours to return. In contrast, the two active sites in track 263 and the single site in 379 yielded a different profile, with a return to baseline values within 30 minutes, and a maximum rise of 0.6°C; Figure 2 illustrates the difference between the two observed patterns. Unlike previous reports of BAT temperature change (e.g. Perkins et al, 1981), we saw no evidence of biphasic patterns.

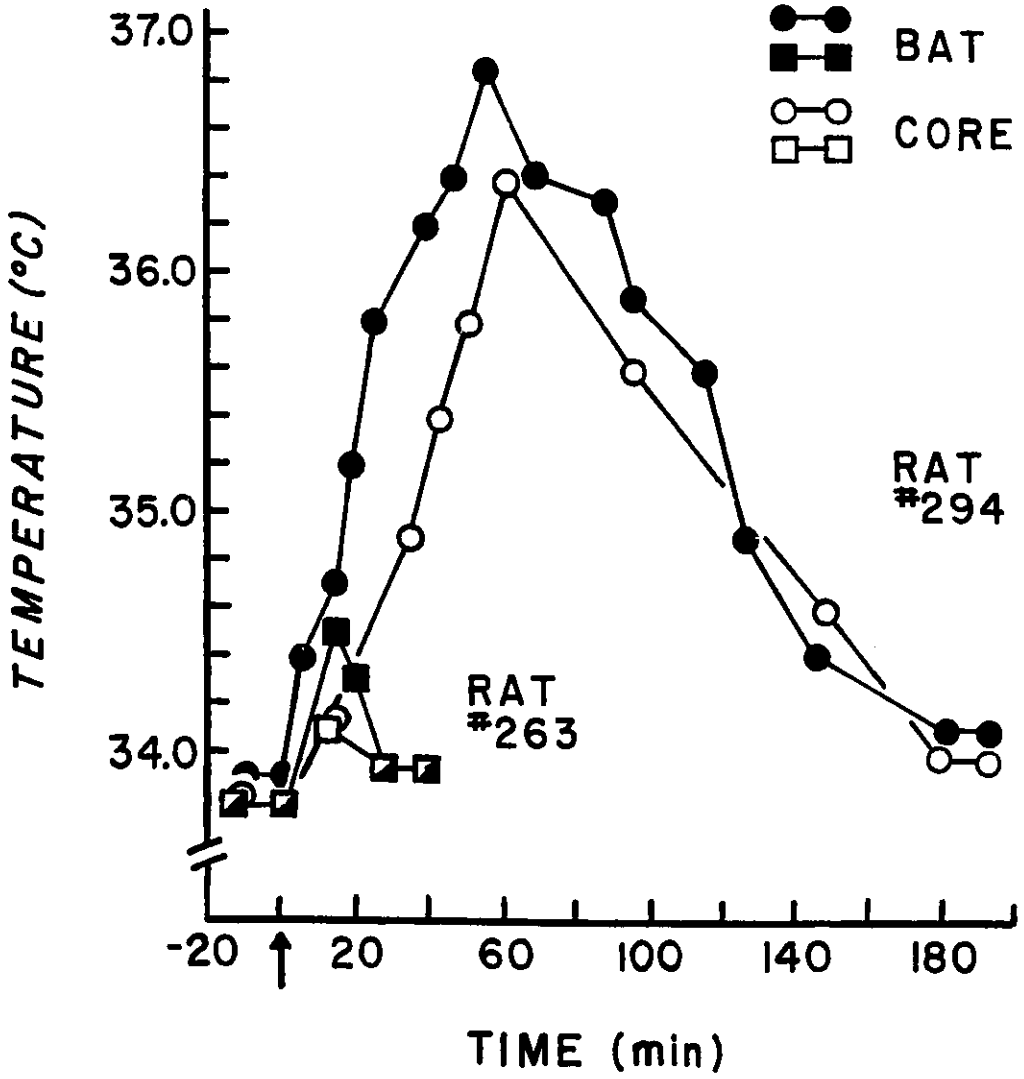
DISCUSSION

The results indicate that the VMH nucleus does give rise to BAT thermogenesis when electrically stimulated, particularly in the mid-anterior regions; this is consistent with the view that the VMH is involved in the central control of BAT. However, this is the first study to stimulate in multiple sites within one animal, and the finding of a number of non-active VMH sites suggests that not all regions of the nucleus participate in the regulation of BAT heat production. Other work from this laboratory corroborates this conclusion; Stenger & Bielajew, (1992) found that only one of 13 VMH sites elicited a BAT temperature increase. The results of this study provide a commentary on the importance of electrode type in mapping studies: the authors used fixed electrodes due to the nature of the study, and like Freeman & Wellman (1987), might well have concluded that the VMH plays little role in BAT thermogenesis.

Figure Caption

Figure 2: An illustration of two BAT responses that were elicited by stimulation of two different hypothalamic sites. The filled symbols in the profiles refer to BAT temperature, while the unfilled ones represent core temperature changes. The arrow indicates the point at which the one minute stimulation trial was administered.

The abbreviated temperature profile (263) corresponds to a stimulation site at the mid-anterior VMH, laterally positioned, and estimated to be near the ventral border; it was the sixth stimulated site, and the second active site in the track. The enhanced temperature rise (294) corresponds to an active site in the dorsomedial hypothalamus; it was the second of eight tested sites.


37.0
36.0
35.0
34.0
TEMPERATURE (°C)
BAT
CORE
RAT
#294
RAT
#263
-20
20
60
100
140
180
TIME (min)

Issues Related to Acute Electrical Stimulation: The experimental design used here, that of acute stimulation of anaesthetized rats, is a well-established paradigm in brown fat research but is encumbered by several contentious issues. Perhaps the most problematic is the influence of the anaesthetic on normal BAT functioning. As has been noted (Himms-Hagen, 1991), anaesthetics may cause core temperature to fall, and thus secondarily invoke BAT thermogenesis, unless the anaesthetic directly suppresses physiological BAT responses (eg. Shimizu & Saito, 1991).

It may not be necessary that the paradigm strictly reflect physiological conditions in order to provide information; the stimulation procedure can clearly reveal distinctive effective regions in the neural tissue, particularly when results from a number of studies are assessed. However, the resultant pattern may be incomplete if brown fat thermogenesis is inhibited. Examining the BAT response in conscious animals represents one alternative, but the VMH also activates a number of additional metabolic changes, such as increased blood glucose, that may in turn affect the BAT response (Himms-Hagen, 1990).

Another concern is the degree of current spread from the electrode tip. Ranck (1975) has reviewed the literature on current intensity and effective excitation, and shown that the radius of excitation, as a function of current expressed in logarithm units, is a straight line with a slope of 0.5. Using his chart, and correcting for pulse duration, we estimate that a 300 µA current of 100 µsec duration will have a radius of

excitation of 300 μ for neurons with conduction velocities greater than 10 m/sec. Since it is likely that the neurons of interest in this report conduct more slowly, probably in the 2 -5 m/sec range, the radius of excitation is in fact expected to be less, and thus appears to be in line with the 0.16 mm moves that were used with the moveable electrodes. In other words, the effective stimulation fields associated with adjacent sites here likely had little overlap.

Finally, there is the issue of the stimulation parameter values. Our frequency was similar to that used in other studies, but the current value was three times that reported by Freeman & Wellman (1986), and six times that used by Perkins et al, 1981. In contrast, the pulse duration that we employed was one fifth that used by either group; our preference is to use shorter pulse durations in order to avoid the possibility of repetitive firing induced by prolonged depolarization (see Matthews, 1977).

Consequently, in order to compare our level of stimulation with others, we calculated the total charge delivered with each trial, the product of frequency x current x duration. We obtained a value of 1.5 μcoloumbs, which is equivalent to the smallest charge values used in other studies (Perkins et al, 1981; Freeman & Wellman, 1987; Halvorson et al, 1990). Parenthetically, the wide range in the values of the reported parameters suggests that little attention has been paid, in the past, to the impact of each stimulation parameter.

Heterogenous VMH Substrates: The observation of both effective and ineffective sites in the VMH, along with the differences in BAT temperature profiles, suggests that VMH elements are functionally heterogeneous. It appears that both medial and lateral aspects of the nucleus can activate BAT, in contrast to previous reports which have implicated the lateral areas as either the most sensitive or even the only active areas (Perkins et al, 1981; Freeman & Wellman, 1987). The finding that there are active sites in the mid-VMH at different ventral planes further suggests that functional regions regulating BAT activity may be confined to discrete areas, and duplicated within the nucleus.

The observation of differing temperature profiles may be interpreted in several ways. The reported range in core temperatures suggests that various degrees of thermoregulatory responses were invoked in the anaesthetized animals; thus, a BAT thermogenic response may have indirectly been invoked. However, BAT temperature was observed to be stable prior to every stimulation trial, and the changes in the tissue temperature all occurred immediately after stimulation. Further, there was no experimental irregularity to account for the different profiles, as ambient temperature and stabilization times were similar; most importantly, initial body temperature was not correlated with subsequent BAT response (see Figure 2 for an illustration).

The metabolic response of brown fat has been assessed through BAT norepinephrine turnover (eg. Young et al, 1982), sympathetic nerve activity (Egawa et al, 1991), as well as the levels of

either mitochondrial GDP-binding (Billington et al, 1991) or uncoupling protein mRNA (Champigny & Ricquier, 1990). There are specific advantages and disadvantages associated with each index; here we measured changes in BAT heat production directly with a thermocouple thermoprobe, emulating a number of other researchers (eg. Hinckel et al, 1983; Freeman and Wellman, 1987; Woods and Stock, 1994). Although the values of brown fat temperature changes may not be tightly correlated with absolute levels of BAT thermogenic response, due partially to the confound of local blood flow, this paradigm, which was first applied to the study of brown fat by Perkins et al (1981), has the important advantage over biochemical measurements that it reveals real-time temporal patterns in acute BAT response to stimulation.

Given that the variety of temperature profiles obtained in this study reflect differing signals from the VMH, it suggests the notion of a heterogenous range of VMH mechanisms for brown fat activation. In line with this idea, neurally-induced lipogenesis and hypertrophy of BAT can be dissociated from BAT thermogenesis, implying that several different neural mechanisms may regulate BAT activity (Glick, 1984; Himms-Hagen, 1985). It would be interesting to pursue this investigation by determining if those VMH sites associated with no increase in BAT temperature were instead activating brown fat in a different way, one that might be biochemically detectable. Finally, the role of stimulation parameters is also relevant in the cases of the non-responsive sites; similar to most other papers, we used only one level of stimulation, which was in the general range of

previously reported levels. Although it is likely misleading to define a CNS region in absolute terms without assessing the effect of several stimulation parameteres, such a systematic experimental design represents an entirely new and rich direction, and is beyond the focus of this work.

Range of VMH Signals: This apparent heterogeneity of VMH effects can be further seen with cold, electrical or dietary stimulation, as each may give rise to different combinations of peripheral and brown fat responses. Electrical stimulation of the VMH invokes sympathetic reactions similar to those elicited by cold stress, including increased blood glucose levels, increased cardiac output, and BAT thermogenesis (Frohman, 1980; Shimazu, 1981; Foster & Frydman, 1979; Morimoto et al, 1986). In contrast, glucose injections directly into the VMH are followed by BAT thermogenic activity, but the peripheral metabolic responses are opposite to those seen with cold and electrical stimulation (Frohman, 1980; Sakaguchi & Bray, 1987; Sakaguchi & Yamazaki, 1988). Finally, the effect of VMH stimulation by 2-deoxy-glucose is different again; peripheral responses are similar to those of cold stress, but the decreased binding of GDP to interscapular BAT mitochondria suggests reduced thermogenesis (Le Magnen, 1985; Storlien et al, 1985; Arase et al, 1987).

This variety in the pattern of peripheral response to VMH signals, possibly modulated by humoral factors, illustrates the range of VMH signals which is necessary to elicit adaptive metabolic responses in the face of diverse energy demands. The

diversity in signal types is also reflected in the complexity of the anatomical organization of the nucleus; H^3 leucine injections into the VMH have been used to trace fibre bundles to their major descending target, the periaquaductal grey area. From there, substantial projections can be detected to centres in the brainstem and spinal cord that regulate the autonomic system, namely the ventromedial reticular formation for the sympathetic system, and the nucleus ambiguus for the parasympathetic one (see Luiten et al, 1986 for review). The fact that the hypothalamic region in general is known to exert its influence through both the motor and neuroendocrine systems further supports the presence of a potentially vast range of signals from the VMH, as does the position of the nucleus as a centre of integration.

The intrahypothalamic connections of the VMH are also of interest here. We report, as do Freeman & Wellman (1986), a substantial contribution to BAT thermogenesis from the medial dorsal hypothalamus. Indeed, the reciprocal connections between that nucleus and the VMH perhaps represent the most significant intrahypothalamic pathway for the VMH, in conspicuous contrast to the regions of the lateral and the paraventricular hypothalamus.

Conclusion: A number of mechanisms might underlie both the observed mosaic of effective and ineffective sites in the VMH, and the apparent variation in signals that can be generated by the nucleus. For example, we hypothesised that VMH activity may be regulated by input from adjoining nuclei, particularly the temperature-sensitive preoptic and anterior hypothalamic areas,

since it has been shown that both stimulation and cooling of the areas evoke BAT responses (Nakayami et al, 1981; Hugie et al, 1992). The application of an anaesthetic to the VMH appears to interrupt the BAT response, as has been reported for a number of other areas, including the SCN (Amir et al, 1989). The acute stimulation paradigm could be exploited to investigate the neural circuitry behind this apparent relay; for example, it would be interesting to evaluate the effect of VMH destruction on the influence of effective sites outside the nucleus. Assessment of the extent of changes in functional connectivity would enrich the current anatomical descriptions of VMH pathways.

The results of a pilot study from this laboratory which showed that a doubling of the stimulation frequency caused previously non-responsive sites to elicit BAT activity suggest a complementary mechanism, one that involves a change in the critical threshold required to activate the neuronal substrate. This idea has been proposed by Preston et al (1988), who were seeking an explanation for observed BAT responses to a strong cold stimuli following VMH destruction. Thus, systematic alteration of the stimulation parameters could assist in identifying these neuronal properties, as well as potentially opening the doors to physical characterization of the VMH neurons through the application of parametric techniques (Gallistel et al, 1981).

Very recently, several groups have explored this avenue and report the change in brown fat temperature response to alteration in the values of the stimulation parameters. Woods and Stock

(1994) applied low intensity, long duration pulses to the VMH, and elicited a sustained drop in BAT temperature; they suggested that the low level stimulation was dissociating the thermogenic response of the parenchyma from the reported vascular changes. Another group (Dib et al, 1994) scaled frequency, intensity and intertrain interval. The authors report a monotonic relationship, in that the magnitude of the BAT temperature response grew with increasing levels of stimulation.

EXPERIMENT 2

Brown fat thermogenesis was first reported as a response to cold exposure (eg. Foster & Frydman, 1979), and subsequent stimulation studies indicated that the VMH is the primary CNS control region for brown fat activation (Shimazu and Takahashi, 1980). Effective sites have since been located in a number of other CNS regions, including the preoptic and dorsomedial hypothalamus, the paraventricular nucleus, and the SCN.

Increases in BAT activity have also been attributed to overfeeding on cafeteria diets (Rothwell & Stock, 1979), low protein or high fat diets (Glick et al, 1981; Gong et al, 1990), and cooling of the temperature-sensitive preoptic hypothalamus (Imai-Matsumura & Nakayama, 1986) or cutaneous thermal receptors (Kurosawa, 1991). The fact that BAT thermogenesis appears to be activated by a variety of manipulations suggests that BAT metabolism is regulated in relation to both thermal and energy balance (Himms-Hagen, 1989).

The results of the first experiment clearly support the idea that stimulation-induced BAT thermogenesis can influence core temperature, but in the course of this work, we observed a small number of sites in extra-VMH regions that were associated with a drop in brown fat temperature. This BAT response does not appear to be well characterized, either in terms of CNS control areas, or underlying mechanisms. The purpose of this study was to precisely map the sites which elict abrupt decreases in BAT temperature; to that end, moveable electrodes were aimed at the ventral lateral thalamic nucleus (VLT) and the zona incerta (ZI.

METHOD

Fifty five male hooded rats of the Long-Evans strain (Charles River, Quebec), weighing 305 to 420 g at the time of surgery, were housed individually at 20-23°C and placed on a 12 hour light/12 hour dark cycle with light onset at 0700 h. Standard lab chow and water were freely available. Surgeries were always begun between 0800 and 1000 h, and conducted at housing temperature range.

Both the surgical and stimulation procedures were identical to those of Experiment 1, with the following modifications:
1) Core temperature was monitored and held at 36.6 - 37.0°C by means of a homeothermic blanket unit (Harvard Scientific Ltd.). In some cases where a BAT drop response was elicited, core temperature was observed to drop below this level by 0.1 to 0.3°C. We defined a temperature drop as a change of at least -0.2°C.
2) Moveable electrodes (Kinetrods, Reg'd) were aimed at either the ZI or the VLT in each rat. The flat-skull coordinates for the ZI were as follows: 2.12 - 3.3 posterior to bregma, 1.5 mm lateral to the mid-saggital suture, and about 7.5 mm below the dura; those for the VLT placement were: 1.8 - 2.8 mm posterior to bregma, 1.5 mm lateral to the midline, and 4.5 - 6.5 mm below the dura (based on the atlas of Paxinos and Watson, 1986)
3) In some animals (for 73 sites of 401), brown fat temperature values were precisely recorded for a 10 minute period prior to stimulation, in order to detect fluctuations in BAT temperature unrelated to the stimulation.

4) In a subset of the 26 VLT rats, effective sites were re-stimulated after the temperature returned to baseline levels, to determine if the drop in BAT temperature was reproducible. Of these animals, seven were treated to an additional procedure in which the site associated with a temperature drop was stimulated three times in total: a second time to replicate the temperature effect, and a third time to observe the BAT response following an injection of either propranolol (a β-noradrenergic blocker, 20 mg/kg i.p.) or phentolamine (an α-adrenergic blocker, 10mg/kg i.p.); the injections were given 15 min. prior to stimulation. The blockers were used to assess whether the SNS is involved in the mechanism of the BAT temperature drop. No further sites were tested in an animal after application of the blocker.

Sacrifice and histology procedures were identical to those of Experiment 1.

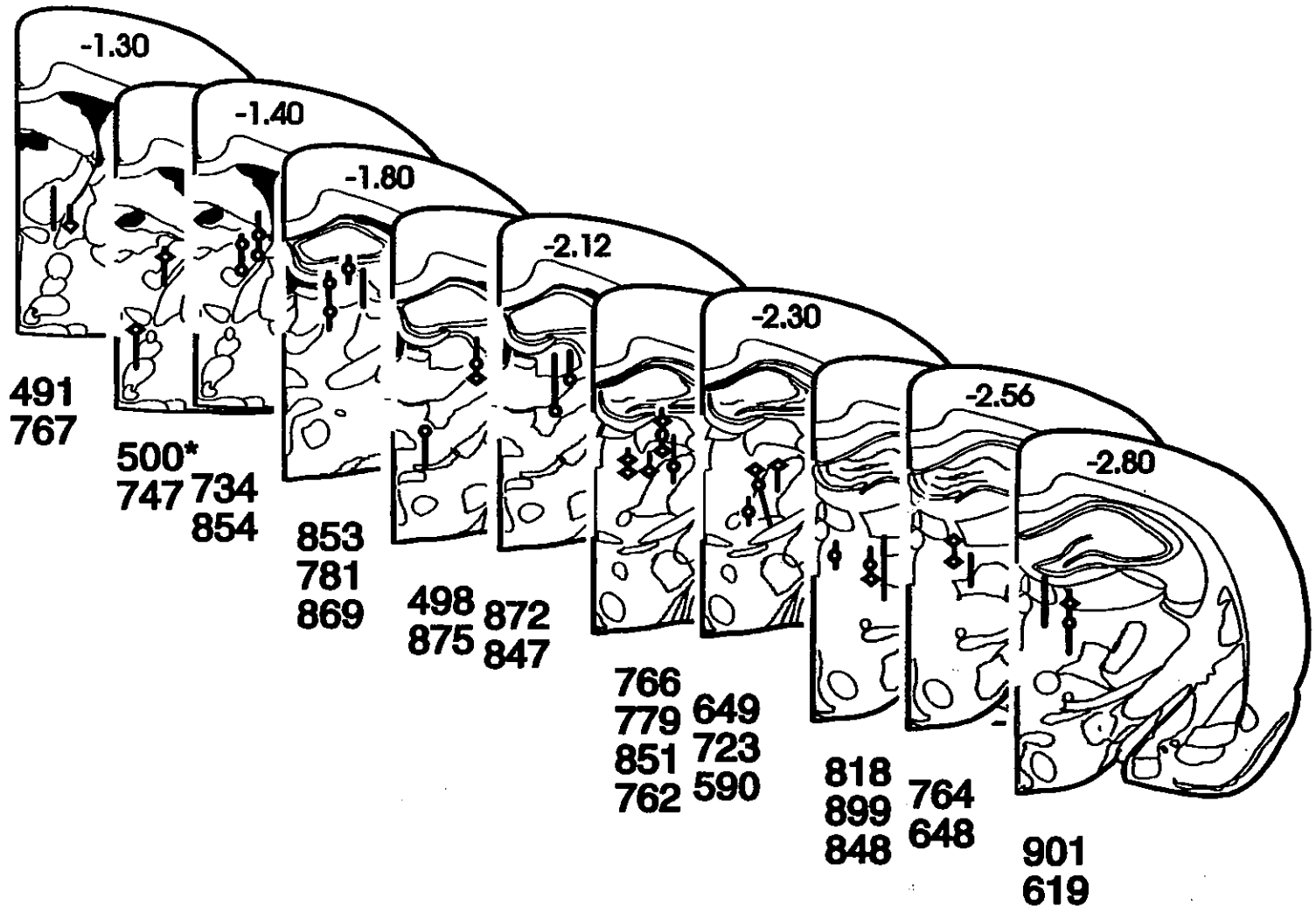
RESULTS

Of the 55 implanted electrodes, 23 either penetrated the ZI or were immediately proximal to it, 26 were in the VLT area, and finally six tracks were found in separate structures more than 0.5 mm distant from either the ZI or the VLT. The plates in Figure 3 detail the histologically verified locations of the VLT electrode placements, with the most rostral sites on the left. The sites effective in eliciting a stimulation-induced drop in BAT temperature are noted as either an open circle or diamond; the diamonds distinguish those which showed a second temperature drop after restimulation of the site, while the circles indicate

Figure Caption

Figure 3. Tracings of atlas plates (adapted from Neurographics, 1993) that best correspond to those sections showing the location of the electrode tips aimed for the VLT. From left to right, the plates lie in a rostral to caudal arrangement, and the numbers on the plates identify the anteroposterior level (coordinates from Paxinos and Watson, 1986); where two plates depict the same level, they are placed in parallel and the coordinates appear on the second or rightmost plate. The numbers below the plates list the identity of the rat associated with each electrode track in that plate; the top number corresponds to the track closest to the midline placement, and so on to the final number in a given column which labels the most lateral track on that plate.

The sites effective in producing a BAT temperature decrease are represented by a diamond or a circle, while the lines indicate sequential sites within one electrode descent which were ineffective. The diamonds denote either those effective sites which showed a second temperature drop when the site was restimulated, or those sites (n = 7) for which restimulation was not attempted. In contrast, the circles identify those effective sites which did not show a repeat temperature drop when restimulated. The asterisk next to 500 refers to the one track shown in the figure that is labelled as a ZI site, thus indicating the location of the only positive site observed in the ZI region which is not directly in the band of the nucleus.


-1.30
-1.40
-1.80
-2.12
-2.30
-2.56
-2.80
491
767
500*
747
734
854
853
781
869
498
875
872
847
766
779
851
762
649
723
590
818
899
848
764
648
901
619

those that did not. The lines note those sequential sites associated with either no change or a rise in BAT temperature.

The ZI electrode placements are not illustrated, as all of the sites that were effective, except for one (see Figure 3, rat 500) were found directly in, or bordering on, the band of the nucleus. Seven additional rats, with electrodes implanted in the region of the medial preoptic area, displayed only the more typical stimulation-induced rise in BAT temperature (Holt et al, 1987; Amir & Schiavetto, 1990; Kelly & Bielajew, 1991); their data are not included in this experiment.

In all rats, a core temperature of 36.6 - 37.0°C was obtained within the first 15 min. of surgery, and did not exceed this range unless a change in BAT temperature was recorded. Of the 56 out of 401 individual sites that evoked brown fat temperature drops (see summary in Table 1), the corresponding core temperature fell in 22 instances, with the decreases ranging from 0.1 to 0.5°C; most of these (n = 18) occurred in animals with electrodes aimed at the VLT area. Although the use of the blanket did not always prevent the core temperature drops, it is likely that it acted to reduce them.

Prestimulation Controls: In order to establish that the BAT temperature changes which we defined as drops indeed represent an active response to stimulation, in contrast to the smaller changes at sites labelled "ineffective", we used the non-specific temperature variations of the prestimulation control trials as a comparison. Table 1 lists the sites associated with temperature

TABLE 1: SUMMARY OF STIMULATION-INDUCED TEMPERATURE CHANGES

SITES	0°C	-0.1°C	DROPS* -0.2°C*	DROPS* ≥-0.3°C*	+0.1°C	≥+0.2°C	TOTAL
ZI	49	36	9	12	45	22	173
VLT	49	43	16	16	39	30	193
OTHER	13	6	1	2	9	4	35
PERCENT OF TOTAL	27.7% (n=111)	21.1% (n=85)	6.5% (n=26)	7.5% (n=30)	23.2% (n=93)	14.0% (n=56)	100% n=401
PRESTIM TRIALS	32.9% (n=24)	20.6% (n=15)	4.1% (n=3)	1.4% (n=1)	38.4% (n=28)	2.7% (n=2)	100% n=73

ZI = 23 electrode tracks, **VLT** = 26 electrode tracks, **Other** = 6 electrode tracks

changes from baseline, ranging from 0.0°C to -0.2 or +0.2°C or greater, for both stimulated and prestimulated control trials

Of the 73 prestimulated trials, 92% showed either no change or a 0.1°C change during the ten minute prestimulation period, while only 4.1% showed a change of -0.2°C; the fact that the -0.2°C changes are much less frequent suggests that they comprise a distinct category from the other changes. This pattern is clearly mirrored in the stimulation findings, where temperature drops of 0.2°C make up only 6.5% of the trials, compared to 72.5% for 0.0°C or 0.1°C temperature changes (ie. ineffective sites).

This consistent result argues for our use of a -0.2°C change as the criterion for defining a temperature drop; Woods & Stock (1994) in fact report a mean of -0.2°C for stimulated BAT temperature drops. In addition, all four of those prestimulation trials in our study which displayed a -0.2°C or -0.3°C change also showed a large drop when the sites were subsequently stimulated; this is further evidence that the -0.2°C drops represent induced changes, in both the stimulated and unstimulated trials. We suspect that the 0.2°C drops in the prestimulation trials were evoked by mechanical pressure as the electrode tip was lowered into the effective area. Finally, it is important to note that of the 26 stimulated sites which elicted -0.2°C changes (see Table 1), ten were immediately above or below sites which yielded larger drops (see Figure 3 for the VLT examples).

Stimulation in the region of the ZI (23 rats): In 15 of the subjects, the electrode tracks penetrated the band of the ZI

nucleus. Nine of these tracks gave rise to individual sites which caused a stimulation-induced drop in baseline BAT temperature, with roughly the same proportion of effective to ineffective descents located in the anterior and posterior areas; the remaining six tracks that penetrated the ZI did not induce any change in BAT temperature.

In one subject, 477, a drop was observed in four consecutive sites; these temperature profiles are illustrated in Figure 4. The other eight descents with positive sites displayed a variety of patterns, from only a single effective site, to either consecutive or non-consecutive effective sites. All tracks included a number of stimulated sites both above and below the nucleus at various lateral-medial positions.

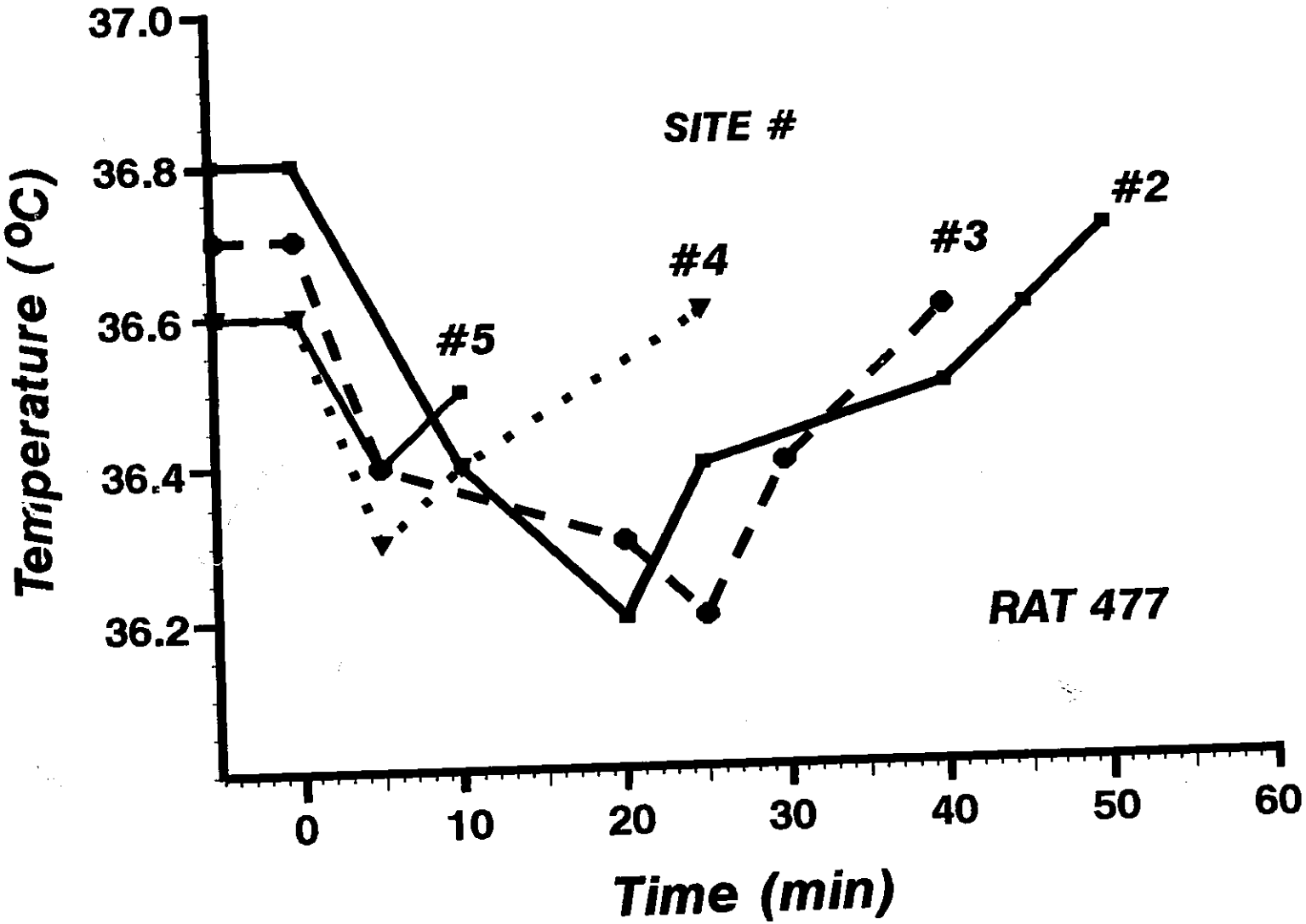
The typical BAT temperature profile showed a drop that ranged from 0.2 to 0.8°C, and lasted 20 to 50 minutes, with no corresponding change in core temperature. All BAT temperature profiles returned to baseline, or within 0.1°C of it. In the case of three of the positive sites, however, core temperature did fall, beginning about six minutes post stimulation and recovering later than BAT temperature. The magnitude of change was always less than that of BAT.

Finally, there were eight electrode tracks that did not penetrate the ZI, but had tips located adjacent to the nucleus, within 0.5 mm; one of these, rat 500, displayed an effective site anterior and midline to the ZI (see Figure 3).

In summary, six of ten anterior ZI tracks, three of five posterior ZI descents, and one of eight descents just proximal to

Figure Caption

Figure 4. The graph shows the temperature profiles obtained from stimulation of four consecutive sites located in the anterior ZI (rat 477); stimulation was begun at time 0 on the abscissa. The number next to each profile indicates the site that was stimulated: second, third, fourth or fifth of the six stimulated sites in the track. The electrode was lowered, and each new site stimulated, only after BAT temperature had remained steady for 20 min. Core temperature remained at 36.6 °C throughout the procedure.


37.0
36.8
36.6
36.4
36.2
Temperature (°C)
SITE #
#2
#3
#4
#5
RAT 477
0
10
20
30
40
50
60
Time (min)

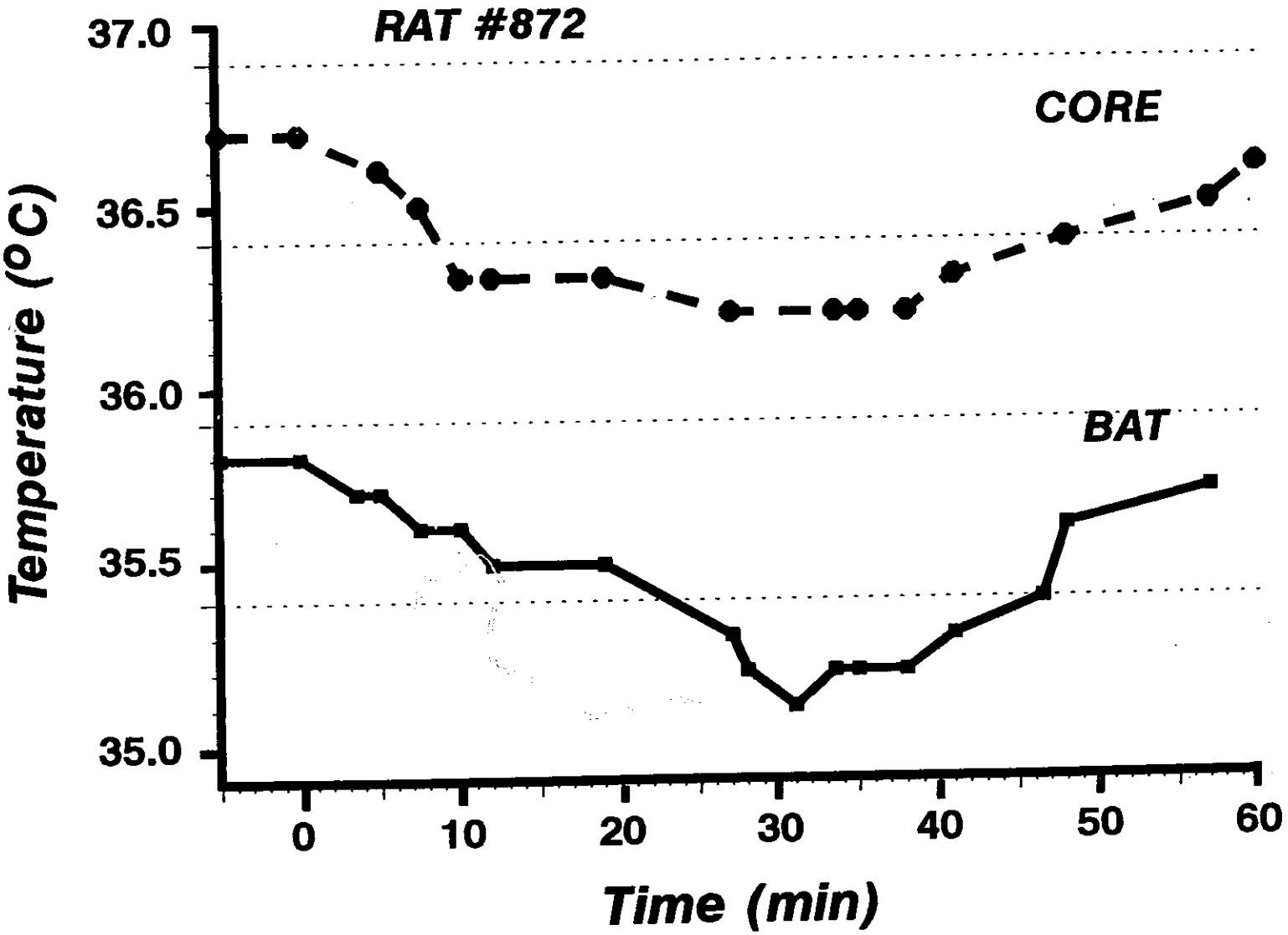
the structure each yielded at least one site which induced a BAT temperature decrease.

Stimulation in the region of the VLT (26 rats): Of the 26 electrode tracks in this area, 21 displayed one or more effective sites (see Figure 3; 32 positive sites in total). There appears to be no particular pattern to the location of the observed effective sites in this region. The profiles of elicited BAT temperature changes were similar to those observed during stimulation of the ZI; the drops ranges from 0.2 to 0.9°C. However, core temperature was observed to decrease in more cases than in the ZI stimulated rats, and faster, as illustrated in Figure 5, taking roughly ten minutes to begin to level out. In contrast, the rare drops in core temperature that were observed with ZI stimulation took up to 20 to 30 minutes to reach nadir.

A group of rats with VLT area implants (766, 767, 779; plus 763, septal area) were given an i.p. injection of the β-adrenergic blocker propranolol 15 minutes prior to restimulation, while three other (851, 875, 899) were injected with phentolamine, a non-specific α-adrenergic blocker. In the first group, the BAT temperature drop persisted despite the presence of the β-blocker, although the drops did begin later, more than six minutes post-stimulation instead of two, with a maximum change of only 0.3°C. In the subset injected with the α-blocker, one animal showed no drop on restimulation, while in two, a temperature drop of 0.1°C was observed. A second restimulation ten minutes later yielded identical results.

Figure Caption

Figure 5. The graph depicts the temperature profile obtained from stimulation of the eleventh site in electrode descent 872; stimulation was begun at time 0 on the graph. Core temperature is indicated by the upper dotted line, and BAT temperature by the solid line. Note the rapid descent of core temperature within the first ten minutes, in contrast to the slower core temperature fall that we observed with stimulation of effective ZI sites.


RAT #872
CORE
BAT
37.0
36.5
36.0
35.5
35.0
Temperature (°C)
0
10
20
30
40
50
60
Time (min)

Stimulation of other regions (6 rats): Six tracks were found to be in nuclei distinct from, and more than 0.5 mm distant from either the ZI or the VLT; two of these (761, 763) yielded effective sites. The first was located anterior and lateral to the ZI in the substantia innominata, while the second was anterior and dorsal in the septofimbrial nuclei. Again, the temperature profiles were similar to those associated with the ZI or the MDT. The remaining four tracks were located in the hippocampal area, and in regions rostral to the ZI (roughly 1 mm posterior to bregma).

DISCUSSION

The results indicate that electrical stimulation of structures in the CNS can elicit two contrasting types of BAT temperature change; in agreement with previous studies, temperature rises were observed for some sites in the MPO area and elsewhere (Holt et al, 1987; Rothwell, 1989; Amir & Schiavetto, 1990; Kelly & Bielajew, 1991), while the temperature drops appeared to be confined to the ZI and VLT regions. Increases in BAT temperature rises were also noted after stimulation of various individual sites surrounding the ZI and the VLT (see Table 1); this additional rich source of data on the neural circuitry regulating the brown fat response will be addressed in a separate manuscript.

In this study, 21 of 26 descents in the region of the VLT, and nine of 15 descents that penetrated the band of the ZI yielded sites effective in eliciting BAT temperature drops. In

contrast, there were only three effective sites of a total of 13 tracks in areas surrounding these nuclei. In fact, if the two other regions that we previously mapped - the VMH and the medial preoptic area - are included, then this value falls to three effective sites in 37 electrode tracks (Kelly & Bielajew, 1991).

Characteristics of the BAT Temperature Decreases: The stable BAT temperatures recorded over the 73 ten-minute monitoring periods clearly show that there is little spurious or non-specific variation, indicating that the temperature changes we observed in this study are indeed induced by the electrical stimulation (see Table 1). The fact that the temperature drops always occurred within six minutes of stimulation also serves to correlate the temperature changes with CNS activity.

However, in 16 of the 24 effective VLT sites which were restimulated, the BAT temperature drop did not reoccur (0.0°C, n = 10; -0.1°C, n = 6); in the remaining eight, the second temperature drop was weaker than the first. Figure 3 shows the location of both the sites that gave rise to a drop following repeated stimulation (diamonds), and those that did not (circles).

The non-repeat temperature drops were associated with identical placements to those which could be re-elicited, and also showed similar temperature profiles. The failure of the second stimulation to reproduce the temperature drop may, in some cases, be related to the same factors that caused stimulation of consecutive effective sites in rat 477 to elicit temperature

drops of decreasing magnitude; possibly some aspect of the BAT response, whether peripheral or central, is not fully recovered before the second stimulation. Finally, it is possible that stimulation of general thermal circuits itself may interfere with the reappearance of the BAT temperature drop. However, this hypothesis remains speculative as it appears that no restimulation of effective sites was attempted in any other studies where BAT temperature drops were elicited with additional thermal responses (Hinckel et al, 1983; Szreder er al, 1994).

The use of the homeothermic blanket to maintain the rats' core temperature at the daytime average of 37°C minimizes any interference due to feedback cycles between core and BAT temperature, although it appeared that the blanket was unable to respond quickly enough in some cases to completely prevent the induced core temperature change. The effect of the stimulation on core could not be properly evaluated due to the presence of the blanket; the most effective method would be to stimulate an awake animal. In those cases where we did observe a small decrease in core temperature, it always followed the drop in BAT temperature. This delay is interpreted as evidence of a direct experimental effect on BAT, thus making it unlikely that BAT temperature is dropping merely as a result of core changes (Imai-Matsumura & Nakayama, 1986; Amir, 1990; Kelly & Bielajew, 1991).

CNS Regions that Regulate BAT Temperature Drops: From a total 401 individual stimulated sites in the present mapping study (193 VLT, 173 ZI and 35 other), we report only three outside of either

the VLT or ZI area that were effective in producing a BAT temperature decrease. Even allowing for histological inaccuracies, the regions controlling the BAT temperature drops appear to be fairly specific. In the case of the ZI, twenty of the 21 individual sites which evoked a decrease in BAT temperature were all found in the band of the nucleus, whereas there was only one temperature drop recorded from the 145 sites surrounding it (see Figure 3). However, these results do not allow us to distinguish whether activation of cell bodies or fibres of passage are responsible for the BAT temperature drops.

While the ZI has not previously been shown to have a functional connection with brown fat temperature changes, several lines of evidence suggest a possible link between the ZI and the regulation of metabolism. Efferent projections from the ZI to the DMH have been demonstrated (Ricardo, 1981), and, in turn, we report that stimulation of the DMH elicits a BAT response, as do others (Freeman & Wellman, 1987). The ZI may also send information indirectly to the autonomic nervous system via projections to structures such as the periaquaductal gray and the reticular formation (Ricardo, 1981). Finally, the ZI nucleus has been implicated in both the glucoprivic and lipoprivic feeding response, based on the findings of various lesion studies (Walsh & Grossman, 1975; Tepper & Kanarek, 1984; Calingasan & Ritter, 1992).

The primary function of the VLT is to relay non-specific sensory information to the cortex, and there is apparently no prominent role for any of the diverse thalamic structures in the

regulation of appetite or body weight. There is evidence, however, that the thalamus participates in thermoregulation (Schönbaum & Lomax, 1990), though it is not considered part of the classic thermal circuit (the spinal cord, lower brainstem, and hypothalamus). Previous reports of BAT temperature drops as part of the thermoregulatory response to brainstem stimulation (Hinckel et al, 1983) fit with the observations reported here; furthermore, we found that many of the VLT stimulations evoked a faster drop in core temperature than ZI ones, and only in the case of VLT sites were BAT temperature drops as small as -0.2°C accompanied by core temperature decreases. These results suggest that VLT stimulation has a direct effect on core temperature, in addition to its effect on brown fat; an assessment of the range of possible thermic responses to VLT stimulation, beyond BAT temperature drops, would be revealing.

Examination of this thermosensitive neuronal circuit does suggest a potential physiological basis for abrupt BAT temperature drops. The cold-sensitive units within the VMH have been shown to respond to both preoptic hypothalamic and peripheral cooling (Nakayami et al, 1981), and are essentially the same neurons which are facilitated by glucose (Imai-Matsumara & Nakayami, 1981). These characteristics suggest a neuronal mechanism for BAT thermogenesis induced by preoptic area and/or ambient cooling, as well as a means for thermal signals to influence the control of feeding. It would be interesting to determine if the warm-sensitive cells of the VMH or the preoptic area are then involved in the control of BAT temperature drops,

perhaps by experimental inhibition of either nucleus during induction of the temperature drops.

Role of SNS in Control of BAT Temperature Drops: Unlike a number of earlier papers that report decreases in BAT activity due to simple reversal of experimentally-induced excitation (eg. Niijima et al, 1984), we observed BAT temperature drops from baseline tht were directly related to CNS stimulation. Such short-term, stimulation-induced drops in BAT temperature have been previously reported in the context of thermoregulatory investigations; one study found that stimulation of the nucleus raphe magnus in awake animals caused a marked decrease in brown fat heat production, as well as attenuating other thermic responses (Hinckel et al, 1983). The temperature drops were attributed to stimulation of warm responsive units in the thermoregulatory circuit.

Intracerebroventricular injections of both neuropeptide Y (Szreder et al, 1994) and insulin (Holt & York, 1989), chemical stimulation of localized regions within the dorsal medial hypothalamus (Yoshimatsu et al, 1993), as well as inductions of elevated blood pressure (Shibata, 1982) have all been shown to cause immediate transient reductions in baseline BAT activity, and it is suggested that this response is due to suppression of sympathetic nervous activity. Calasso et al (1993) report interscapular BAT temperature drops during desynchronized sleep, which they attribute to both decreased sympathetic input, and to an increased flow of cooled blood through the BAT deposit.

Finally, Woods and Stock (1994) found that very low-level stimulation of the VMH elicited acute BAT temperature drops that persisted until the stimulation was switched off. At that point, BAT temperature abruptly rose and surpassed the initial baseline level. The authors suggest that lipolysis, which provides fuel for heat generation, is in fact stimulated during the drop phase of BAT response, but an inhibitory response specific to the low-level stimulation prevents the cells from utilizing the fatty acids until the stimulation is turned off.

Overall, the range of BAT temperature values observed in other studies is similar to ours, from the typical drop of 0.2°C reported by Woods & Stock (1994) to the -1.0°C change seen by Hinckel et al (1983). It appears that acute BAT temperature drops are likely due to one, or a combination of, four mechanisms: 1) decreased firing rate of intercostal nerves, thereby suppressing sympathetic input; 2) constriction of BAT blood vessels due to α-stimulation (Flaim et al, 1977; Foster, 1985); 3) redirection of blood through arteriovenous anastomoses, preventing enough oxygen from reaching the cells to allow the thermogenic response, despite lipolysis (Himms-Hagen, personal communication; Nnodim & Lever, 1988; Woods and Stock, 1994) and 4) cooling of blood entering the BAT deposit, due to such factors as an induced thermodilatory effect in the tail (Calasso et al, 1993).

The first step is to determine whether SNS activity regulates decreases in brown fat temperature as it does the production of heat (eg. Seydoux et al, 1977). Thus, a subset of four animals

were injected with the β-blocker propranolol prior to stimulation, and as expected, the presence of the β-blocker did not interfere with the induced drops. However, earlier studies of the biphasic BAT temperature response to VMH stimulation implicate α-adrenergic-induced vasoconstriction as the cause of the initial temperature drop (eg. Flaim et al, 1977; Perkins et al, 1981); we investigated this possibility by restimulating the site after systemic application of phentolamine, a non-specific α-blocker.

Intraperitoneal injections of phentolamine have been shown to lower body temperature in awake animals (eg. Kent et al, 1990); however, our anesthetized, warmed animals showed no BAT or core temperature change due solely to injection of phentolamine. The severely attenuated drop which was observed after restimulation in the presence of the α-blocker is difficult to interpret, for several reasons.

The sample size is small, but was restricted due to the difficulty in finding sites which yielded a temperature drop of -0.2°C or greater on restimulation. Thus, the problem was that restimulation itself did not consistently give rise to a second temperature drop, and further, almost all second drops were of lesser magnitude than the initial one. It could be significant that a weak decrease persisted in two cases of stimulation after phentalomine application; this finding is compatible with a prominent, but possibly not exclusive, role for α-induced vasoconstriction in the regulation of BAT temperature drops.

The severe attentuation of the drops is a concern. Due to the

problems getting repeat BAT responses, no sites were formally tested for the ability to induce a response on a third restimulation; however, the consistent size of the attenuated responses, along with the fact that a fourth stimulation in the presence of the blocker induced an identical attentuated response, suggests that the change in BAT temperature response was due to the application of the blocker rather than to simple tolerance. In order to further examine the participation of the SNS in the control of BAT temperature drops, it would be useful both to record the firing rate of BAT sympathetic nerves when brown fat temperature decreases are evoked, and to directly examine the changes in blood flow.

Interestingly, during two of the three trials with the α-blocker, core temperature dropped by 0.2°C after restimulation, regardless of the size of the BAT temperature drop; this further supports the idea that VLT stimulation, possibly unlike ZI stimulation, has a direct, distinct effect on both core and BAT temperature.

Conclusion: Taken together, the observation of induced bidirectional changes in brown fat temperature from these first two studies led us to speculate on the possibility of a circadian profile for BAT thermogenesis. Indeed, diurnal variations in BAT sympathetic firing rate have been reported (Sakaguchi et al, 1988), as well as circadian changes in brown fat GDP-binding (Redlin et al, 1992). It is important to determine if these varied levels of BAT metabolism in fact reflect a cyclic pattern

in BAT heat production, as the daily pattern of brown fat thermogenic activity could reveal significant interactions of BAT temperature with feeding, or with core temperature, and thus establish a possible role for short-term BAT temperature decreases in metabolic regulation.

In summary, this study demonstrates that an abrupt, transitory drop in brown fat temperature can be induced by electrical stimulation of central structures. We observed decreases in BAT temperature following stimulation primarily of sites in the ZI and the VLT areas. An injection of β-adrenergic blocker did not prevent the BAT temperature drop; however, the response to the α-adrenergic blocker was inconclusive, with two of the three cases associated with an attenuated temperature decrease.

EXPERIMENT 3

In the previous two experiments, we reported the induction of abrupt drops in BAT temperature after stimulation of either the ZI or the VLT, in addition to the classic thermogenic response of the tissue to VMH stimulation. It was speculated that if such opposing events are represented in the daily temperature profile of brown fat, then the pattern would resemble the peaks and troughs displayed by core temperature profiles. Through the circadian patterns, the relationship between the two temperature series could be assessed in order to provide information about the contribution of BAT heat production to core values. However, the evidence regarding a possible circadian profile of brown fat heat production is contradictory.

Recent studies report the presence of a circadian rhythm in BAT metabolism, but several different indices of BAT activity were used and the results are not in agreement. Brown fat levels of the sympathetic transmitter NE are higher in the evening compared to morning values (Davidovich & Petrovic, 1981) but the levels of GDP-binding, as well as the basal firing rate of the sympathetic nerves innervating BAT, have been reported to appear at a maximum in the illumination period (Rothwell et al, 1983; Sakaguchi et al, 1988). Others, however, have observed no systematic change in GDP-binding levels (Park and Himms-Hagen, 1988a). In addition, it has been proposed that the rising levels of corticosterone seen at the end of the dark phase act to inhibit BAT activity, with the opposite pattern prevailing at the end of the light phase. This hypothesis is in line with the idea

that brown fat thermogenesis is activated at night, coincident with a number of ingestion and energy deposition processes (Le Magnen, 1985; Bray, 1990).

However, the indicators of BAT metabolic activity - NE turnover, GDP-binding, SNS firing rate, concentration of uncoupling protein, lipid turnover - can apparently be dissociated from each other or from actual heat production or BAT O_2 consumption (eg Luboshitzky et al, 1983; Sakaguchi et al, 1988; Ma & Foster, 1989); thus, it is not known whether reported diurnal changes in fact reflect the presence of a cyclic pattern in BAT thermogenesis. So this study was designed to directly examine the daily profile of brown fat heat production using radio-frequency transmitters.

GENERAL METHOD

Male Sprague Dawley rats (Charles River, Quebec) were housed individually, under identical conditions to those described in the previous study. In addition, all rats from which core temperature data were to be collected were continuously exposed to the sound of clicks from a CB receiver in order to acclimatize them to the noise.

Rats weighed from 300 to 395 g at the time of surgery. The animals were anaesthetized either with an injection of sodium pentobarbital (Somnotol, 65 mg/kg, i.p.) and rompun (Xylazine, 3mg/kg, i.m.), or by exposure to graded concentrations of fluothane (Halothane) gas and oxygen through a nose cone. Surgeries lasted no longer than one hour; if supplemental

anaesthesia was necessary, the inhalant methoxyflurane (Metofane) was used, rather than the barbituate, in order to lessen the disruptive effect of the longer-acting injectable anaesthetic on the circadian rhythms.

BAT Temperature Profiles: surgical procedure and data collection

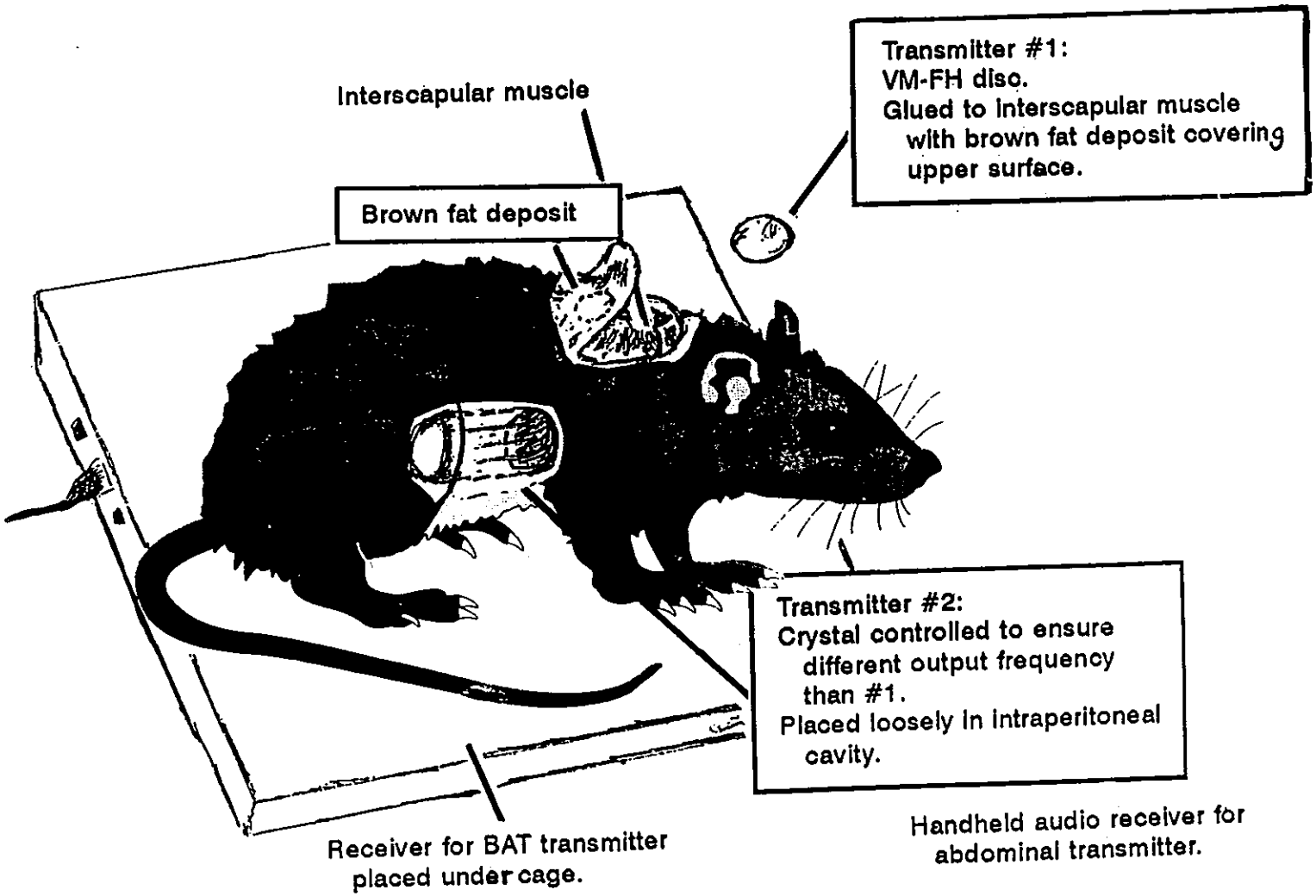
An XM-FH disc radio-frequency transmitter (Mini-Mitter Co., Inc.) was implanted beneath the interscapular BAT deposit in each of ten rats. The wax-coated transmitters are about 1.5 cm in diameter, weigh 5 g each, and are shaped like flat discs. Each transmitter was sterilized in a solution of Benzadine, and all instruments and gauze were sterilized in an autoclave. The shoulder muscle and the BAT deposit of the anaesthetized rat were exposed by cutting and pulling back the skin of the interscapular area, and gently teasing the tissue apart with forceps to clear a quarter-sized opening (see Figure 6 for illustration).

The transmitter was held in place beneath the brown fat in one of two ways. In most animals (up to and including rat 881), the transmitter was glued directly to the shoulder muscle; in others, it was glued to a small piece of sterilized surgical teflon, and the teflon tag then sewn at two ends to the muscle. In both procedures, the brown fat deposit lay on top of the transmitter once it was in place. The wound was closed up, and the animals were allowed a minimum of five days to recover from the procedure.

The surgical aftercare routine varied. For all rats numbered

Figure Caption

Figure 6: Two different implantation sites - beneath interscapular brown fat, and inside the abdominal cavity - are depicted in this figure; the rat drawing is courtesy of Corel Clipart. Each flat disc transmitter was implanted into ten rats; it was bonded into place below the BAT deposit in one of two ways, either glued directly to the shoulder muscle (rats 700, 757, 745, 771, 799, 819, 881), or glued first to a piece of surgical teflon which was then sewn to the muscle (rats 890, 897 and 903). The transmitter output was collected by a receiver placed under the cage, and then sent to an automated system. The second transmitter, which was implanted in four of the ten animals, was placed loosely in the intraperitoneal cavity in order to record core abdominal temperature. The data was collected via a VCR and then manually recorded off the tape. All transmitters were purchased from Mini-Mitter Co., Inc.



Transmitters purchased from Mini-Mitter Co.

819 and below, post-surgical care was identical to routine care, while all animals after 819 received acetomenophen in the drinking water (flavoured liquid Tylenol; 160 mg/ml; 1 ml Tylenol/100 ml water) for three days to minimize pain and encourage weight gain. The final set of animals (numbered 881 and above) received a three day regime of both acetamenophen and enrofloxin (Baytril; 5mg/kg/day) in the drinking water, in order to assist weight gain and to prevent swelling due to foreign body reaction. Any animal that showed signs of swelling, low weight gain, lethargy or fever was excluded from the study (10 of 22 rats).

Once the animal showed a weight gain for three consecutive days, data collection began. Receivers were placed under the cages, and the automated system recorded BAT temperature every 30, 60, or 90 sec throughout the 24 h cycle, for up to eight consecutive days. Cages were cleaned, and the rats weighed, between 0900 and 1100 hours on every second or third day.

At the end of the data collection period, each rat was injected i.p. with a lethal dose of sodium pentabarbitol. We verified that the BAT deposits had physically adhered to the transmitters in each animal. Some subjects were removed from the study at this point due to swelling which had not been detected earlier, or the appearance of thickened tissue envelopes surrounding the transmitters.

To ensure accuracy, the output of the transmitters was manually calibrated with temperature change using a water bath, both before and after the data collection.

Interscapular brown fat samples were removed from four animals at sacrifice, in order to determine the level of NE found in intact tissue. Brown fat tissue was removed quickly in subdued lighting to minimize catecholamine degradation due to light exposure. The samples were frozen on dry ice and stored in a -80°C freezer. To prepare for analysis, the thawed samples were added to 1 ml of a 4% perchloric acid solution which also contained 0.5 mg/ml EDTA and 0.05 mg/ml sodium bisulphate; the mixture was homogenized with a micro ultrasonic cell disruptor. To perform the acid extract of the NE, 10 mg of alumina and 400 µl of tris/EDTA (pH 8.5) were added, as well as 40 µl of DHBA as an internal standard. Tubes were shaken for 20 minutes, centrifuged, and the supernatant aspirated.

The remaining alumina pellet was washed three times with 0.2% tris/EDTA (pH 8.1), and then a low pH solution of acetic acid/10% sodium bisulphite was added to elute the NE from the pellet. The pellet was shaken and centrifuged; 20 µl of the resultant supernatant was used for the electrochemical HPLC detection, and the remainder was frozen in case of future need. All supernatants were diluted 100-fold; the control samples required further dilution to bring the NE content down to measurable levels. A Waters 460 electrochemical detector was used; the stationary phase is a C18 hydrocarbon chain bonded to a 5 µl silica particles, while the mobile phase is a mixture of water, methanol, and buffer salts. Results were recorded in ng NE/g BAT tissue.

A time-series analysis, also called Fourier frequency

analysis or spectral decomposition, was performed to determine the length of any repeating cycles within the temperature profiles.

Specific Groups or Subsets

i) Simultaneous Core and BAT Temperature: surgical procedure and data collection

The first part of the surgical procedure was identical to that described above, with one additional step; a second transmitter was placed loosely in the abdominal cavity after closure of the BAT area incision. The muscle and skin were sewn with absorbable suture. The wax coated transmitters - crystal controlled TM-discs from Mini-Mitter, Inc. - are cylindrical in shape, about 3 cm long, 1.5 cm in diameter, and weigh 15 g. Each transmitter was sterilized in a solution of benzadine before implantation.

The same range of post-surgical care routines was applied as described earlier, but the animals generally took three days to begin gaining weight rather than the two days typical of animals that received a single transmitter. No swelling or discolouration of the abdominal incision was observed in any animal.

The receiver was placed beneath the cage as before for BAT temperature collection, and the additional core temperature data were collected by a VCR. A hand-held, partially disabled CB receiver ("walkie-talkie", Model CH3, Mini-Mitter Co., Inc.) was placed near the cage, and a microphone next to the receiver picked up the audible clicks. The microphone output was fed into

a tapedeck, and the subsequent signal into a VCR, which allowed continuous collection periods of up to eight hours before a tape change was required. The temperature data were later taken directly off the VHS tapes by hand; the time duration required for 20 clicks was recorded at each 10 min. interval, and temperature increases were represented by proportional decreases in time/20 clicks.

ii) Temperature Profiles from Sympathectomized Rats: surgical procedure and data collection

All procedures were identical to those initially described, except that the BAT deposit was surgically denervated before the transmitter was glued or sewn in place. A T-shaped incision was made, and the two flaps of skin held back with clips while the brown fat lobes were gently lifted up to expose the intercostal nerve bundles. The four most anterior nerves were severed, and a 2-3 mm piece removed to prevent regrowth. The fifth nerve was left intact, as it is believed to play little role in BAT innervation (eg. Foster et al, 1982b; Himms-Hagen et al, 1990). The transmitter was then placed beneath the brown fat, and the rest of the procedure carried out. For both denervated animals, the transmitter was glued to a teflon tag, and the tag sewn to the shoulder muscle.

When the animals were sacrificed, the BAT deposit was quickly removed and placed on dry ice for storage in a -80°C freezer, taking care to minimize exposure to light. The samples were later analyzed by HPLC methods to determine NE levels; the

procedure is described on page 89.

iii) Temperature Profile from Interscapular area with BAT Removed: surgical procedure and data collection

In these animals, the additional step occurred before the placement of the BAT transmitter. The shoulder muscle was exposed and all visible brown fat removed, taking care to leave as much white adipose tissue as possible to ensure consistent tissue influences on the transmitter output. The rest of the surgical and data collection procedures were the same as above.

Methodological Controls

iv) Room Temperature Profiles

In order to monitor room temperature fluctuations, a second receiver collected ambient temperature for two days during the study. An XM-FH transmitter was placed on the second receiver, and temperature readings collected by an automated system.

v) BAT Temperature Profile following NE Injection

Two rats from the main study, and therefore with XM-FH disc transmitters implanted beneath the BAT deposit, were exposed to an additional acute procedure after the data collection was completed, and just prior to sacrifice. Each rat received an injection of Somnotol (65 mg/kg; i.p.); once a sufficient level of anaesthesia was achieved, the rats were placed on the surface of a receiver, and a homeothermic blanket used to monitor and maintain core temperature. An i.p. injection of NE was given,

and the temperature output of the BAT transmitter was recorded. The recording took approximately 15 minutes, after which the animals were given a lethal injection of Somnotol. The transmitters were retrieved, and the calibration verified, as in the initial procedure.

vi) Comparison of Transmitter output to Thermocouple Thermoprobe output

Two additional rats, not part of the main study, were subject to the acute stimulation procedure described in Experiments 1 and 2, with the modification that an XM-FH transmitter was placed beneath their BAT deposit, along with the thermocouple thermoprobe. Each anaesthetized rat was placed directly on the flat receiver, with the homeothermic warming blanket between the rat and the plastic surface to maintain core temperature at about 37°C. Successive VMH or ZI sites were stimulated with a moveable electrode until a BAT temperature change was induced; the output of both the thermoprobe and the transmitter were read simultaneously throughout the procedure.

RESULTS AND DISCUSSION

Part A: Interpretation of Temperature Profiles

EXAMINATION OF GENERAL FEATURES:

In this study, the BAT temperature data were collected from a total of ten animals: eight with intact tissue, one with the tissue denervated, and one with the BAT deposit removed. Four of these subjects also contributed simultaneous core and BAT

temperature data, and in two cases the room temperature was monitored during the same period in which the BAT data were collected. In total, 41 x 24h periods of brown fat temperature readings were recorded, and 8 x 24h and 4 x 12h periods of core temperature.

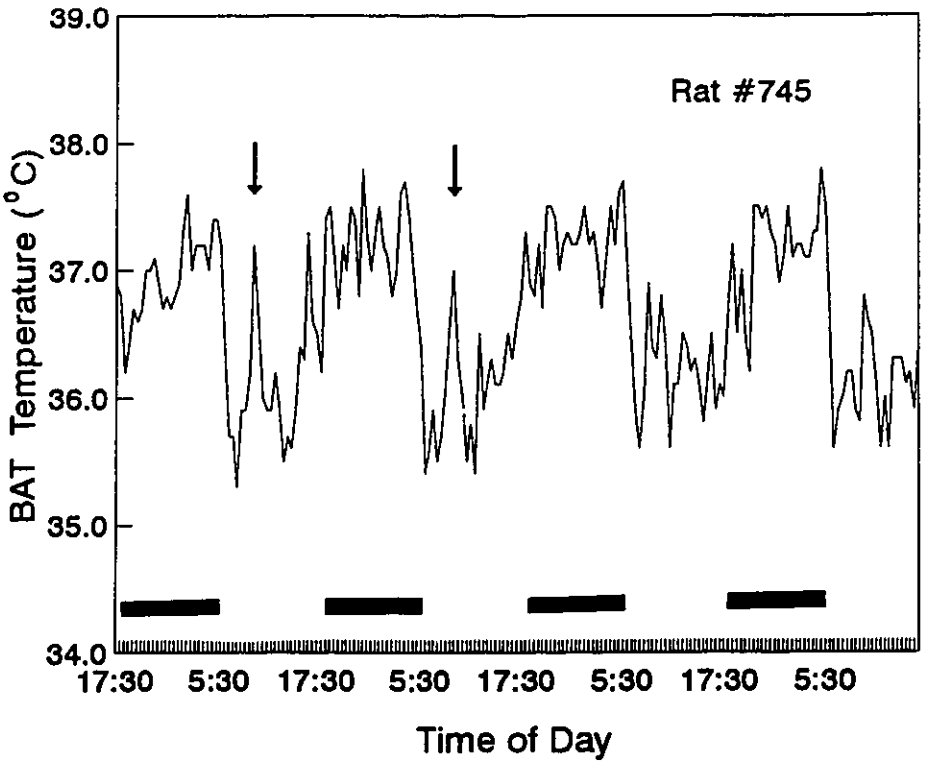
The upper graph of Figure 7 shows one example of temperature data collected in 30 minute intervals over 4 days (rat 745); the black bars indicate the daily 18:00 to 06:00h lights out period. Each half hour observation is based on the average of the readings from a six minute span, three minutes on either side of the time point. Data were in fact collected at intervals as frequent as 30 seconds, but averaging the observations across the entire half hour interval is not recommended because smoothing obscures the original shape of the temperature profile and the information contained in it (A. Dabrowski, personal communication).

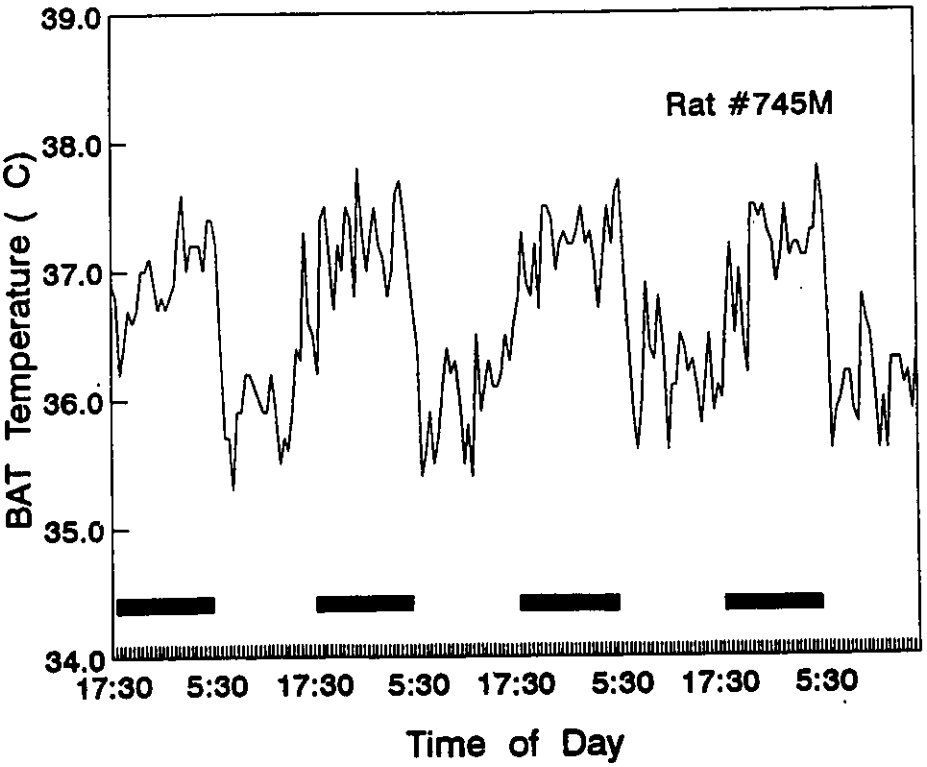
The most prominent characteristic of the graph is the elevated nocturnal baseline that defines the circadian rhythm of BAT temperature; the daytime temperature mean is 35.8±0.2°C, and the night mean 37.0±0.3°C. Numerous peaks and valleys throughout the 24 h periods are also visible, and these continuous oscillations are superimposed on the primary pattern, the nocturnal elevation. The nightly cycles appear to be of larger amplitude than those of the daytime.

The third major feature of the BAT temperature profile is the apparent difference between those slopes that appear at light onset, and the ones at light offset. In three of four instances,

Figure Caption

Figure 7: The upper graph shows the raw BAT temperature profile recorded from one rat for four consecutive days (745). The black bars denote the 12h dark periods (18:00 to 06:30 h daily). The values on the graph represent half hour intervals; these were determined by averaging the readings from the six minute period surrounding every half-hour time point, three minutes either side. The arrows point to the temperature rises that occurred during activity around animal care procedures. These "outlier" values were replaced with averaged estimates for the purposes of spectral analysis; the lower graph illustrates the same profile with the modified values.


Rat #745
BAT Temperature (°C)
39.0
38.0
37.0
36.0
35.0
34.0
17:30
5:30
17:30
5:30
17:30
5:30
17:30
5:30
Time of Day


Rat #745M
BAT Temperature (C)
39.0
38.0
37.0
36.0
35.0
34.0
17:30
5:30
17:30
5:30
17:30
5:30
17:30
5:30
Time of Day

the temperature rise at light offset occurs in a jagged, gradual pattern, and contrasts the abrupt drop that is observed at all four points of light onset. There the values appear to fall directly from a maximum late in the dark period to an immediate trough at lights-on. The use of abrupt lighting transitions, rather than the gradual dimming and brightening of natural settings, likely plays a role in the shape of the temperature pattern; it appears that the animals' behaviour reflects the lighting schedule in that they exhibit sharp changes in activity level at both light switches, although the slopes of the temperature profiles correspond to this behavioural profile only at light onset, not offset.

Finally, note the narrow temperature peaks indicated by the arrows; the rises are coincident with documented activity in the room due to animal care. These systematic outlier values were replaced with the mean of the six surrounding values for purposes of the spectral analysis, in order to avoid any distortion in the results.

The three major characteristics - nocturnal elevation, 24h peaks and troughs, and the contrasting slopes at light switch points - are evident in all temperature profiles from intact animals; the profiles are presented in Column A of Figure 8. The individual patterns of daily temperature changes for interscapular brown fat show an overall range of 34.0 to 38.2°C; the mean of the collapsed daytime values is 35.9±0.02, compared to 37.0±0.02 for the dark period (based on 768 values for each period).

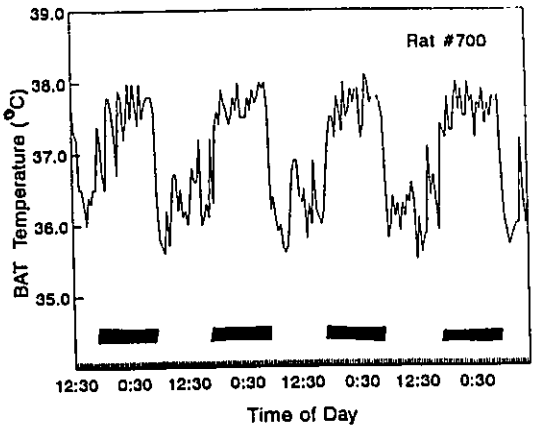
Figure Caption

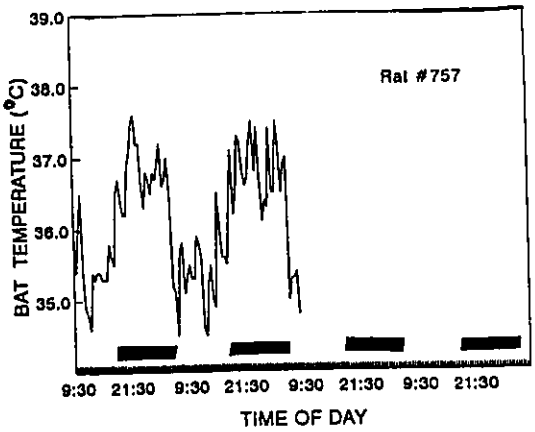
Figure 8: Column A depicts the BAT temperature profiles for six different rats; some series are longer than the 4 days shown, and those data were included in the spectral analysis. The black bars denote the 12h dark periods (18:00 to 06:00h daily). The values on the graph represent averaged readings from half hour intervals. Peaks due to animal care are visible, but were modified for the spectral analysis.

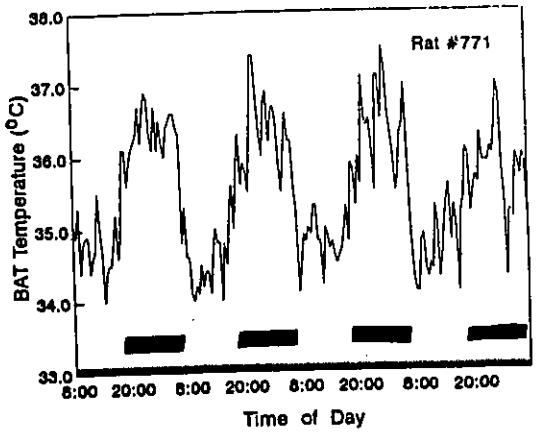
Column B shows the spectral density graphs that resulted from the Fourier analysis of each temperature series; each periodgram is placed opposite the temperature series on which it is based. The periodgrams indicate the frequency of any repeating cycles which might underlie the temperature profile.

Each series was trimmed, before analysis, to represent precisely a set of 24 h periods, or 48 observations (1 each half hour); therefore, the circadian frequency, for example, is 1/48, or 0.021.

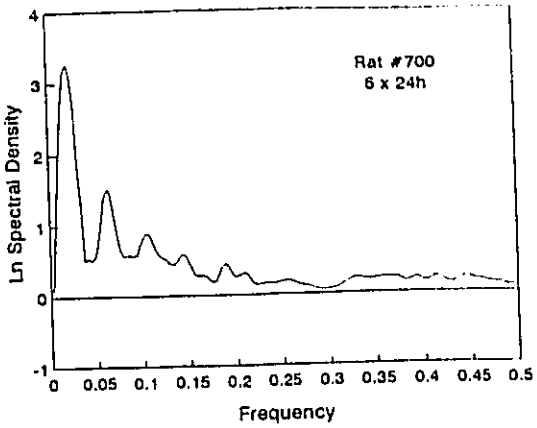
Column A

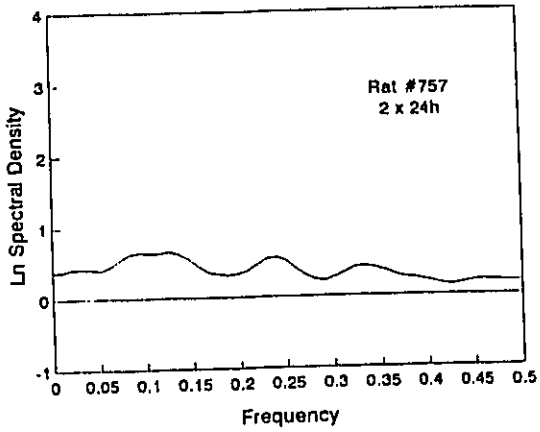


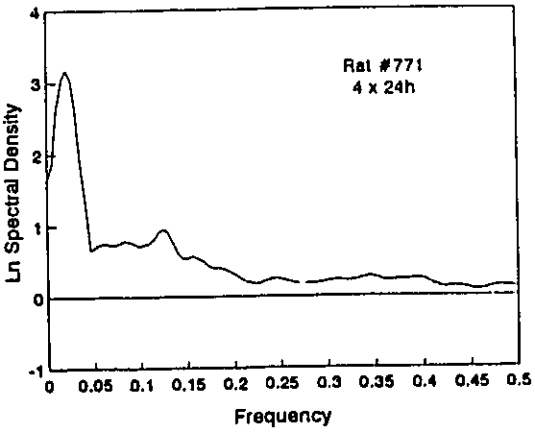




Column B

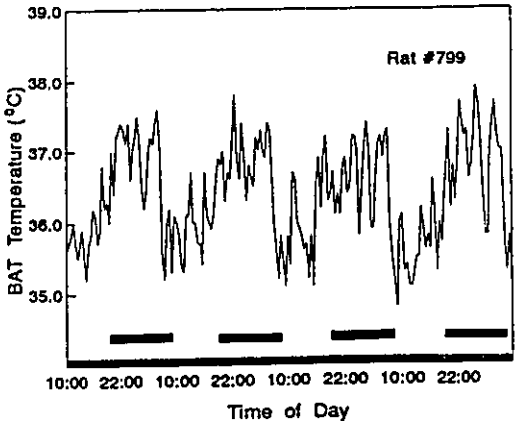


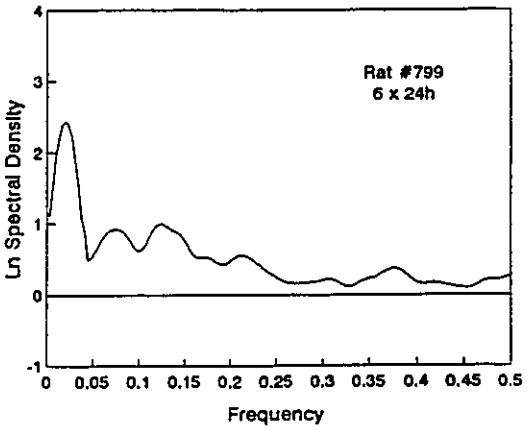


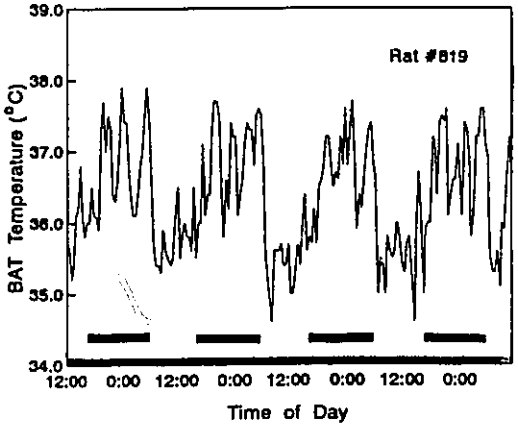


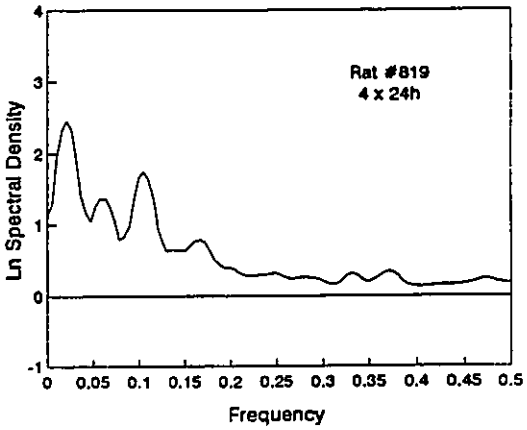
Column A

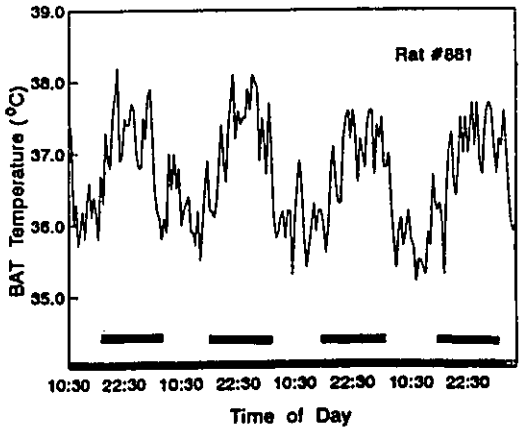
Column B

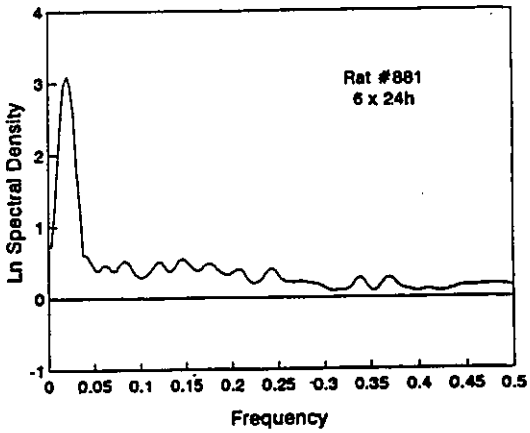












The overall mean, also termed the mesor, of the BAT temperature rhythm is 36.4±0.02; this value is 1.5°C lower than that reported by Closa et al (1993). In that study, daily BAT temperature changes were measured with thermoprobes sewn in place and attached to Harvard jackets. The simplest explanation for the difference in the baseline values is the smaller size of a thermoprobe, which allows it to be completely covered by the BAT deposit and thus limits other tissue influences on the output. In contrast, the transmitter used here protrudes from the BAT deposit. We did directly compare thermoprobe readings with the simultaneous transmitter readings within subjects; the results are discussed later, in the section on methodological factors that influence transmitter output.

Presence of Anticipatory Rise in BAT Temperature: The three predominant characteristics of the BAT temperature profile are similar to those reported in core temperature studies, in both rats and monkeys (see Fuller et al, 1985; Refinetti & Menaker, 1991; Zerath et al, 1994). Gordon (1991) describes the several distinct phases of core temperature patterns in the rat: a stable daytime mean, a rising phase that begins approximately two hours before lights out, a stable and elevated nocturnal mean, and finally, a phase in which the temperature drops abruptly and shows a narrow trough before levelling out to daytime values. This results in a consistent saw-tooth shape, which can also be seen here in the BAT temperature patterns: a gradual rise at light offset with an abrupt drop at lights on. Examination of

the individual graphs in Figure 8, and the one that displays the hourly means computed from all of the data (Figure 9), indicates that the BAT temperature rise begins roughly three hours before light offset, between 15:00 and 16:00h.

Interestingly, the sawtooth pattern that results from this anticipatory rise is most obvious in the temperature profile from rat 890 (see Figure 10). Here the transmitter was implanted by bonding it to teflon first, instead of directly to the shoulder muscle, and the consequent isolation from the muscle appears to have accentuated the gradual daytime temperature rise. While the daytime oscillations are still present in the temperature profile of rat 890, the pattern is altered. In the previous graphs of data from the intact animals, the peaks and troughs appear to be cycling through a stable baseline value, until about 15:00h when the baseline slopes upward; however, in the graph of 890, the baseline begins to slope upward immediately after light onset. Perhaps the lower temperature of muscle (Closa et al, 1993) acts to dampen the transmitter output in the intact animals during the day, and thereby prevents the expression of any temperature increase until later in the day.

The graph of the averaged data (Figure 9) also reveals an early morning dip in temperature values that may be a robust feature of the individual temperature series. We speculate that this dip may be related to a consistent change in the oscillation pattern of each profile, whereby a downward slope appears in the baseline value of the ultradian peaks and troughs; in contrast, the baseline temperature value of individual profiles appears to

Figure Caption

Figure 9: This curve depicts the averaged BAT temperature profile that was obtained by collapsing half hour observations across eight rats; a total of 36 x 24 h periods were pooled. The SEM's were no larger than 0.1°C. The daily 12h dark period runs from 18:00 to 06:00h.

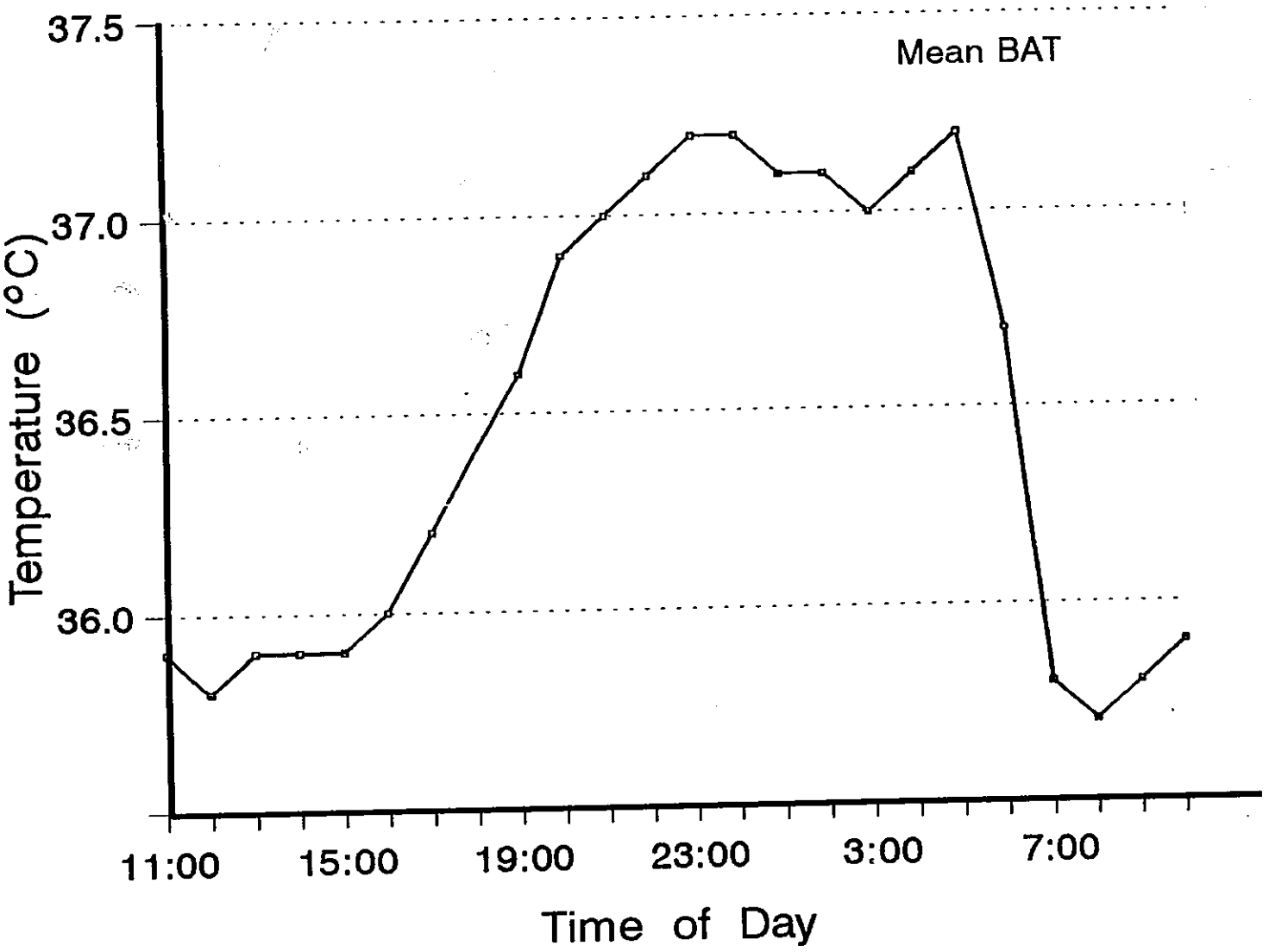
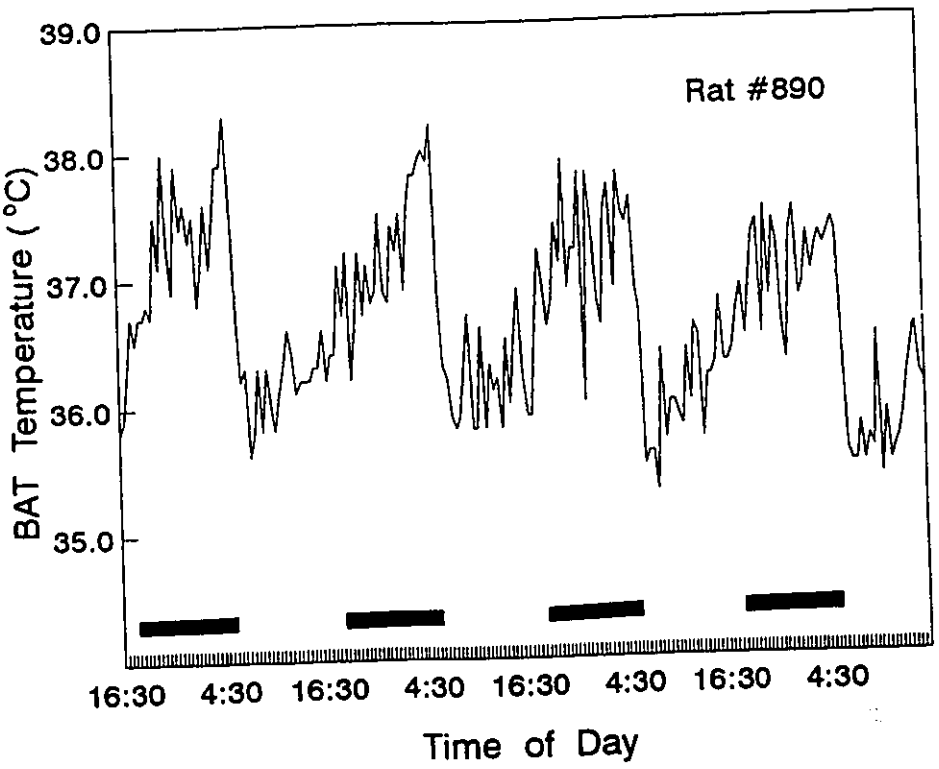
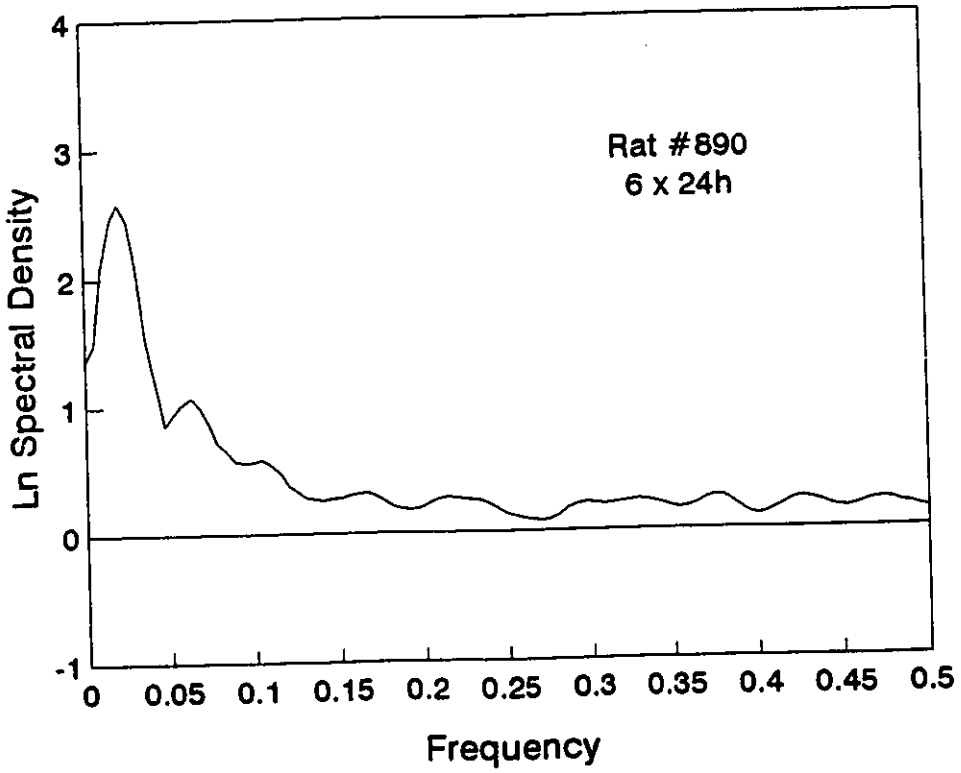

Mean BAT
37.5
37.0
36.5
36.0
Temperature (°C)
11:00
15:00
19:00
23:00
3:00
7:00
Time of Day

Figure Caption

Figure 10: The upper graph shows the BAT temperature profile recorded from one rat (890) for four consecutive days; in this case, the transmitter was glued to a piece of surgical teflon before placement under the BAT deposit, unlike the procedure which generated the earlier seven graphs (see Methods section). The black bars denote the daily 12h dark periods (18:00 to 06:30h).

The lower graph illustrates the spectral density graph that results from the Fourier analysis of the upper temperature series.


Rat #890
BAT Temperature (°C)
39.0
38.0
37.0
36.0
35.0
16:30
4:30
16:30
4:30
16:30
4:30
16:30
4:30
Time of Day


Rat #890
6 x 24h
Ln Spectral Density
4
3
2
1
0
-1
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
Frequency

slope upwards before midnight or so. However, the obvious variability in the duration and the period of the ultradian cycles, both within and between rats, must also contribute to the wide shape of the dip in the averaged waveform.

To further investigate both the profile characteristics and the underlying causes, the optimal procedure would be to collect simultaneous individual recordings of both BAT temperature patterns and those of a potential factor, such as food intake or plasma/CNS NE levels. The averaged data, by contrast, are less applicable here, and instead are important because the shape of the resulting profile (eg. Figure 9) represents the "circadian waveform" of the collapsed data; therefore, it can be used to mathematically subtract the circadian component from a temperature series.

METHODOLOGICAL FACTORS THAT INFLUENCE TRANSMITTER OUTPUT

As a consequence of the placement of the disc-shaped transmitter in the interscapular area, the temperature readings in this study may represent the combined influence of several tissue types and physiological processes. First, approximately one third of the transmitter protruded rostrally from the overlying BAT deposit, and therefore contacted the subcutaneous white fat. Second, in the case of four rats (700-881), the transmitter was bonded directly to the shoulder muscle; however, in the remaining three (rats 890, 897, 903), the transmitter was bonded to a piece of teflon first, and in that way isolated from muscle temperature. Third, it is known that brown fat activity

is sensitive to ambient temperature; given that the transmitter is not in a deep body position, it is important to ensure that temperature patterns are not due simply to room temperature changes.

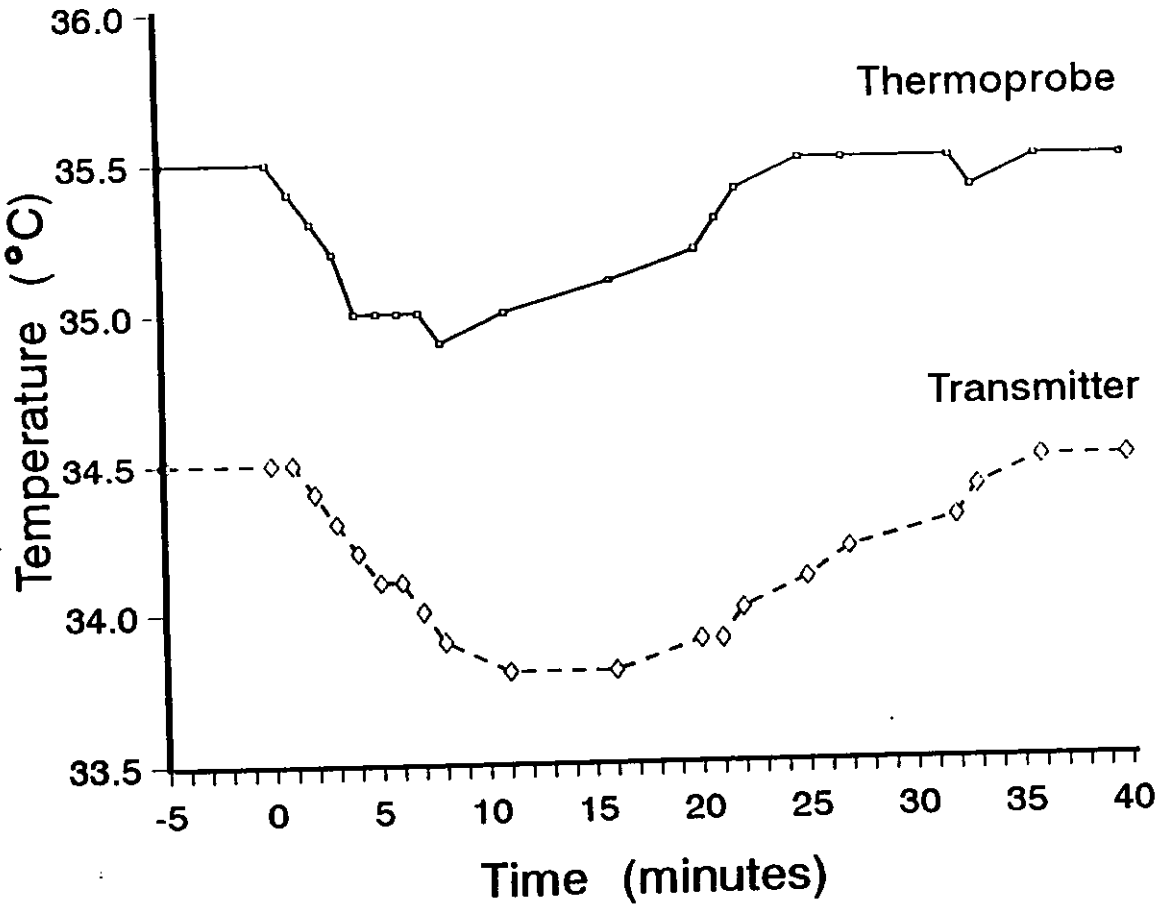
Results of Thermoprobe Comparison: As Figure 11 shows, the BAT transmitter readings are consistently almost exactly 1.0°C lower than the thermoprobe values throughout the recording period. This sytematic difference is most likely due to the additional input to the larger transmitter from cooler tissues in the interscapular region, and clearly contributes to the consistently higher BAT temperature values reported by Closa et al (1992; 1993) that were discussed earlier.

In addition, response time is slower in the case of the transmitter, although the magnitude of changes in the recorded temperature patterns are identical. The delay is particularly evident during calibration of the transmitter, when the temperature of a water bath rises by 1°C in 15 seconds; the transmitter takes approximately 60 seconds longer to register a stable temperature than the thermoprobe when the instruments are calibrated simultaneously. However, it is important to note that temperature changes do not occur that quickly in normal physiological settings.

Overall, the transmitter appears sufficiently accurate to reliably record BAT temperature changes, particularly as any observation reported here has been averaged over a six minute span of readings. Finally, the thrust of this work is to examine

Figure Caption

Figure 11: The graph depicts a comparison of the temperature readings from a thermocouple thermoprobe and XM-FH disc transmitter; the temperature profile represents the response of interscapular BAT to CNS stimulation. The stimulation was given at time 0, and the baseline values collected for five minutes prior to stimulation. Temperature values were monitored for 40 min, that is until baseline was reestablished. The thermoprobe readings of core temperature were displayed continuously on an LED output, and the transmitter readings shown every 3 seconds on a computer screen. Values were recorded whenever a change was noted.


36.0
35.5
35.0
34.5
34.0
33.5
Temperature (°C)
Thermoprobe
Transmitter
-5
0
5
10
15
20
25
30
35
40
Time (minutes)

BAT temperature profiles, hence relative changes, which appear to be faithfully recorded, are more important than absolute values.

NE Injection Control: Figure 12 illustrates one example of the BAT transmitter output in response to an injection of NE (rat 796): BAT temperature values increased by 1°C in ten minutes, while core values started to rise some three to five minutes after BAT values had begun to change. A second animal showed an identical response. These results are very similar to those obtained when the BAT response to NE injection is measured with thermoprobes, as reported in the previous section, thus conforming that the disc transmitters do index BAT thermogenic activity. In our set-up, and indeed in all BAT studies where the recording instrument is anchored on muscle, the temperature signal can be influenced by muscle. However, skeletal muscle is not considered a significant site of sympathetically mediated thermogenesis (Dulloo et al, 1988; Thurlby & Ellis, 1986).

In total, the two procedures described above provide evidence that increases in BAT thermogenic activity and blood flow are reflected in the temperatures recorded by the XM-FH disc transmitters.

Room Temperature Controls: Figure 13 shows the BAT temperature pattern obtained in two different rats (819, 890) during which period room temperature was simultaneously monitored. The transmitter output shows no obvious relationship to ambient temperature; there are neither parallel shifts, nor an

Figure Caption

Figure 12: The two profiles compare the temperature changes of the interscapular BAT area to that of core body temperature in response to systemic NE administration (4 mg/kg i.p.). Brown fat temperature was measured by a disc transmitter (Mini-Mitter, Co., Inc.) and core temperature by a rectal thermoprobe. The injection was given at time 0 on the abscissa.

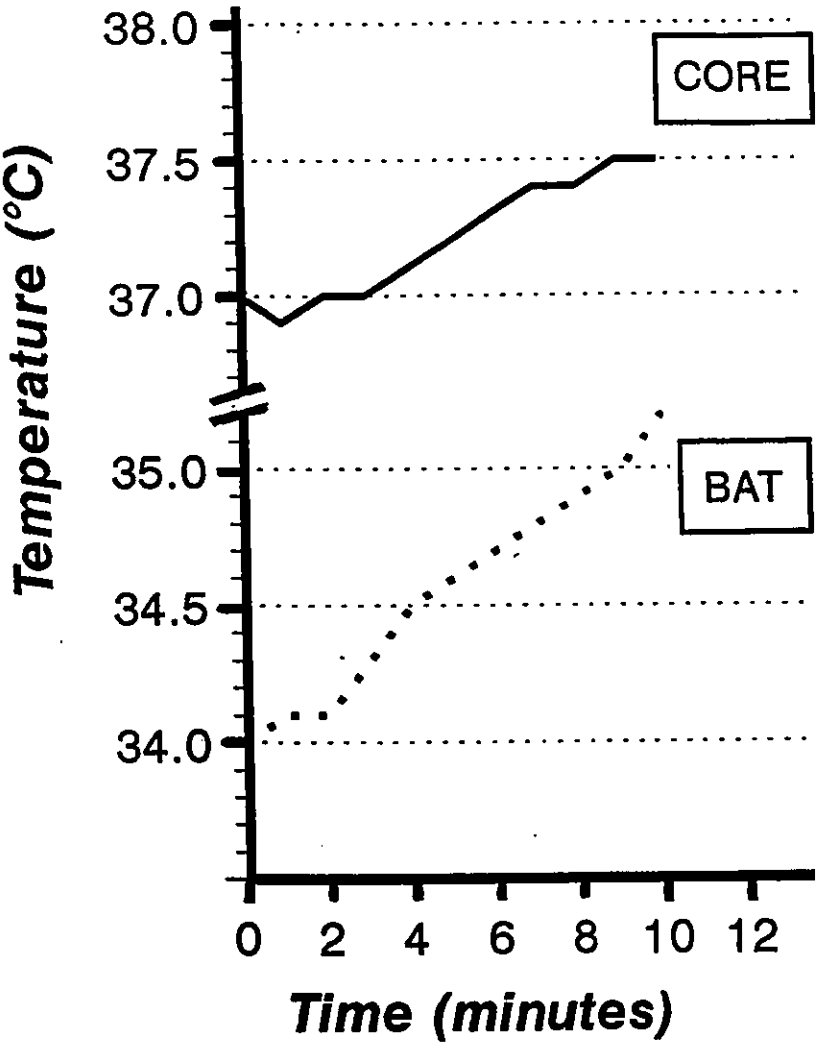
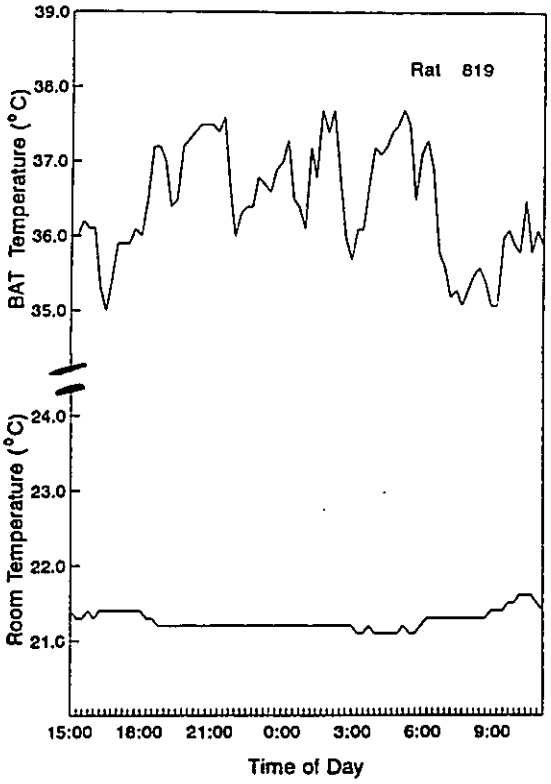
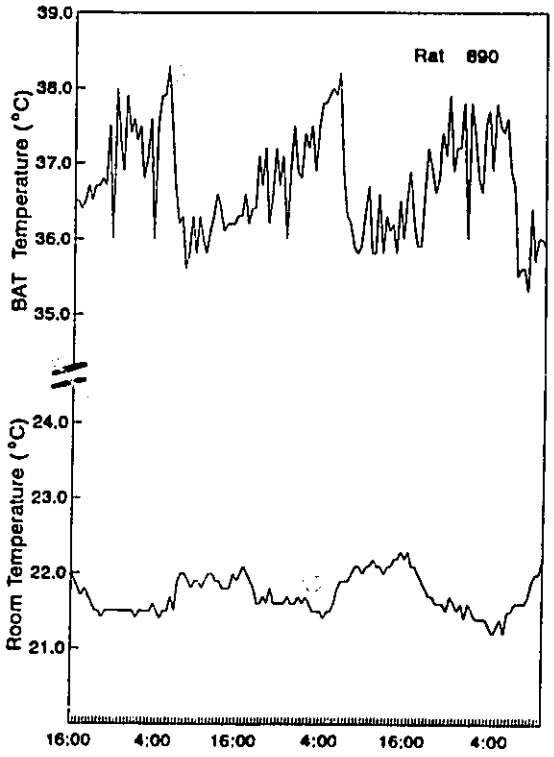

38.0
37.5
37.0
35.0
34.5
34.0
CORE
BAT
Temperature (°C)
0
2
4
6
8
10
12
Time (minutes)

Figure Caption

Figure 13: The graphs depict the BAT temperature profiles of two different rats against the corresponding room temperature record from the data collection period. In the case of 819, upper graph, temperatures were monitored for 21 h; for 890, lower graph, data were collected for 72 h. The BAT temperature values represent half hour intervals, averaged across the six minute period surrounding each 30 min. time point.


Rat 819
BAT Temperature (°C)
39.0
38.0
37.0
36.0
35.0
Room Temperature (°C)
24.0
23.0
22.0
21.0
15:00
18:00
21:00
0:00
3:00
6:00
9:00
Time of Day


Rat 890
BAT Temperature (°C)
39.0
38.0
37.0
36.0
35.0
Room Temperature (°C)
24.0
23.0
22.0
21.0
16:00
4:00
16:00
4:00
16:00
4:00

acute cold-induced response in BAT. In the upper graph, room temperature is very steady at 21.2 to 21.7°C, while it ranges from about 21.5 to 22.5°C in the lower example, but neither graph suggests that BAT temperature values respond to room temperature changes.

Conclusion from Results of Methodological Controls: The comparison between the thermoprobe output and that of the transmitter demonstrates that the two are highly correlated, although there is potential for as much as a three minute lag between temperature change and transmitter output if the change is large and rapid. In view of the fact that much of the analysis uses observations averaged across six minutes, this aspect should have a negligible effect on the results. Norepinephrine injections show that the transmitter does respond to changes in the heat output of interscapular BAT, and the room temperature data indicate that the ambient temperature does not significantly contribute to the changes in transmitter output. Finally, when each transmitter was removed, it was observed that the brown fat deposits had adhered to its surface. Together these results constitute strong evidence that the transmitter output is primarily influenced by temperature changes in the brown fat tissue.

SOURCE OF HEAT OUTPUT PATTERN

Beyond the methodological issues, however, is the more challenging question of the underlying cause of the changes in

BAT temperature. Temperature values represent the net gain or loss of heat from a region; in this study, the transmitter output is clearly responsive to heat production by brown fat, but is also inevitably influenced by other tissues, such as white fat, and by the range of circulatory factors.

Circulatory Factors: The BAT vasculature comprises vessels, capillaries, arterioles, and AVAs (Flaim et al, 1976; Nnodim & Lever, 1988; Norman et al, 1988). Although regulation of the sympathetic vasoconstriction of this vasculature is not completely understood, there is evidence, in some stimulation situations, for separate CNS regulation of the various components (eg. Engel et al, 1992; Woods and Stock, 1994). Chemical vasodilators may further regulate the arterioles or AVAs, through possible axon reflexes, or simply by nerve terminals that can act as both afferent and efferent fibres (see Lisney & Bharali, 1989). For example, the heat released by thermogenesis may act on nerve terminals, and cause them to release substances which act on vessels to induce further dilatation; this in turn allows more fuel and oxygen for thermogenesis, and removes metabolic waste products.

As described earlier, distal vasomotor tone is regulated cyclically by the thermoregulatory system in order to modulate core temperature; however, this circulatory aspect should not affect our transmitter output because, first, the transmitter is isolated from skin circulation by the WAT deposit above it, and second, the interscapular skin can be considered a proximal area

and the temperature patterns of proximal skin regions are distinct from those of distal areas. Proximal temperature patterns have been reported to be similar to those of core, but either slightly phase delayed (Fuller et al, 1985) or phase advanced (Krauchi & Wirz-Justice, 1994). This is in contrast to distal areas which are commonly observed to be phase advanced, and show a mirror image waveform (Smolander et al, 1993; Krauchi & Wirz-Justice, 1994).

When all known BAT control mechanisms are considered, it is highly unlikely for an increase in BAT blood flow to occur without concurrent thermogenesis, thus allowing the conclusion that any recorded rise in transmiter temperature can be related to a genuine BAT temperature rise. The exception may be those increases related to increased cardiac output (Yahata et al, 1983; Thornhill & Halvorson, 1993), but it appears that such events do not cause either an increase or decrease in temperature recordings from the interscapular BAT area.

However, while studies on anaesthetized rats allow direct measurement of both temperature and blood flow in various stimulation situations, the results may have limited relevance for understanding physiological changes in conscious animals. The most problematic factor in the interpretation of transmitter output is the possibility that the transmitter may not show a rise in temperature even when thermogenesis is occurring, because the heat produced is being removed too fast by the blood flow (Benzi et al, 1988; Himms-Hagen, 1991; Closa et al, 1993). This discrepency is most likely to occur at the start or the cessation

of a particular stimulated thermogenic response. However, even though BAT temperature readings can not be strictly correlated with thermogenic activity, it has been demonstrated in this study that when enough heat is released by BAT, the transmitter does reflect that change, owing both to its position, and the speed and accuracy of the response.

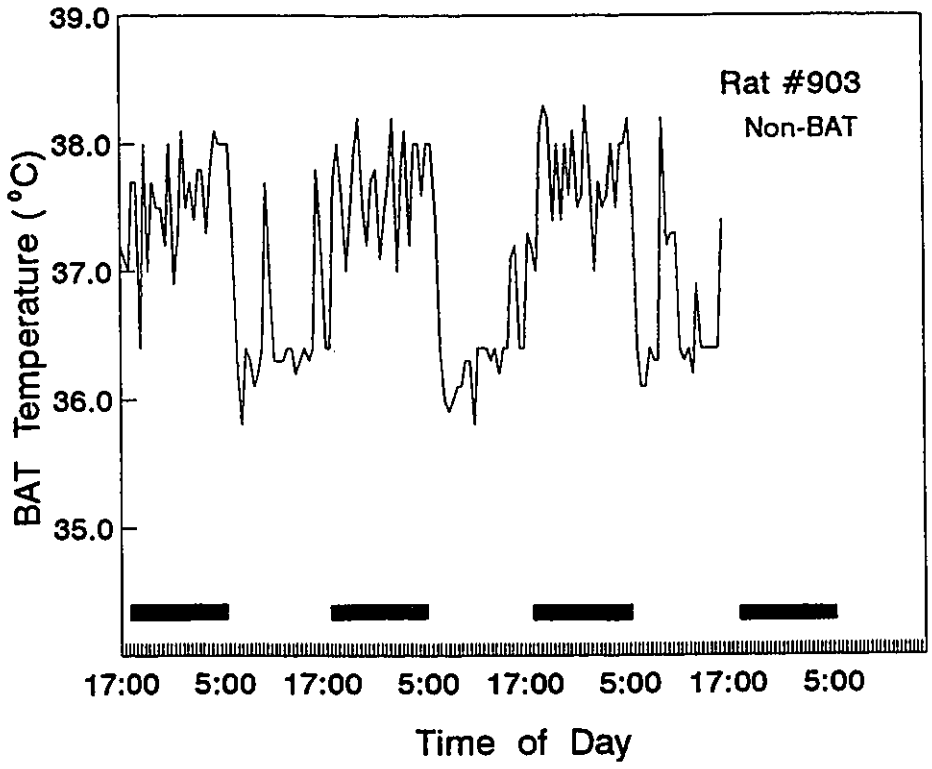
In our study, these issues were addressed by means of two manipulations. The BAT deposit was removed in one case, with the white fat left as intact as possible in order to ensure that any sympathetic influence on WAT remained unchanged. The output was compared to that of normal conditions, where brown fat tissue is in contact with the surface of the transmitter. In the second manipulation, the interscapular BAT deposit was denervated to determine the role of sympathetic input on the temperature patterns recorded by the transmitter.

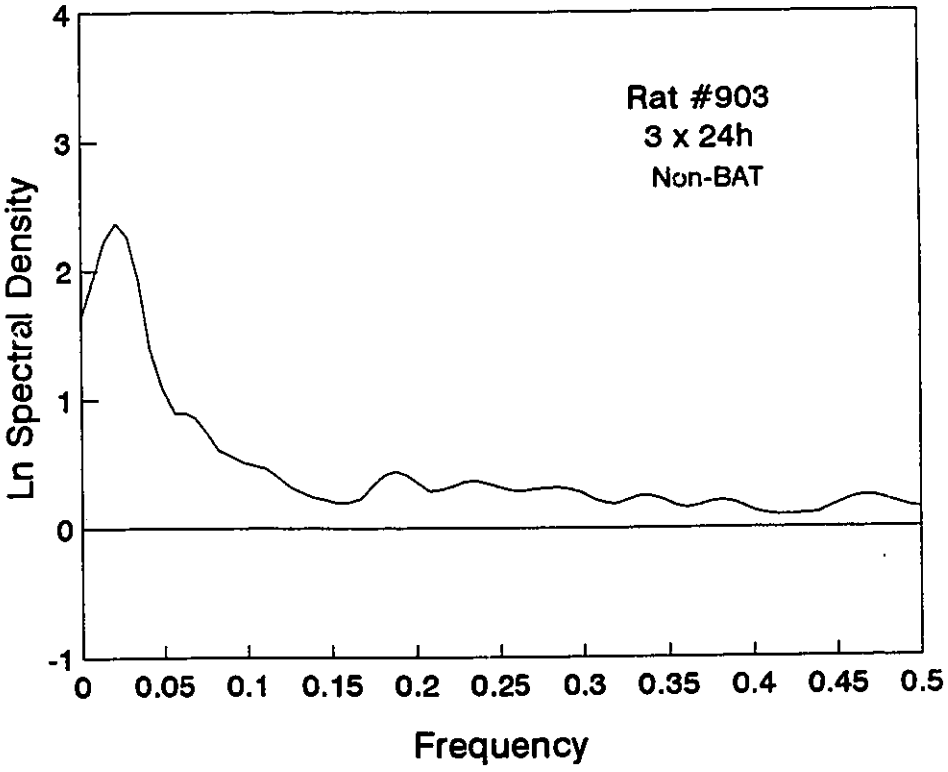
Excised BAT: The data from the animal that had the BAT deposit removed prior to transmitter placement are a vivid demonstration that brown fat heat output plays a somewhat subtle role in transmitter output. Indeed, at first glance, the brown fat temperature pattern in Figure 14 (upper graph) is very similar to that of the intact subjects. There is an elevated nocturnal baseline, with continuous peaks and valleys superimposed on the circadian rhythm. What distinguishes this pattern from the ones that were described earlier is the absence of a clear, consistent sawtooth pattern. Instead of the gradual, jagged slope observed at light offset in the intact animals,

Figure Caption

Figure 14: The upper graph illustrates the temperature profile obtained from a rat which had all visible interscapular brown fat removed before the transmitter was bonded in position beneath the remaining white fat. The black bars denote the 12h dark periods.

The lower graph show the results of spectral analysis performed on the above temperature series; the abscissa indicates the Fourier frequencies examined in the analysis, while the ordinate shows the power of each observed spectral peak.


Rat #903
Non-BAT
BAT Temperature (°C)
39.0
38.0
37.0
36.0
35.0
17:00
5:00
17:00
5:00
17:00
5:00
17:00
5:00
Time of Day


Rat #903
3 x 24h
Non-BAT
Ln Spectral Density
4
3
2
1
0
-1
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
Frequency

there is an abrupt, sheer rise, so that the slopes at both light changes are similar. This contrasts most strikingly with that of rat 890 (see Figure 10), where the BAT temperature rises steadily from the time of light onset and thus prevents the appearance of any stable daylight baseline. In both of these animals, 890 and 903, the transmitter was first glued to a piece of surgical teflon and then implanted, so that it was isolated from the effects of muscle activity. As a result, the temperature pattern in the subject with BAT deposit excised is an appropriate comparison for distinguishing between BAT input and that of other tissues.

The mean BAT temperature for rat 903, 37.1±0.06°C, is higher than that of the intact animals, 36.4±0.02°C. Since both sets of values displayed similar maximums, the disparity in means appears to be a direct function of the observed minimum values; the lowest reading in rat 903 is 35.8°C, while that of the intact group is 35.1°C. As a result, the daytime pattern also appears to be different than that of the intact animals, with fewer ultradian oscillations, and a smaller amplitude to the cycles. The effect of disturbance due to animal care can be seen only in first lights-on period.

As there was no apparent behavioural difference between the animal with BAT excised and the intact ones, either in activity level or food intake, the flattened daytime series in the non-BAT subject suggests that the typical daytime pattern of transmitter output in intact animals is indeed related to heat released by brown fat. In contrast, the persistence of the general shape of

nightly ultradian cycles suggests that they are due to factors other than BAT heat; these could include motor activity, WAT circulation, or general changes to proximal body temperature in response to inner core changes. It is also possible that residual BAT tissue, not visible during extraction, contributed to the observed pattern.

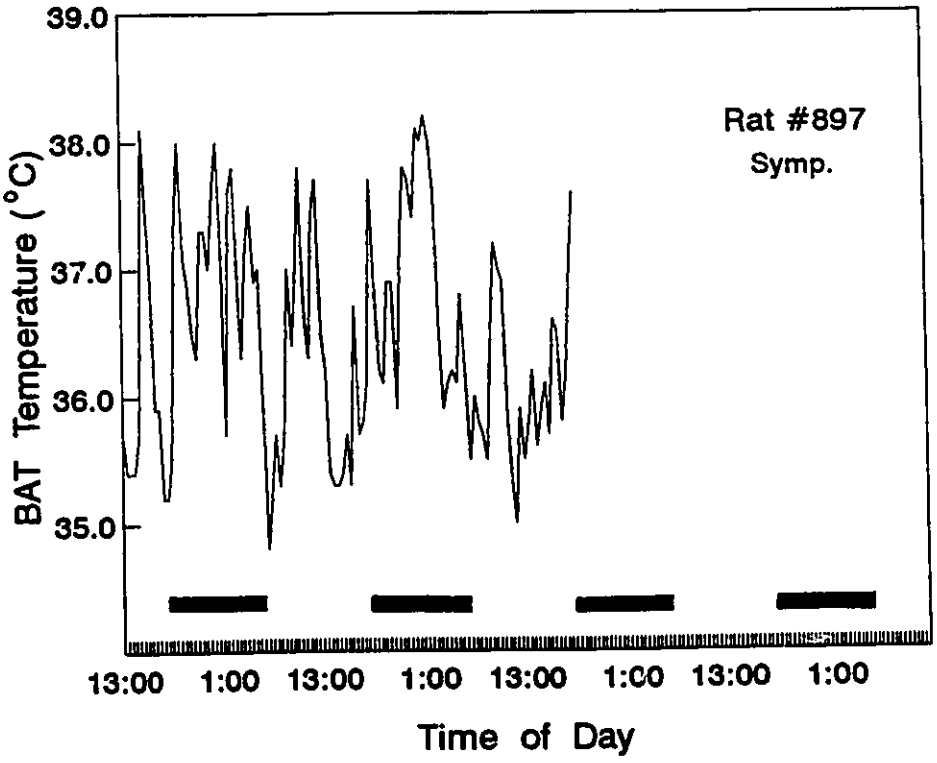
Considering that the temperature profile of a transmitter sitting loosely in the interscapular area displays a similar, clearly circadian pattern to rat 903 (data not shown), the results from the animal with the BAT deposit removed are consistent with expectations, and agree with the finding that BAT heat production preceding the dark phase contributes to nocturnal core temperature; the remaining inner body BAT deposits continue to contribute heat to the core, and in fact, are likely more important for that purpose due to their mass and position (Himms-Hagen, 1976; Matthias et al, 1994). Further data collection is needed to confirm this finding; to our knowledge, this important control condition has not been evaluated in any other study of chronic BAT temperature.

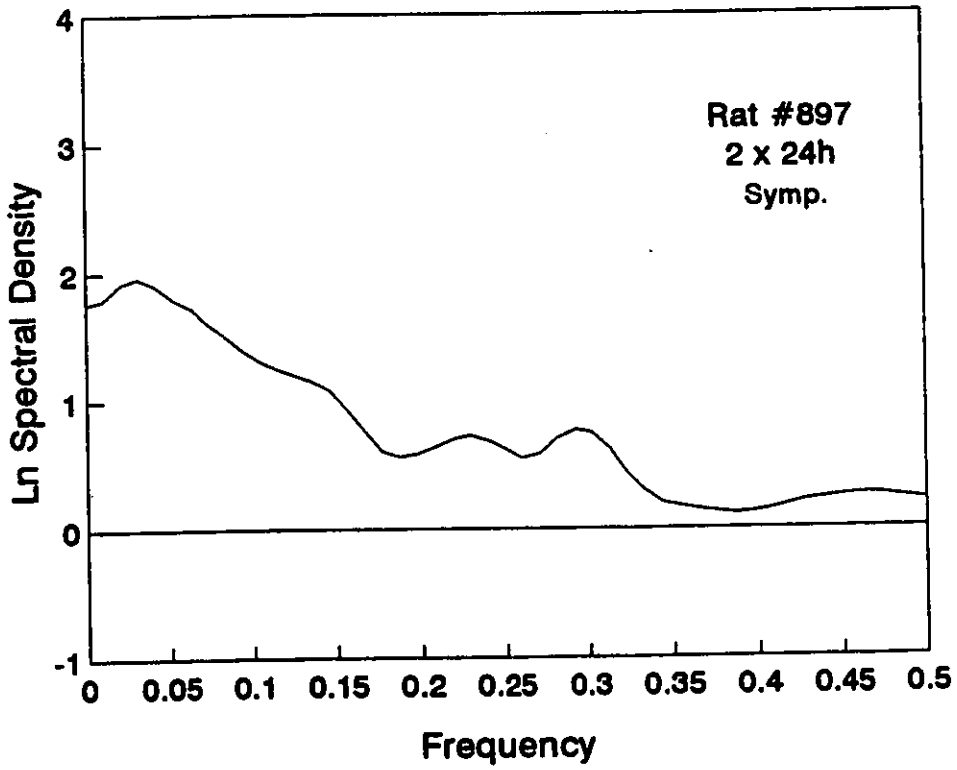
Denervated BAT: In contrast to the removal of the BAT deposit, the surgical denervation of BAT does appear to evoke a striking alteration of the daily temperature profiles, as Figure 15 illustrates. We can report on only one animal, however, as the denervation procedure, perhaps in combination with pressure from the transmitter, apparently produced irritation of the skin surrounding the implant site. The animals scratched the area,

Figure Caption

Figure 15: The upper graph shows the BAT temperature profile recorded from a rat which had undergone surgical denervation of the interscapular deposit immediately before the transmitter was bonded in place. The black bars denote the daily 12h dark periods (18:00 to 06:30 h), and the values on the graph represent half hour intervals.

The lower graph shows the results of the spectral analysis of the above series; the abscissa indicates the Fourier frequencies which result from the analysis, while the power or relative magnitude of the spectral peak at each frequency is depicted as height on the ordinate.


39.0
38.0
37.0
36.0
35.0
BAT Temperature (°C)
Rat #897
Symp.
13:00
1:00
13:00
1:00
13:00
1:00
13:00
1:00
Time of Day


4
3
2
1
0
-1
Ln Spectral Density
Rat #897
2 x 24h
Symp.
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
Frequency

and thereby induced swelling; it is likely that there was no cutaneous feedback to inhibit the scratching behaviour since nerves to the skin were severed with the denervation (Foster et al, 1982b; Engel et al, 1992).

The BAT tissue was assayed for NE levels using HPLC analysis, as was the tissue of several other denervated and control animals. The results are summarized in Table 2, and confirm that the denervation procedure was successful. Both the control and denervated levels are in line with previous reported values (Foster et al, 1982a and b; Minokoshi et al, 1986).

The primary difference in the temperature pattern from the denervated animal is that any existing nocturnal elevation of temperature is obscured by both large amplitude ultradian cycles, and the sustained periods of elevated temperature in the daylight hours. Values appear to lack typical daytime or nighttime restrictions; note the peak of 38.0°C in the daytime, and one of 36.0°C at night. Neither pattern seems to be the result of spurious values, as similar ones precede and follow the peaks. Room temperature was between 21.3 and 21.9°C during collection period.

The characteristic jagged, gradual rise at lights-out and the steep drop at lights-on are reversed in several instances, and therefore appear to be inconsistent. The transmitter was bonded to teflon, similar to both animals 890 and the one with the BAT deposit removed, 903, but the denervated animal showed the lowest temperature values. Similar to the outcome of all implantations where the denervation procedure was performed, the rat discussed

Table 2: Norepinephrine levels of brown fat tissue: control and denervated

RAT ID#	ng NE/g TISSUE	MEAN ± SEM
Control		
#890	851.5	
#900	1206.0	
#902	560.5	
#903	870.0	
		872.0± 132.0 ng/g
Sympathectomized		
#897	25.3	
#898	3.6	
		14.5± 10.9 ng/g

here did scratch the shoulder area; the data collection period was kept short, and because no swelling was observed when the transmitter was removed, the data were retained.

It is difficult to interpret the role of the SNS in the BAT temperature profiles from the large amplitude swings of the denervated animal, other than noting the fact that denervation did substantially alter the transmitter output. It is possible that the scratching itself influenced the temperature profile even in the absence of a swelling reaction.

CONCLUSION: TEMPERATURE PROFILES

In summary, two essential and substantive questions are addressed in this section: what do the temperature patterns of interscapular BAT look like and what do they represent. Methodological studies confirm that the transmitter output is responsive to BAT changes and that it accurately mirrors the output of a thermoprobe. Room temperature is shown not to be a significant contributing factor, and finally, in all cases, the transmitter is in physical contact with the BAT deposit.

The primary contributing factors to the transmitter output are considered to be one, the level of BAT thermogenesis, and two, the related increases in blood flow. Any changes in the circulation of the surrounding WAT due to sympathetic regulation (Youngstrom & Bartness, 1995) may also play a role in the transmitter signal, as may the general temperature changes that a proximal body area is likely to exhibit as a straightforward consequence of changes to deep body temperature. Muscle

temperature is not likely to make a strong contribution to the temperature oscillations, as it is reported to be lower than that of BAT throughout the 24 h (Closa et al, 1993).

The most significant finding from the profiles of the temperature data is the presence of an anticipatory rise in BAT temperature prior to light offset. The data from intact animals all show the distinctive sawtooth profile, but this shape is demonstrated most clearly in the case of rat 890 (see Figure 10) where the transmitter was isolated from the muscle by a piece of teflon. The ultradian peaks and troughs are still visible, but they oscillate through a mean value that steadily increases from the point of light onset, in contrast to the apparently stable baseline observed in the other profiles.

While almost all tissues in the body demonstrate a circadian temperature pattern (eg. Fuller at al, 1985; Closa et al, 1993), the precise values and shape of the profiles vary systematically, as do their phase relationships to core temperature. The temperature profiles of various tissues can also be categorized by the presence or absence of anticipatory rises prior to light offset; the appearance of a rise prior to light offset is indicative of endogenous control of core temperature by a pacemaker, and refutes the notion that nocturnal rises are merely responses to cyclic environmental conditions or to muscle movement. Thus, the significance of anticipatory rises in BAT temperature is that they may contribute to the cyclic elevation in core temperature.

Thermal Regulation and BAT: The precise mechanisms underlying the nightly rise in core temperature in rats are not known, although it has been demonstrated that the change is not simply a consequence of motor activity (Honma & Hiroshige, 1978; Refinetti, 1994). Brown and coworkers (1991) suggest that the nightly supplementary heat in rats maintained at room temperature may be due to muscle tone from the huddled postures that the animals are observed to adopt. Closa directly addresses the role of brown fat in altering core temperature, by demonstrating that BAT blood flow increases during the warming portions of the peaks and troughs visible in core temperature profiles (Closa et al, 1993). Core temperature has been shown to be influenced by BAT heat production, in studies of both CNS-stimulation induced and cold-induced thermogenesis, and in both anaesthetized and conscious animals (eg. Foster & Frydman, 1979; Amir et al, 1989; Fyda et al, 1991). Taken together with the evidence for thermal regulation of BAT thermogenesis, and the finding of anticipatory increases in the BAT profiles, these results argue for a role for BAT in the nocturnal increases in core temperature.

Our temperature profiles, along with those of Closa's group (1992; 1993) support the hypothesis that BAT thermogenesis is more active at night. This is in line with the associated nocturnal elevation of both food intake and sympathetically mediated O_2 consumption (Le Magnen, 1985). However, studies of the daily changes in BAT activity have reported conflicting results (eg. Rothwell et al, 1983; Sakaguchi et al, 1988). Some of the discrepancy in the literature is the result of the various

measures used to assess sympathetic activity (Sakaguchi et al, 1988). The measurement of the SNS firing rate, for example, considers only input to brown fat tissue, not output; plasma NE is known to be a poor index of sympathetic activity due, first, to the large number of factors that affecting the spillover of NE from cleft to bloodstream and, second, to the variable contribution of the adrenal gland to plasma NE (Fillenz, 1990).

Finally, regulation of the sympathetic system is clearly complex and highly differentiated among tissues, so that the various branches may show different levels of excitation simultaneously. For example, the mass discharge of SNS output associated with the emergency flight or fight reaction leaves certain targets untouched (Guyton, 1991). And as discussed in the first study, the tissues that respond to VMH stimulation may display a variety of response patterns (eg. Shimazu, 1981; Morimoto et al, 1986; Arase et al, 1987; Kelly & Bielajew, 1991).

Our study, which directly measured brown fat temperature changes, supports the hypothesis that BAT heat production is increased at night, and also demonstrates that the temperature increase anticipates light offset. This pattern provides additional evidence that the transmitter output is not a simple reflection of heat from motor output or core temperature changes, but, more significantly, it suggests that BAT thermogenesis is driven by an endogenous pacemaker, similar to core temperature, motor activity level, and other processes. In order to further explore the hypothesis that BAT heat production cycles underlie those of core temperature, whether circadian or ultradian, a

a description of the periodicities in BAT temperature pattern is required.

Part B: Fourier Analysis of BAT Temperature Rhythms

STATISTICAL BACKGROUND

The following section presents a brief description of the basic principles underlying spectral analysis; discussions of the various strengths and weaknesses of this technique appear in each section as the results are presented. This summary is drawn from a number of sources (Bloomfield, 1976; Kendall, 1976; Chatfield, 1980; Fuller, 1976; Dowse & Ringo, 1989; Diggle, 1990; Christensen, 1991) which should be consulted for more detailed information.

Time series spectral analysis, or Fourier transformation, represents the mathematical equivalent of separating light into its component colours by a prism, hence the name. The procedure decomposes a series of events (eg. hourly temperature data) into its underlying cycles, which are based on sine and cosine waveforms. The analysis was designed for a specific type of series, and the main characteristic is that the series presents a stochastic, rather than deterministic process. Thus each sequential observation is viewed as a random variable whose value is selected according to a Gaussian normal probability distribution; in other words, the series could be described by specifying the joint probability distribution associated with each observation. In spectral analysis, the series is characterised by its mean and covariance. This leads to the

mathematical requirement that the mean and covariance remain stationary. For this to be true, they must not increase or decrease in the long run; however, short series of biological data tend to violate this requirement due to individual variability, and the it appears that spectral analysis can accommodate a moderate level of non-stationarity (Ortega et al, 1994).

Economic analysis is done largely in the more intuitive domain of time, rather than through frequencies. The time domain generally uses an autocorrelation method to decompose the data, whereby the series is correlated with itself after offsetting by specific lags; this allows an analysis of the underlying cycles, and thus the development of a specific model to forecast future patterns. Spectral analysis seeks to describe similar data, but does it in terms of underlying frequencies, or periods, instead of comparing the observations at offset time 1, and 2 and so on. While the information is mathematically equivalent, the two types of analyses bring out different features of a series; for example, spectral analysis is most often used to search for hidden periodicities when there are no expectations about underlying cycles. Spectral analysis has been likened to moving a radio dial and noting where the signal to noise ratio is the strongest (Kendall, 1976).

Just as the sums of squares represent a measure of the variability in ANOVA, so the variability of a time series is decomposed into cosine functions representing each possible period length (or frequency). The frequencies examined are

limited to whole numbers: the first is 1 cycle per total number of observations, the second is 2 cycles per total number of observations, and so on. These cycles are termed "Fourier frequencies", and they appear on the abscissa of the periodgram produced by spectral analysis (see Figure 8, column B for examples).

The shortest possible cycle in every series is described by the final Fourier frequency of 0.5 - for example, 24 cycles/48 observation, 72 cycles/144 observations, and so on. This is illustrated by the periodgrams in Figure 8, as all of those series are based on either 2 x 48 observations, 4 x 48, or else 6 x 48. Thus, the final Fourier frequency on the x-axis of these periodgrams is, respectively, 48/96, 96/142, and 144/268.

The ordinate of the periodgram displays the value of the term "X_T" for each Fourier frequency, or cycle. This term describes the amplitude and the phase of the cosine function, although the terms are then rewritten using the cosine law in order to involve sinewaves as well. Each ordinate term X_T is defined by the following formula

$$X_T = \frac{2}{n} \{(\sum X_T \cos(\frac{2\prod k}{n})t\}^2 + \{(\sum X_T \sin(\frac{2\prod k}{n})t\}^2$$

which uses the original observations to denote the "power" or importance of each Fourier frequency in the overall pattern. Thus, each frequency on the abscissa is represented as power or height on the ordinate (See Figure 9 for examples).

The BAT circadian cycle (1 cycle/24 h, or 1 cycle/48 observations, etc) is visually obvious due to the consistently

elevated baseline of the dark period temperatures; however, the ultradian rhythms tend to be more difficult to see, for a number of reasons. First, it is likely that several different ultradian frequencies of varying strength make up the final waveform, and the eye is unable to separate these. Second, biological time series data are well known to contain a high level of noise (Mercer, 1965; Dowse & Ringo, 1989; Diggle, 1990) due to short-term imprecision in the regulation of metabolic processes, as well as in recording procedures. Thus, spectral analysis is useful a procedure to uncover "hidden" ultradian periodicities (Dowse & Ringo, 1989). The presence of infradian cycles, which repeat less than once per day, is also possible but there is little evidence for endogenous cycles longer than a day, except for seasonal changes related to changing levels of light (Saunders, 1977).

RESULTS OF FOURIER ANALYSIS

Fourier analysis was performed on all ten of the BAT temperature series described in the previous section. Recall that sharp, narrow temperature peaks occurred during documented animal care periods; these were replaced with interpolated values only for those series based on six days, as preliminary analysis indicated that the presence of the peaks did not change the results in shorter series. There is no satisfactory method of dealing with outliers in time series analysis, but because they can have a tremendous distorting effect on the results, the replacement of the outliers with values closer to the mesor of

the series is suggested (SPSS, Inc., 1990; Christensen, 1991; Refinetti, 1992). The ideal solution is simply not to have any outliers (A. Dabrowksi, personal communication); for example, housing which can be maintained with only weekly cleaning may be used (eg. Wollnick et al, 1987).

The lower panel of Figure 10 shows an example of a periodgram, based on six days of observations (rat 890). The largest spike can be seen at a frequency of 0.02, which corresponds to 1 cycle/48 observations, or 1 cycle per 24h. The results of all spectral analyses can be seen next to the corresponding temperature profiles, both in Column B of Figure 8 and in the lower panels of Figures 14 and 15. All periodgrams are based on 30 minute intervals; values were obtained by averaging the readings from the six minute period surrounding each half hour time point. Smoothing, or windowing, was then applied to each spectral graph in order to assist in distinguishing peaks from noise in the periodgram spectrum (Chatfield, 1980; SPSS, Inc., 1990; Refinetti, 1993).

Smoothing: The process of smoothing a periodgram means that the terms comprising the ordinate values for each Fourier frequency on the abscissa are averaged with one or more values on either side. This procedure causes the peaks in the transformed periodgram to appear broader and more blunt than those of the original; the larger the span across which the original values are averaged, the more rounded the spectral peaks become.

Smoothing eliminates small, spurious peaks by reducing noise;

it does this at some cost to resolution of exact peak position, but the ability to detect cycles, or sensitivity, is unaltered (Chatfield, 1980; SPSS, Inc, 1990; Refinetti, 1993). The aim in applying the procedure is to find the span that prevents the appearance of double peaks, without smoothing the periodgram so much that no peaks are visible (Bloomfield, 1976). In this study, a window of span size 9 was typically applied, because larger spans tended to obliterate all peaks except the circadian one. Therefore, those peaks remaining after "windowing" were counted as representing a cycle.

Circadian Cycles: A summary of the spectral peaks observed in each analysis (Figures 8, 10, 14 and 15) is presented in Table 3; note that the dotted line separates those animals that had the BAT transmitter implanted with a teflon tag (890, 897, 903) from those that had the transmitter bonded directly to the muscle. The pattern of each series, except for the one that represents the denervated animal (897), was dominated by the spectral peak at the circadian frequency of 0.02, as was anticipated from visual inspection of the profiles of the individual temperature series. One series displayed a less distinct, but still strong, circadian spike; this was the short series associated with rat 757 that had only 2 x 24h periods of data collection. The total number of Fourier frequencies in the periodgram is, of course, less in the abbreviated series, and consequently the peaks show leakage, or less resolution, between the frequencies, as compared to periodgrams generated from 4, or 6, x 48

Table 3: Frequency peaks from spectral analyses of all BAT temperature data

Rat ID#	24h	8h	6h	4.8h	4h	1.7h	Series Length
700	P	P	--	--	P	--	6 x 24h
757	P	--	--	--	--	--	2 x 24h
745	P	--	--	--	--	--	4 x 24h
771	P	--	--	--	P	--	4 x 24h
799	P	--	P	--	P	--	6 x 24h
819	P	P	--	P	--	--	4 x 24h
881	P	--	--	--	--	--	6 x 24h
890	P	P	--	--	--	--	4 x 24h
897	--	--	--	--	--	P	2 x 24h
903	P	--	--	--	--	--	3 x 24h

P : Spectral Peak appears at corresponding Fourier frequency

--- Only animals below line had transmitter implanted using surgical teflon

observations (Mercer, 1965; Diggle, 1990). However, the underlying circadian cycle remains strong, as evidenced by the distance of the peak above baseline noise levels.

In the case of the data from the denervated rat, 897 (Figure 15), the circadian pattern is harder to visualize in the temperature graphs as it is overwhelmed by the large amplitude ultradian swings. Further, while the largest spike on the spectral periodgram appears to be circadian, it is actually located at a frequency of 0.31 instead of the circadian frequency of 0.21. This represents a cycle of 16h, making the denervated series the only one to show any deviation from a strictly circadian pattern. The significance of this, if any, is unknown.

Ultradian Cycles: The summary presented in Table 3 also indicates the ultradian cycles that were visible in the ten periodgrams. It can be seen that no single ultradian cycle length is pervasive across the series, although it should be noted that, in any case, the results from the longer time series are more reliable. Only the long series from rat 881 failed to show a peak at any ultradian frequency, and there are no behavioural or environmental observations to account for this. Given the appearance of continuous peaks and troughs superimposed on all temperature profiles, we had expected to see evidence of short, high frequency cycles in the analysis of the data from intact animals, similar to those reported by Closa after analysis of core temperature data (Gomez-Sierra et al, 1993). However, only the periodgram of the denervated animal showed statistical

evidence of such a cycle.

The findings from the two animals in which brown fat was either denervated or extracted are presented first, followed by a discussion of a number of subtle aspects of the analysis which will facilitate interpretation of the results from the intact animals.

Denervated rat: The periodgram of the data from the denervated rat is the only one to show a cycle smaller than 4h, despite numerous reports of 60 to 120 minute ultradian cycles in other systems (Hildebrandt, 1988; Stupfel & Pavely, 1990; Gomez-Sierra et al, 1993). The most obvious difference between the profiles generated from the denervated rat and the intact animals is the large amplitude of the ultradian oscillations in the former; a single cycle swings from 35 to 38°C in the denervated animal, in strong contrast to the 0.3°C range from peak to trough in the intact subjects (for comparison, see Figure 8, Column A vs. Figure 15).

It is possible that a large amplitude is required in order for the ultradian oscillations to appear as a formal cycle in mathematical analysis, especially in light of the clearly overwhelming influence of the circadian cycle in the intact animals. Alternatively, the amplitude of the cycles may play no role at all, and instead, there is simply too much variation in the oscillations of each temperature profile to allow spectral analysis to discern them, unlike the situation with the denervated animal.

Brown Fat Excision: The findings from the animal with the BAT deposit removed indicate no loss of the circadian cycle, as corroborated by visual inspection of the temperature graph. There are no peaks present at any ultradian frequencies in the periodgram (Figure 14, lower panel). However, the apparent absence of ultradian cycles may be misleading because 1) the series is short and 2) there are periodgrams based on data from intact animals which also lack ultradian peaks. Given that the only visible change in the temperature graph from this animal was the loss of the gradual temperature climb prior to lights-out, the spectral results were within expectations.

ISSUES IN SPECTRAL ANALYSIS

The suitability of spectral methods for statistical evaluation appears to be a point of debate (Diggle, 1990; Enright, 1990; Dowse & Ringo, 1991), but in studies where the thrust of the design is to determine if circadian cycles have been eliminated by a particular manipulation, the apparent absence of the circadian peak in the periodgram can be statistically confirmed by chi-square analysis (Sokolove & Bushell, 1978; Mitsushimi et al, 1994). In this case, because we are investigating trends in ultradian periodicities, and because series of varied lengths are being compared, statistical tests are inappropriate.

Chi-square analysis could be applied to the data associated with the denervated rat, but the series is too short (Sokolove & Bushell, 1978; Diggle, 1990) for the procedure to be meaningful.

Furthermore, since the predominant cycle is not circadian, it would be important to collect more data to ensure that the result was not specious. Fisher's test for hidden periodicities (Brockwell & Davis, 1987) has been suggested as a measure of whether the periodgram contains a value substanitally larger than the average value (ie. white noise); however, again the teest requires a minimum data set, and further, the computer program used for the entire analysis (SPSS Inc., personal communication) is unable to perform this test, nor any other test of signifiance for individual cycles.

One of the potential difficulties in interpreting spectral graphs is the possibility of "aliasing" (Koopmans, 1974; Kendall, 1976); this occurs when observations are collected at intervals that are too wide to allow the expression of an underlying cycle. For example, if there is a cycle that repeats every 15 minutes, but data are collected only every half hour, then the appropriate frequency cannot even be part of the Fourier analysis. This will force the cycle to appear as a peak elsewhere, at another frequency, and may lead to an incorrect interpretation. To avoid this problem, a representative series should first be analyzed at the smallest interval possible, to demonstrate that no high frequency cycles are present.

It is difficult to argue the existence of cycles of less than half an hour duration in metabolic systems, given the inherent looseness of minute by minute thermal regulation (Gordon, 1990; Stupfel & Pavely, 1990), but nonetheless, the series for one subject (rat 745) was reanalyzed using 10 minute rather than 30

minute intervals. The resulting profile was indistinguishable from that shown in Figure 8, third panel, indicating that there is no evidence of cycles less than 30 minutes duration. In addition, the periodgrams themselves provide support for this conclusion. Inspection of each one prior to the smoothing process reveals that the spectral density is close to zero where the ordinate and abscissa meet; this suggests that the choice of sampling interval was sufficiently small (Chatfield, 1980).

Confound of Harmonics in Ultradian Analysis: Before discussing the observed spectral peaks, one further issue needs to be addressed. "Harmonic" frequencies often show up as mathematical fallout when the strongest cycle - in this case circadian - is non-sinusoidal in shape. As spectral analysis is based on the decomposition of a pattern into waveforms, or cycles, that are each defined using sine and cosine waves, even non-sinusoidal patterns have to be described in sinusoidal terms. This means that a non-sinusoidal pattern will not be broken down into a simple representative cycle; rather it will often appear as a combination of peaks at distinct frequencies, with only the strongest peak providing information on the true underlying frequency of the pattern.

The remaining peaks are termed harmonic frequencies, and it is the mathematical combination of all peaks, the harmonics and the primary one, that ultimately creates the best approximation of the original non-sinusoidal waveform (for illustration, see Diggle, 1990). As much of the literature in the area

concentrates on the circadian aspect of temperature patterns, there is little focus on ultradian cycles, and so the significance of any peaks at the higher ultradian frequencies is rarely addressed (eg. Ruis et al, 1987; Fuller et al, 1981).

The periodgram of rat 700 (Figure 8) best illustrates the effect of harmonic frequencies. The straight line between the first three peaks betrays the probability of a harmonic frequency, because the computational relationship between the neighbouring frequencies does not allow for the influence of natural noise. Such crisp resolution of the peak transitions can also be seen in periodgrams created from artificial series with no noise (Diggle, 1990; SPSS, Inc., 1990). If adjacent peaks represent genuine independent cycles, then biological noise would likely cause the base of the peaks to be less precise and more rounded, as it does in the other periodgrams of Figure 8.

One approach to determining which peaks represent true ultradian cycles and which represent harmonics is to limit the influence of the non-sinusoidal circadian waveform by statistically subtracting it from the data (Dowse & Ringo, 1989; SPSS, Inc., 1990). In this study, it is clear from the BAT temperature profiles that the overall shape of the circadian pattern is not pure sinusoidal, but instead resembles a sawtooth shape; this is caused by the gradual slope at light offset combined with the abrupt slope at light onset. Even in cases where the most powerful cycle is in fact sinusoidal, it is still recommended that it be subtracted from the data after the initial analysis because its mere presence can prevent weaker, higher

frequency peaks from appearing (Kendall, 1976; Chatfield, 1980). Once the predominant cycle is removed, spectral analysis is reapplied to the now "deseasoned" or "prewhitened" data.

SPECTRAL RESULTS FROM DESEASONED DATA

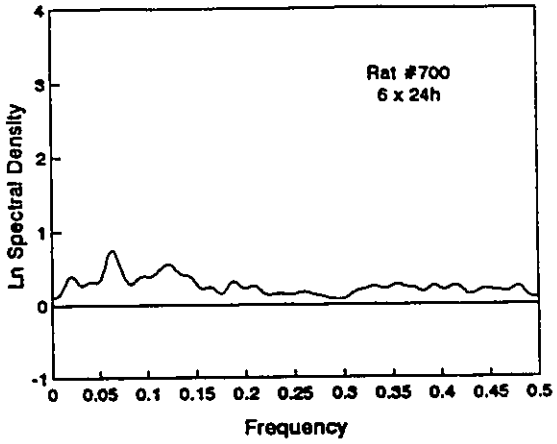
The method of least squares, which entailed the pooling of all data into 60 minute intervals, was employed to deseason the data. Alternative approaches to deseasoning that consider each series individually can be used, but the procedure of pooling data across animals ensures that the most faithful description of the non-sinusoidal circadian waveform is computed, thus resulting in the most effective subtraction of the circadian cycle from the profiles (A. Dubrowski, personal communication).

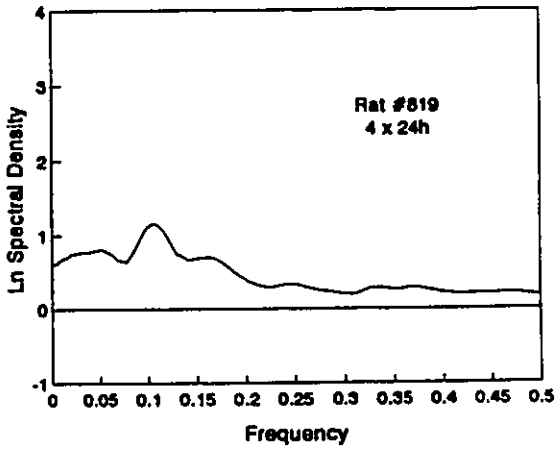
The group means were based on data from seven of the eight animals with intact BAT deposits. These series were included because they were all based on recordings from transmitters that were implanted the same way, by bonding directly to the muscle. In the case of the eighth rat, 890, where the transmitter was first bonded to teflon, the temperature profile was sufficiently different to warrant excluding it from the pooled data. Instead, hourly means were calculated on the lone series, and resulting waveform served to deseason the profile of 890 only.

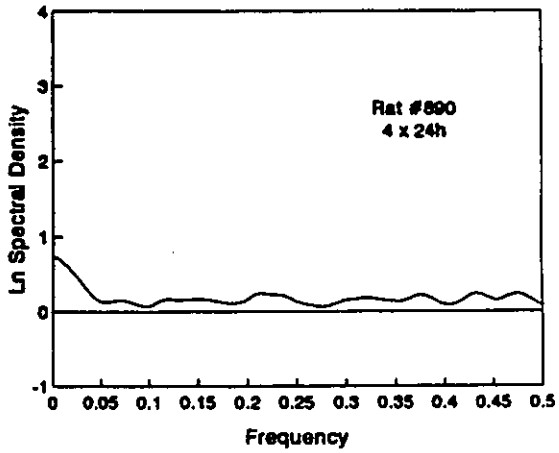
Several examples of the results of spectral analysis applied to deseasoned data can be seen in Figure 16. A comparison back to the corresponding periodgrams of the original, or non-deseasoned data (Figure 8, Column B) reveals that the deseasoning procedure has indeed altered the spectral graphs. A final

Figure Caption

Figure 16: The three panels illustrate three examples of periodgrams obtained from the spectral analysis of temperature data that was deseasoned prior to analysis (rats 700, 819 and 890); deseasoning refers to the statistical subtraction of the circadian waveform from each temperature profile. The peaks in each periodgram indicate the presence of an underlying cycle in the pattern of the corresponding temperature series; cycle length is determined from the frequency at which the peak is found. The data in the series are based on observations collected at 30 min intervals (24h period = 48 observations).


Rat #700
6 x 24h
Ln Spectral Density
4
3
2
1
0
-1
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5
Frequency


Rat #819
4 x 24h
Ln Spectral Density
4
3
2
1
0
-1
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5
Frequency


Rat #890
4 x 24h
Ln Spectral Density
4
3
2
1
0
-1
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5
Frequency

summary of all peaks that were observed at ultradian frequencies is presented in Table 4; the table includes the peaks that appeared in the original spectral analysis (+) from Table 3, as well as those observed in the periodgrams of the deseasoned data (D). Only spectral results from animals with intact BAT deposits are included in this table because there was not enough data in the remaining two series (897, 903) to deseason them individually, as there was for 890.

Ultradian Cycles: As can be seen in Table 4, the most consistent ultradian cycles to appear in the original, non-deseasoned data analyses (+) are the 8h and the 4h rhythms; 3 series show a peak at the 8h frequency, and 2 at the 4h frequency. Two other cycle lengths, 6h and 4.8h, were also observed in the periodgrams of the original data. However, two of the 8h peaks disappeared with deseasoning (D), as did the single 6h peak and also one of the 4.8h peaks. Only in the case of the 4h cycle do all peaks persist after deseasoning.

Harmonics Revisited: This section returns to the issue of harmonics in order to clarify how they affect the specific data presented here. Table 5 illustrates the harmonic frequencies that may arise from a circadian cycle of 1 cycle/24h. This translates into a different set of frequencies for different observation intervals, but as can be seen from the table, the harmonic frequencies are always whole number multiples of the original daily cycle. As Table 5 indicates, harmonic frequencies

Table 4: Frequency peaks from spectral analysis of original and deseasoned BAT temperature data

RAT ID#	24h	8h	6h	4.8h	4h	1.7h	Series Length
700	P	P /D	--	P	-- /D	--	6 x24h
757	P	--	--	--	--	--	2 x24h
745	P	--	--	--	--	--	4 x24h
771	P	--	--	--	P /D	--	4 x24h
799	P	--	P	--	P /D	--	6 x24h
819	P	P	--	P /D	--	--	4 x24h
881	P	--	--	--	--	--	6 x24h
890	P	P	--	--	--	--	4 x24h

D Spectral Peak appears at corresponding Fourier frequency in analysis of deseasoned data

--- Only animals below line had transmitter implanted using surgical teflon

Table 5: Harmonics of the circadian cycle, with corresponding frequencies for 30 and 10 min. intervals of data collection

			30 min. intervals (48obs /24h)		10 min. intervals (144obs /24h)	
Harmonic	Cycles /hr	Frequency	Cycles /obs	Frequency	Cycles /obs	Frequency
Circadian	1 /24h	1 /24h	1 /48	0.021	1 /144	0.007
1	2 /24h	1 /12h	2 /48	0.042	2 /144	0.014
2	3 /24h	1 /8h	3 /48	0.063	3 /144	0.021
3	4 /24h	1 /6h	4 /48	0.083	4 /144	0.028
4	5 /24h	1 /4.8h	5 /48	0.104	5 /144	0.035
5	6 /24h	1 /4h	6 /48	0.125	6 /144	0.042
6	7 /24h	1 /3.4h	7 /48	0.145	7 /144	0.049
7	8 /24h	1 /3h	8 /48	0.167	8 /144	0.056
8	9 /24h	1 /2.7h	9 /48	0.188	9 /144	0.063
9	10 /24h	1 /2.4h	10 /48	0.208	10 /144	0.069
10	11 /24h	1 /2.2h	11 /48	0.229	11 /144	0.076
11	12 /24h	1 /2h	12 /48	0.250	12 /144	0.083
12	13 /24h	1 /1.8h	13 /48	0.271	13 /144	0.093

of the circadian cycle are clearly represented by many of the possible ultradian cycles in temperature data. Hence, a peak at any given "harmonic" frequency could potentially be due to the presence of either a harmonic or a genuine cycle.

As discussed earlier, this is a serious concern in the analysis of biological series, and no single approach can overcome it. On the one hand, the use of long series represents a tool, as this provides more Fourier frequencies on which to build the periodgram, and thus can better isolate any harmonic frequencies that appear as peaks where there is no genuine cycle. On the other hand, the lability of ultradian cycles to environmental influences means that longer series may only serve to introduce more variability and thus reduce the mathematical strength of any specific ultradian cycle (Diggle, 1990; Stupfel & Pavely, 1990). At the moment, there appears to be no solution to this dilemma.

Performing spectral analysis on deseasoned data is also a highly useful procedure, as mentioned earlier, since the removal of the circadian cycle from the original data should eliminate the harmonics due to it. The necessity of this procedure is made clear by a comparison of the harmonics chart (Table 5) to the results of the Fourier analysis of the original data ("+" in Table 4); each of the frequencies where peaks were observed in the spectral analysis are also potential harmonic frequencies.

Interpretation of Ultradian Results: The 4.8h cycle is the most suspect frequency, largely because it does not produce a

whole number of cycles when divided into 24h and thus could not occur within the dominant structure of a circadian cycle; it is unlikely that such a period would show up unless it were only a mathematical fallout of the analysis. In contrast, the persistence of the 4h cycle in three of the periodgrams based on deseasoned data makes it the strongest candidate for a true ultradian rhythm; importantly, the cycle is displayed in two of the three longest series, unlike the 4.8h one which appears only in a shorter 4 x 24h series. Moreover, the 4h cycle can be accommodated within the framework of the 24h circadian cycle, supporting the hypothesis that the cycle is present in our BAT temperature data.

The presence of the 8h cycle is the most difficult to interpret. Considering the confound of harmonics, the appearance of the 8h peak in three periodgrams carries less weight than the fact that the peak did not persist in two series after deseasoning. In contrast, the findings on the 4h cycle present the opposite story, as one temperature series yields a 4h cycle only after deseasoning, making the 4h cycle the only one which shows up in deseasoned data after failing to appear in the analysis of the original data. As the circadian cycle is known to prevent the appearance of existing ultradian cycles, this result provides a strong argument for the presence of a 4h cycle one that is absent in the case of the 8h cycle. However, on the basis of the current data, it cannot be definitively stated that BAT temperature profiles lack 8h cycles.

CONCLUSION: FOURIER ANALYSIS

The combined results of the Fourier analysis on both the original and deseasoned data confirm that the BAT temperature pattern shows a strong circadian rhythm. The analysis provides evidence that a 4h ultradian cycle, and possibly an 8h one, underlie the BAT temperature rhythms. The following section will examine whether these mathematical findings are consistent with the limitations and characteristics of the biological system that produced them.

Background Studies on Ultradian Cycles: A vast spectrum of cycle lengths have been reported for biological ultradian rhythms, from 10^{-3} seconds for nerve impulses, to 16h for metabolic processes. Oxygen consumption, food intake and digestion, cardiovascular function, hormones, and sleep phases have all been found to exhibit ultradian cycles in the 2 to 16h range (see Stupfel & Pavely, 1990 for review). Studies conducted on human infants before they become entrained to environmental cues, such as feeding schedules or parental attention, have led to the proposal of a fundamental ultradian rhythm, the 60-120 minute basic rest activity cycle (Angeli & Carandente, 1988). This cycle appears to underlie the patterns of a large variety of systems, and is believed to be a hard-wired characteristic of the CNS, in contrast to the circadian cycle which is dependent on maternal hormones (Hoppenbrouwers, 1986).

It is interesting to note here that a 4h feeding cycle has been reported for human infants (Morath, 1974), which fits well

with both our finding of a 4h ultradian cycle in BAT, and with Himms-Hagen`s proposal that ultradian BAT thermogenic cycles underlie periodic feeding bouts (Himms-Hagen, 1995). Furthermore, one important structural component of this proposal is the description of the timing and magnitude of short cycles in liver temperature; these are suggested as underlying effectors of feeding bouts in rats (Devries et al, 1991), and it can be seen that the 24h BAT temperature patterns we report here appear strikingly similar to those recorded from liver lobes (Devries et al, 1991; Closa et al, 1991).

Motor activity levels appear to have been the most thoroughly examined for ultradian cycles, with inconsistent results. For example, Wollnick and coworkers (1987) reported periods of 12h, 6, 4.8 and 4 h in a study comparing several strains of rats, while Jilge et al (1986) found ultradians of 6.1, 8.2 and 12.3h in the rabbit. Note that in both cases, all reported ultradian cycles are also potential harmonics, although this aspect is not addressed in the papers.

There is evidence of a cross-species ultradian rhythm of approximately 90 minutes in both plasma NE (Tapp et al, 1981; Levin et al, 1978) and hypothalamic NE (Dietl et al, 1993) which may represent a fundamental rhythm in SNS tone that is similar to the basic rest activity cycle. Cyclic sympathetic input is presumed to underlie the BAT thermogenic response, but the complexity in the regulation of the humoral and neuronal aspects of the autonomic system limits our information on ultradian patterns. The denervation manipulation was intended to provide

insight into the role of the SNS in the daily temperature pattern of brown fat, but because the final experimental group consisted of only one animal, speculation is inappropriate. It is possible that the large-amplitude ultradian oscillations that we report represent plasma rhythms of NE, for example, but it is also possible that they simply reflect scratching behaviour.

Currently, the source of ultradian rhythms is unknown. The controversy centres around whether or not ultradian cycles are endogenously driven, as the circadian cycles are. The consistent finding that ultradian temperature cycles remain after SCN lesions (Eastman & Rechtschaffen, 1983; Ruis et al, 1987; Stupfel & Pavely, 1990) has been interpreted to mean that they are not generated by a master pacemaker, unlike circadian rhythms; however, endogenous control by a neural centre cannot be ruled out, since there is evidence of an alternative pacemaker located outside of the SCN (see Mistlberger, 1994 for review)

It has been proposed that ultradian cycles are not directly driven by any specific neural circuit, but rather are the consequence of the lack of absolute equilibrium of metabolic homeostasis (Stupfel & Pavely, 1990). The short term swings in biological systems that are necessary to maintain longterm consistency may themselves induce ultradian oscillations, and bestow the inherent irregularity and non-stationarity that are characteristic of non-circadian metabolic rhythms. This results in cycles that are not truly periodic, because contrary to circadian cycles, consecutive ultradian oscillations may present episodes with varying periods, or transient phase relationships,

and even aperiodicity.

This inherent variability may help explain why the apparent hourly peaks and troughs in the BAT data did not show up as very short circadian cycles in the statistical analysis. While this characteristic renders ultradian cycles less amenable to statistical analysis, it has the important advantage to organisms of bestowing a plasticity in responding to unexpected environmental challenges, just as the very regularity of circadian cycles allows animals to maximize effective use of such predictable environmental changes as day to night (Stupfel & Pavely, 1990).

Regardless of origin, ultradian cycles apparently exhibit a complex temporal hierarchy, such that the cycles consistently appear in preferred frequency bands; certain whole number divisors of the 24h period are preferentially represented (Hildebrandt, 1988). In fact, rhythmic functions will respond to interference by first changing their amplitude or phase, rather than their frequency. The preferred frequencies of the metabolic system are 16, 12, 8, 6, 4, 3, and 1.5h, and thus any frequency change that occurs will be limited to a jump from one to another of these preferred values. Our results are compatible with this apparent hierarchy, as we found only a limited number of frequencies represented in the temperature profiles of the eight intact animals, and they are all among the reported frequencies.

As a means of further investigating the mechanisms that underlie the circadian and ultradian cycles of brown fat, the third study compared individual BAT temperature profiles to the

corresponding core temperature. The correlation between the patterns can then be evaluated, and the graphs examined for evidence that one profile leads the other.

Part C: Core and BAT Temperature Comparison

COMPARISON OF TEMPERATURE PROFILES

Figures 17a,b and 18a show the daily patterns of BAT (bottom line) and core temperature (top line) collected from three rats (890, 881, 799). Although the graphs represent the same period of data recording as seen earlier, they differ from previous figures in that each value represents an averaged observation now based on ten rather than 30 min. intervals; as such, each of the BAT profiles represented here is based on a subsection of the complete data described in part A of this experiment.

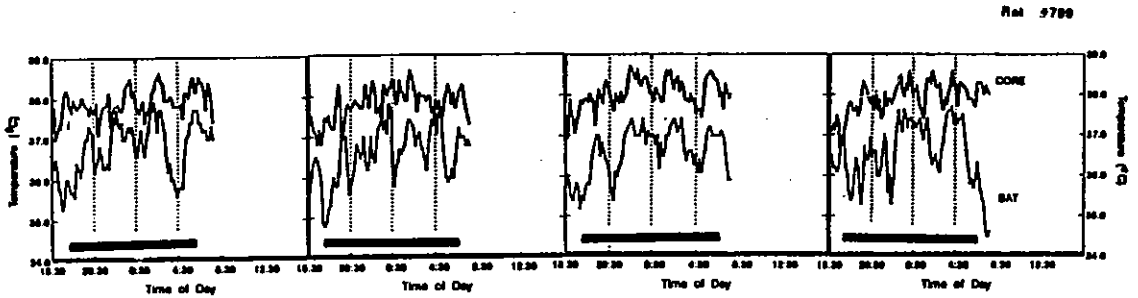
The three revisited series illustrate several predominant features of the relationship between BAT and core temperature. While the brown fat values are consistently lower than those of core temperature, both series exhibit ultradian peaks and valleys that are generally in phase throughout the 24h period, and also show the consistent nocturnal elevation that is typical of a circadian rhythm. This elevation is proportionately larger in the case of the BAT profile, which gives rise to a smaller difference between the two profiles at night; in addition, the ultradian oscillations of the two profiles appear to be more in phase during the dark period.

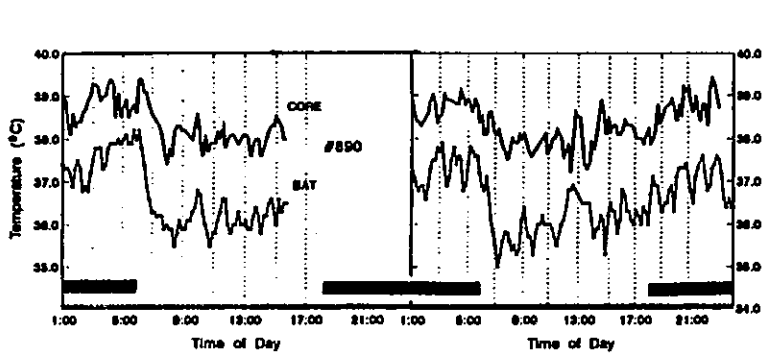
The average core temperature for the daylight period was 37.0°C±0.02, compared to 35.7°C±0.02 for the corresponding BAT

Figure Caption

Figure 17: The three panels show the original, untransformed temperature data obtained from three different rats; BAT and core profiles were collected simultaneously in each case. Core temperature is represented by the dark upper line, and BAT temperature by the lower line. The temperature values depict 10 min intervals; each one was calculated by averaging the observations for four minutes across each 10 min time point, two min on either side. The black bars represent the 12h dark portion of each 24h period (06:00 to 18:00h).

The top graph (A) shows the profiles collected from rat 799; observations were recorded from 16:30 to 07:30 each day. The middle graph (B) illustrates the temperature patterns for rat 890; static noise interfered with the core data collection during the first overnight period. Finally, the bottom graph (C) depicts the profiles collected from the denervated subject, 897; again, static noise interrupted the recording of core temperature, this time during the day.


Rat #789
A
CORE
SAT
Temperature (°C)
Time of Day
Time of Day
Time of Day
Time of Day


B
CORE
#890
SAT
Temperature (°C)
Time of Day
Time of Day

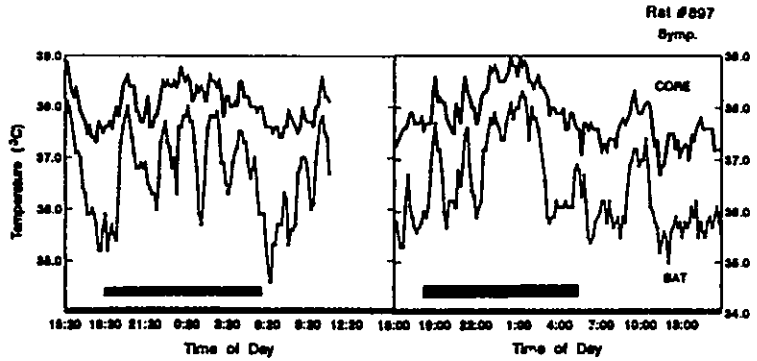
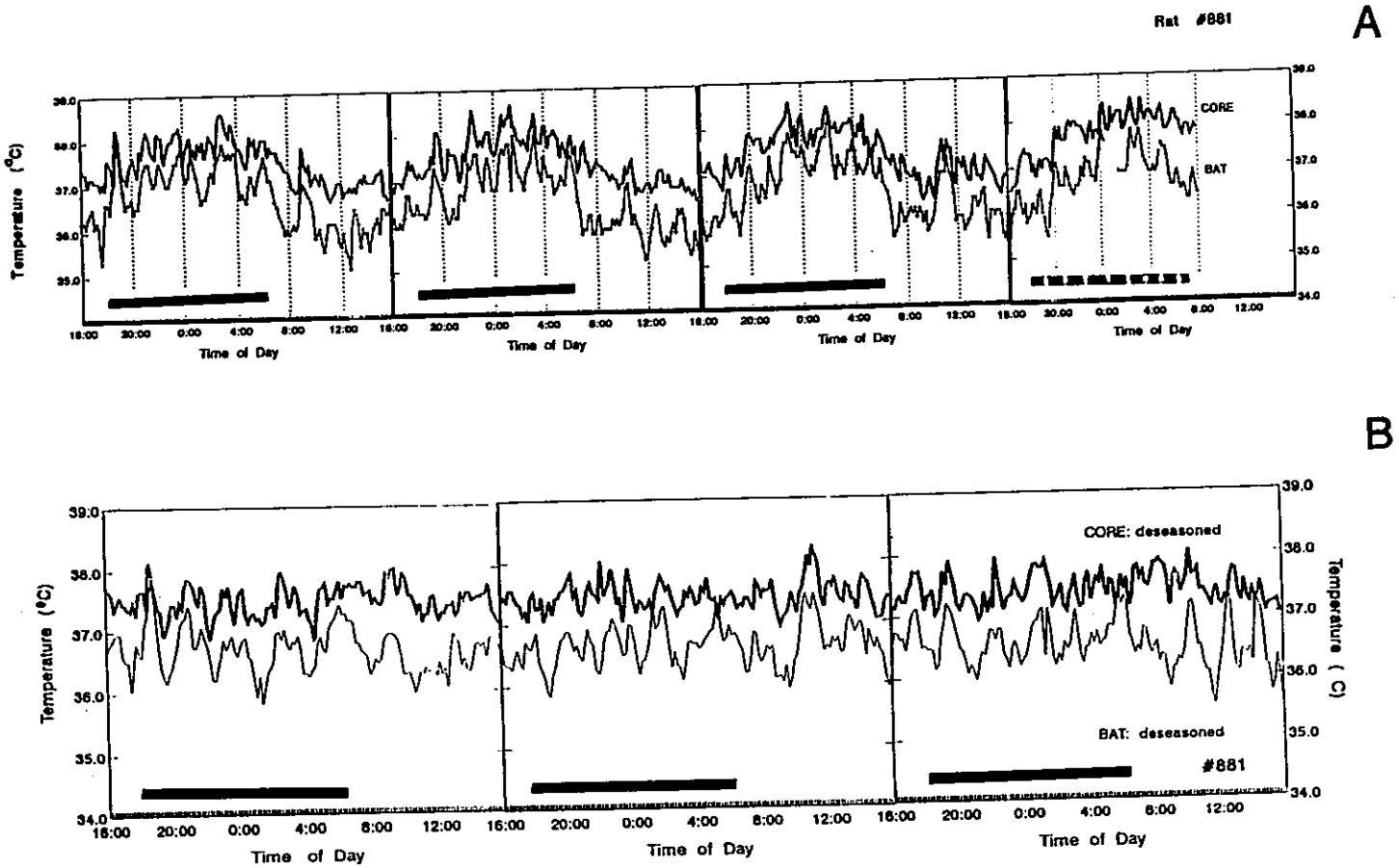

Rat #897
Symp.
C
CORE
SAT
Temperature (°C)
Time of Day
Time of Day

Figure Caption

Figure 18: Both panels depict the simultaneous BAT and core temperature profiles from one rat (881); both series are shown in 10 min intervals, and both represent untransformed data. The upper panel (A) shows four days of data, while the lower panel (B) illustrates the first three days of the same profiles, as they appear after deseasoning (statistical subtraction of the circadian rhythm). The dark topmost line represents core temperature, and the light line, BAT temperature. The rightmost section of the upper panel (A) depicts the BAT and core temperature profiles that were collected during the condition of "24h lights on"; the striped bar represents the period during which the lights are normally extinguished.


A
Rat #881
CORE
BAT
Temperature (°C)
Time of Day
B
CORE: deseasoned
BAT: deseasoned
#881
Temperature (C)
Time of Day

series (see Table 6). During the dark period, the values rose to a mean of 38.0°C±0.1 and 37.0°C±0.02, respectively; thus, the increase was greater proportionately in the case of BAT temperature. Both profiles show anticipatory gradual rises prior to light offset and abrupt temperature drops at light onset, characteristics that are consistent with the temperature profiles based on 30 min. intervals. As noted earlier, rapid behavioural changes coincide with the abrupt light switches at both 0600 and 1800h; thus, the temperature profiles are not a simple consequence of the increased motor activity at night.

Additional insight into the regulation of this nocturnal increase in brown fat temperature can be gained from observing core and BAT fluctuations in the condition where lights remained on for 24h hours. The final section of the top graph in Figure 18, where the dark period bar is striped, illustrates the profiles obtained in this condition. Both core and BAT profiles retained the temperature increases that represent a circadian rhythm; furthermore, synchronous peaks and valleys are still visible, although the gap between the BAT and core values is consistently larger than that which occurs during the normal dark periods. The fact that the circadian pattern persists despite the change in lighting schedule supports the notion that, like core, BAT temperature is driven endogenously by a neural oscillator (Gordon, 1990).

Finally, panel C of Figure 17 depicts the BAT and core temperature profiles recorded from the denervated rat, 897. It has been suggested that remaining BAT deposits compensate for

Table 6: Mean values for corresponding BAT and Core temperature profiles (rats 799, 881 , 890)

	OVERALL MEAN	Light Period Mean	Dark Period Mean
BAT	36.7 °C±0.02	35.7 °C±0.02	37.0 °C±0.02
Range	34.8 - 38.2		
CORE	37.6 °C±0.02	37.0 °C±0.02	38.0 °C±0.1
Range	36.5 - 39.0		

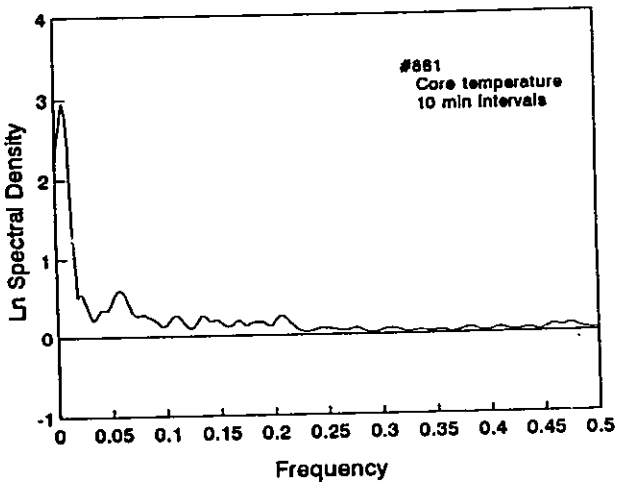
excised or sympathectomized interscapular tissue (Rothwell & Stock, 1992) and that such preparations do not give rise to any apparent thermoregulatory impairment (Himms-Hagen, 1976). This implies that if the profile of denervated BAT is unusual, it will appear out of phase with core temperature oscillations, as the latter should be left relatively undisturbed. However, it can be seen that the core temperature profile in fact mirrors the atypical BAT temperatures observed in this case, making it likely that some disruption other than the denervation is responsible for this effect; possibly, the scratching behaviour of the rat induced a thermal response. The fact that the BAT and core series continue to show a high correlation despite the denervation of BAT casts doubt on the significance of the denervation findings.

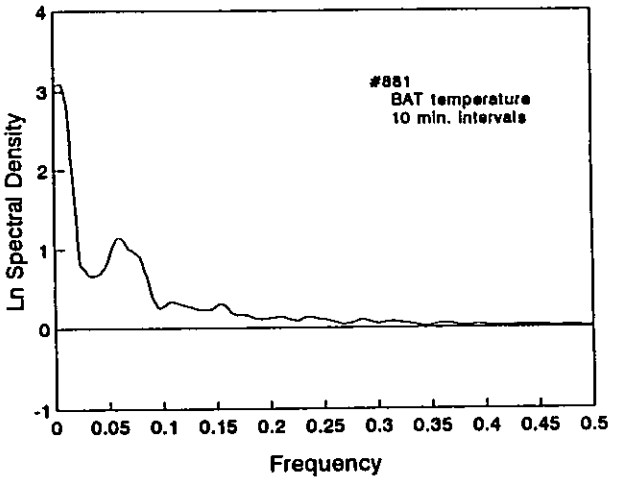
SPECTRAL ANALYSIS

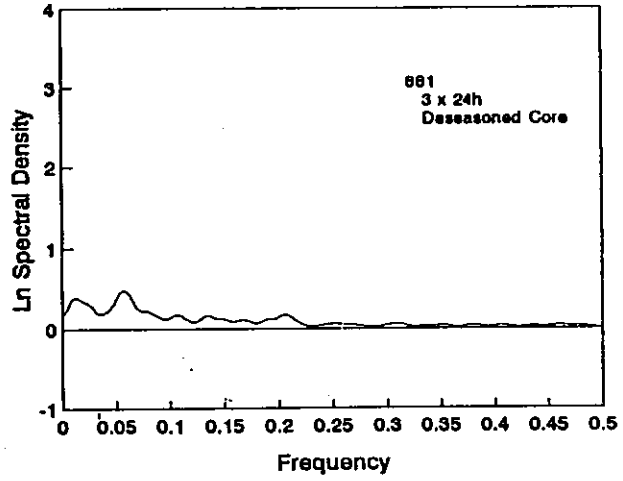
The results of the spectral analyses performed on the revisited temperature profiles are shown in Figure 19; the upper graphs are derived from analysis of the original ten-minute data, while the lower graphs are based on deseasoned data. Only the series from rat 881 had enough continuous observations to allow the time series analysis to be executed. Both BAT and core periodgrams show a large peak at a frequency of 0.007, indicating the presence of a circadian component. However, ultradian peaks were observed at slightly different frequencies in each case; the spectral analysis of the core temperature profile shows an underlying cycle of 3h (frequency peak = 0.056), while the BAT

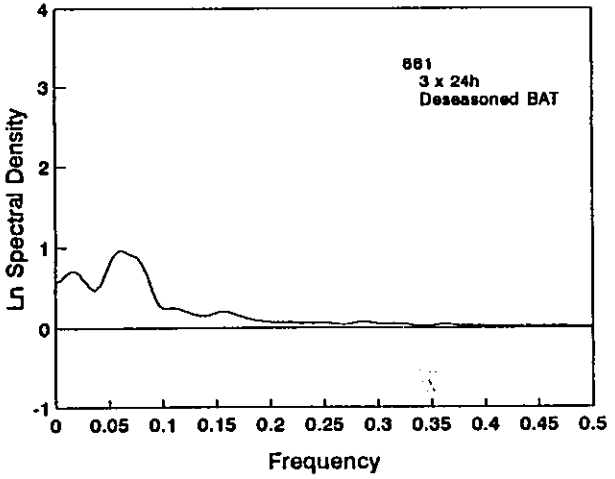
Figure Caption

Figure 19: The graphs show the periodgrams resulting from the separate Fourier analysis of BAT and core temperature data (rat 881); the temperatures were recorded simultaneously. Analysis based on core temperature data are shown on the left, while those based on BAT temperature are on the right. Spectral analysis of the data before deseasoning are shown above the periodgrams based on deseasoned data. All spectral analysis were performed on series represented by 10 min intervals; both the BAT and core series consisted of 3 x 24 h periods (3 x 144 observations)


#881
Core temperature
10 min intervals
Ln Spectral Density
Frequency
4
3
2
1
0
-1
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5


#881
BAT temperature
10 min. intervals
Ln Spectral Density
Frequency
4
3
2
1
0
-1
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5


881
3 x 24h
Deseasoned Core
Ln Spectral Density
Frequency
4
3
2
1
0
-1
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5


881
3 x 24h
Deseasoned BAT
Ln Spectral Density
Frequency
4
3
2
1
0
-1
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5

temperature series displays an ultradian cycle of 2.7h (frequency peak = 0.063). The discrepancy between the ultradian rhythms found in the BAT and core temperature series is surprising, given the apparent high correlation between the profiles, but the frequencies are similar enough to suspect that their difference may represent only leakage, or the effect of variability in ultradian cycling.

Further interpretation of the periodgrams requires consideration of the specific observation interval used. In the previous section, we reported the results of a spectral analysis done on BAT temperature data from rat 881, where the interval between observations was 30 minutes (Figure 8, Column B); in contrast, the periodgrams in Figure 19 shows an analysis of a subsection of the same data series now represented by observations at ten min. intervals. The appearance of ultradian cycles in the second analysis which were absent in the first periodgram may be due to the higher resolution that comes from using intervals of 144 observations/24h instead of 48, or to the fact that the 10 min. series comprises only a subsection of the complete 30 min. series.

Comparison to Existing Literature: The average core temperature value in this study is 37.6°C±0.02, which is comparable to published values (see Gordon, 1990, for summary); for example, Closa and coworkers (1993) reported a mean core value of 37.7°C±0.02. However, the values here show a much wider range, as their temperatures span only from 36.8 to 38.6°C while

our minimum and maximum temperatures were 36.5 and 39.0°C respectively (see Table 6). They reported aortic values rather than intraperitoneal ones as they better satisfy the definition of core temperature, in that they exhibit no change with ambient temperature or circulation alterations (IUPS, 1987). Thus, it is expected that their temperature values would show much less variation than abdominal ones.

To our knowledge, the Spanish lab (Closa et al, 1993; Gómez-Sierra et al, 1993) is the only other group that has used a chronic paradigm to examine BAT and core temperatures collected simultaneously in conscious animals. Other groups have recorded the temperatures in an acute design, as Fyda and coworkers (1991) did for example, in a study assessing brown fat and abdominal temperature changes after application of various pyrogens and antagonists. The chronic data reported by the Closa group represent recordings over discontinuous 24h periods; thus, in no other study was spectral analysis performed on the BAT data.

The most obvious difference between our results and those of the Closa group is the value of the average BAT temperature, as we report a mean of 36.7°C±0.02, in contrast to an average BAT temperature of 37.9°C±0.1 for them. A similar discrepancy was also noted earlier, on page 98, when their mean value was compared to the mean calculated from all of the BAT temperature data collected here (rather than a subsection) and based on 30 minute intervals.

The results of a direct comparison of thermoprobe and transmitter temperatures in anaesthetized rats (see Figure 11)

demonstrate that although transmitter values are approximately 1°C below thermoprobe ones, the temperature patterns derived from the transmitter recordings accurately mirror those of the thermoprobe; thus, systematic technical differences, rather than any true functional disparities, likely account for the gap between the brown fat temperature values reported in this paper and by Closa et al (1993). However, the findings of Fyda and coworkers (1991) do not appear to fit with this conclusion, as their thermoprobe temperature readings showed a mean BAT temperature value of 34.3°C; one explanation for their lower BAT readings is that they reflect a slightly different thermoprobe position from that of Closa's group.

Studies which directly examine the ultradian cycles underlying core temperature profiles are limited in comparison to the number investigating food intake or activity cycles. Lefcourt (cited in Stupfel & Pavely, 1990) found ultradian cycles of 12 ,6 and 1.5h in cows, and Gomez-Sierra and coworkers (1993) report core temperature rhythms of 8h, 4 and 2.2h in rat, based on ten min. intervals; this compares to the observation in this study of a 3h ultradian cycle underlying core temperature rhythm. While the results are varied, perhaps due partially to different series lengths and group size, they do nonetheless conform to the preferred frequencies described in the previous section, page 152 (Hildebrandt, 1988).

BAT Contribution to Core Temperature Rhythm: Closa and coworkers (1993) discuss the significance of finding BAT values

that are higher than core if heating functions are to be attributed to BAT. However, given the circulation and tissue conduction confounds inherent in temperature recordings, we believe that the strength of temperature measurement lies almost exclusively in the interpretation of patterns, and not absolute values. Indeed, a comparison of the findings of Closa et al (1993), and Fyda et al (1991) supports this proposal, as both sets of thermoprobe readings clearly reflect themogenic changes, but yet there is a large difference in the mean BAT temperature values. Thus, the two most reliable tools for measuring BAT contribution to core temperature changes are temperature patterns, as we examine here, and direct measurement of blood flow.

Accordingly, brown fat blood flow is reported to increase about 200 fold during the warming periods of core temperature ultradian cycles (Closa et al, 1993); this provides evidence for a contribution of brown fat thermogenesis to core temperature rises. The authors further showed a 64% coincidence index, as determined by a sign test, between the slopes of the BAT and core temperature patterns at corresponding time points, another finding that fits with the idea that brown fat temperature contributes to core warming periods. However, neither result suggests that brown fat heat production may serve to initiate the core changes.

Fyda et al (1991) suggest that BAT is an important effector organ in mediating core temperature rise during fever-induced hyperthermia. Since fever based mechanisms have been proposed as

the basis of circadian oscillation in core temperature (Ucar et al, 1983; Scales & Kluger, 1987), this finding supports the hypothesis that BAT thermogenesis contributes to daily core temperature changes. Finally, it is well established that BAT thermogenesis promotes core warming during cold exposure (Foster & Frydman, 1979, Kurosawa, 1991; see Himms-Hagen, 1990 for review). This combined weight of indirect evidence led to an examination of the temperature data for indications that the BAT profile leads core, or is phase advanced to core, and not simply parallel to it.

Visual inspection of the profiles from this study revealed no obvious indication that one temperature series leads the other in either circadian rhythm or ultradian cycles. However, this is not surprising, given the overwhelming influence of the circadian cycle on the profile, and the difficulty of distinguishing the various underlying ultradian rhythms in either series (Chatfield, 1980). An examination of Closa's 1993 data suggests a similar difficulty in visualizing any consistent lag between core and BAT profiles. That group did not attempt an ultradian analysis on their BAT temperature series, although the results of their BAT blood flow studies strongly support the hypothesis that BAT contributes to core temperature upswings.

Subsequent to the spectral analyses, cross-correlational procedures were applied to the profiles presented here in order to uncover any consistent phase difference between the BAT and core patterns.

CROSS-CORRELATION ANALYSIS

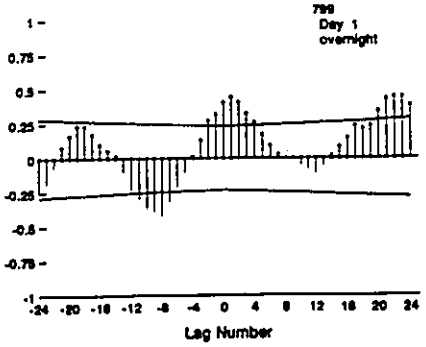
This analysis involves a systematic comparison of the degrees of correlation between two profiles for a number of lags or offsets (eg. Gómez-Sierra et al, 1993). For example, if the correlation between the two series is observed to be highest when one profile is positioned 30 min. in advance of the other, then the interpretation is that the leading series influences the profile of the succeeding one. While mathematical correlation by no means implies causation, it may be argued in the case of comparisons between temperature profiles from different tissues, that both the nature of thermoregulatory mechanisms and the inherently strong degree of influence between various profiles imply a casual role if a phase difference is observed.

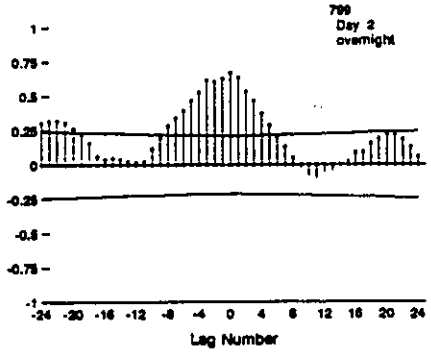
The weakness to the analysis is that no conclusion can be drawn when there is failure to show high correlations at offsets or lags other than 0, because the procedure may simply be unable to detect a phase relationship if more than one ultradian rhythm underlies the profiles. As always, the presence of an overwhelming circadian cycle also represents a potential confound. While there currently exists no procedure to remove ultradian rhythms, the cross correlational analysis can be made more powerful by performing the correlation on deseasoned data (data from which the circadian waveform has been statistically subtracted).

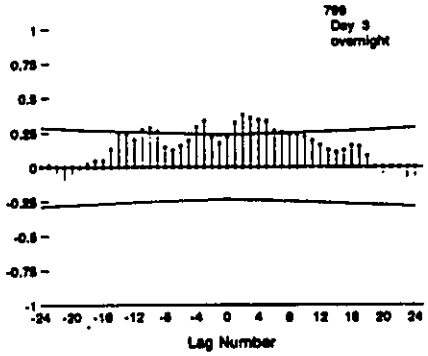
Original, Non-deseasoned Data: Figure 20 shows the results of the cross correlational analysis on the BAT and core

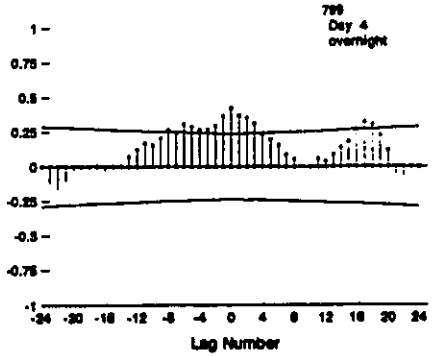
Figure Caption

Figure 20: The graphs depict the results of the cross correlational analyses performed on the BAT and core temperature profiles for each of four consecutive 12 h dark periods (rat 799); the temperature data were the original, untransformed values. The values on the abscissa indicate the number of 10 minute offsets used to determine each correlation coefficient, starting with 0 (no offset) to 24 lags (a 4h offset). Positive lags indicate that the offset was produced by shifting the BAT profile so that it was in advance of core, and negative lags indicate the reverse, core in advance of BAT. The solid lines at approximately $r = \pm 0.25$ indicate the coefficient value required for significance at $p \leq 0.05$.


799
Day 1
overnight
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number


799
Day 2
overnight
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number


799
Day 3
overnight
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number


799
Day 4
overnight
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number

temperature data from each overnight period for 799. The changes in the relationships from night to night are apparent from the variety of shapes presented in the four graphs. For example, in days 1 and 2, there is a smaller or secondary group of correlation peaks at an offset of 20 to 24 intervals, which suggests an underlying cycle of 3.3 to 4h in both temperature series. However, the remaining two graphs do not show evidence of any cycles. Most importantly, in all four overnight periods, the strongest correlations appear when the lag number is about 0, which is interpreted to mean that neither temperature series consistently leads the other. There is some suggestion in the first two graphs that the precise peak may be at lag +2 or +3, the implication being that BAT leads core temperature by 20 or 30 minutes. However, again the finding is not consistent. More data collection is needed to explore this potentially important finding.

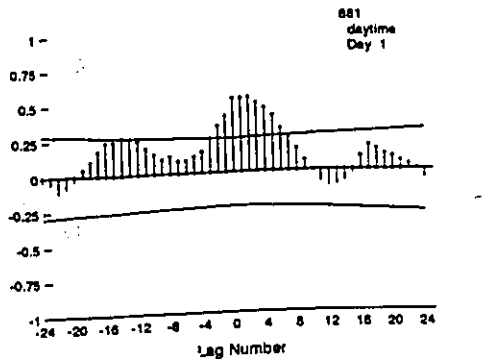
Figure 21 and the upper panel of Figure 22 shows the results of cross correlational analyses on data from rat 881, based on non-deseasoned data. Although observations were collected continuously for three days, the analyses were first performed on each individual day and night period (Figure 21), and then on the overall 3 x 24h period (Figure 22, upper graph).

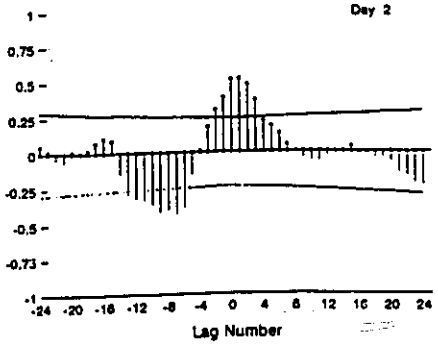
The most prominent feature can be seen when comparing day graphs to night ones; the primary group of coefficients (around lag 0) have consistently higher values in the graphs representing the overnight data (Column A), than in the daytime graphs (Column B). The finding bears out the visual impression given by the

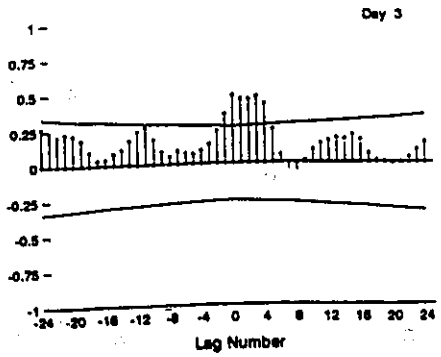
Figure Caption

Figure 21: This figure displays the six separate cross correlation functions derived from analyses of simultaneous BAT and core temperature data from rat 881. The graphs are based on 3 x 24h periods, which were divided into three light portions (Column A) and three dark portions (Column B). See caption of Figure 20 for an explanation of the abscissa units.

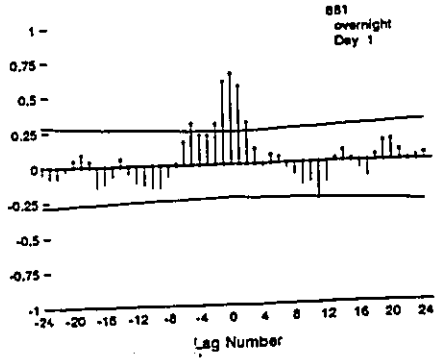
A

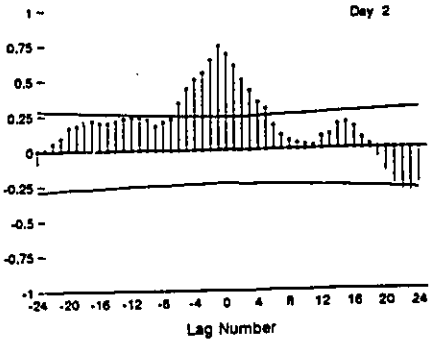

881
daytime
Day 1
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number


Day 2
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.73
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number


Day 3
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number

B


881
overnight
Day 1
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number


Day 2
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number

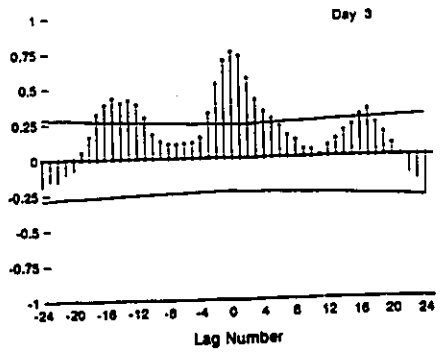
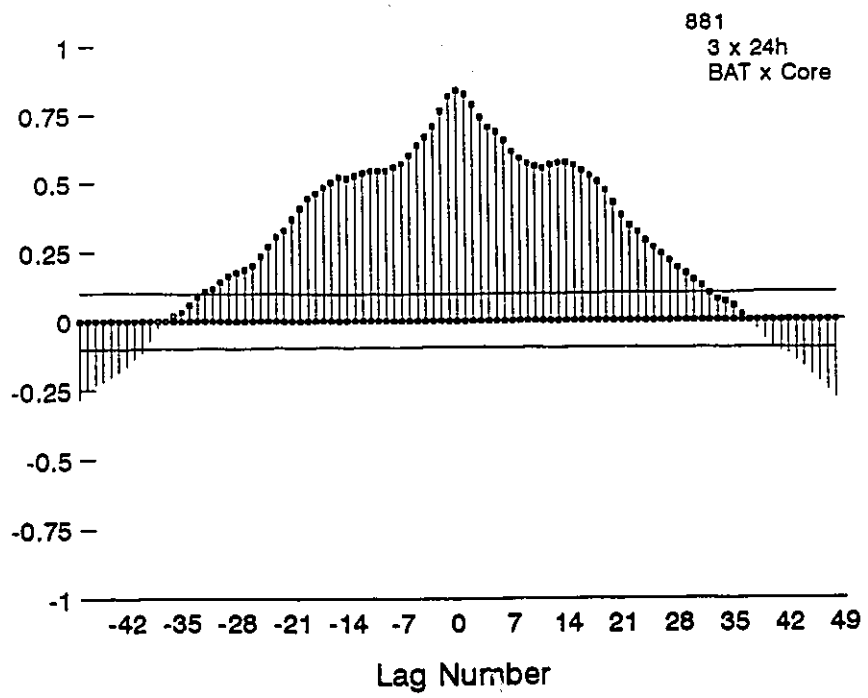

Day 3
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number

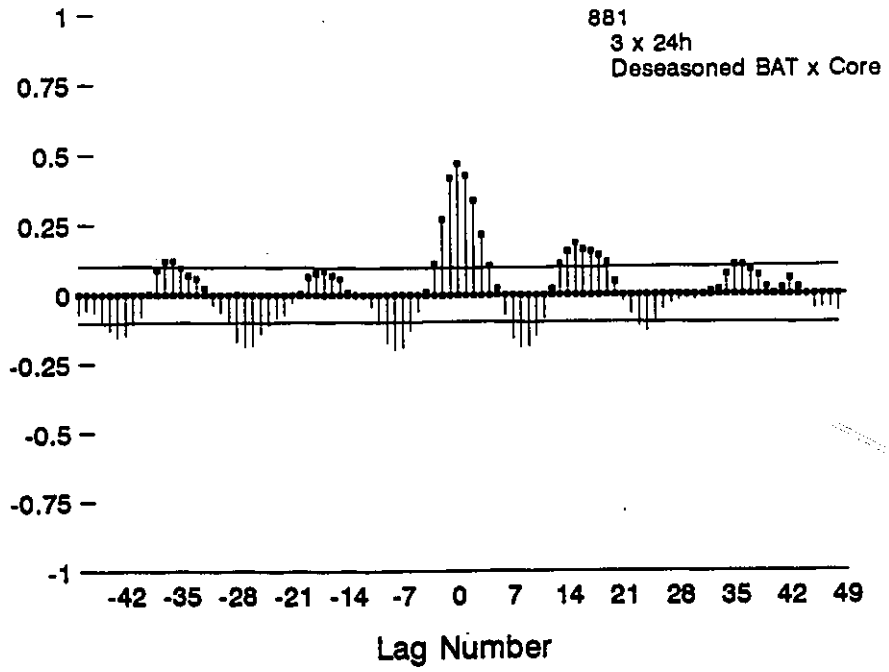
Figure Caption

Figure 22: The graphs display the results of two different cross correlational analyses on the BAT and core temperature profiles of rat 881. Both functions are based on the same data from a three day period, but the upper graph displays the results of the correlational analysis on the original temperature profiles, while the lower graph depicts the cross correlation results after application of the deseasoning procedure to the temperature profile. For both graphs, the correlation coefficients were calculated at each 10 min lag offset, up to a lag number of 48 (8 hours). See caption of Figure 20 for more information on abscissa units.

A


881
3 x 24h
BAT x Core
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-42 -35 -28 -21 -14 -7 0 7 14 21 28 35 42 49
Lag Number

B


881
3 x 24h
Deseasoned BAT x Core
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-42 -35 -28 -21 -14 -7 0 7 14 21 28 35 42 49
Lag Number

temperature profiles, that of more synchronous peaks and valleys in the dark phase than in the daytime.

The potential day-night distinction is relevant to the earlier discussion on the results of the spectral analyses of the BAT and core temperatures. Recall that a shorter ultradian cycle was found for BAT than for core temperature (2.7h vs. 3h). If the strongest correlation between the profiles does indeed occur at night, and the phase relationship is effectively reset every night by the consistent timing of the light switch, then a slightly faster cycle for the BAT profile could cause temperature rises to appear just ahead of core temperature rises throughout the night; the daily resetting would serve to prevent the shorter cycle of BAT from sending the two profiles increasingly out of phase. However, as only one of six cross-correlation functions displays secondary correlation peaks that reach a level of significance (Day 3 at lag 24 to 27), the patterns are simply too inconsistent from day to day to draw any conclusions about underlying cycles. This within-subject variation is similar to that seen in the results of the analysis of rat 799, and confirms the conclusions of previous studies regarding the extreme variability of ultradian temperature rhythms.

The overall cross correlation function for all three days combined is shown in the upper panel of Figure 22; correlation peaks for any existing ultradian cycles are obliterated by the effect of the circadian cycles, as expected. In other words, the strong symmetry of the graph, as well as the placement of the correlation peaks, suggests that the cycle revealed by the cross

correlational function is merely the circadian rhythm of 1 cycle /144 observations. The first trough will occur at approximately offset number 35, after the crossover point, and the secondary peak will then likely appear a further 70 lags away, placing it in the vicinity of offset 144. Therefore, an analysis of deseasoned data is required to overcome the influence of the circadian cycle.

Deseasoned Data: Only subject 881 provided enough continuous data on which to perform the deseasoning function. The lower panel (B) of Figure 18 depicts the temperature profiles of both deseasoned series; while the ultradian oscillations are visible in the data before application of the deseasoning procedure (upper panel), the subtraction of the circadian rhythm serves to highlight the appearance of simultaneous peaks and troughs in the BAT and core profiles.

The results of the cross correlational analysis on the overall, deseasoned 3 x 24h period can be seen in the lower panel of Figure 22. It provides an even more striking illustration than do the temperature profiles themselves of how the removal of the circadian rhythms from such data can thrust the ultradian cycles into prominence. The position of the secondary peak, which indicates the offset where the profiles are again in phase, suggests the presence of an underlying cycle of very close to three hours for both temperature profiles. Finally, the highest correlation coefficient of the primary peak appears at lag +2 or +3; thus, the analysis based on the pooled series of deseasoned

data supports the results of the cross correlational analysis on the data from rat 799 (Figure 21): that the pattern of brown fat ultradian cycles leads the corresponding core temperature pattern by 10 to 30 minutes.

Figure 23 presents the 6 x 12h individual cross correlation functions on the deseasoned data from subject 881; the day and night graphs are again represented in Columns A and B respectively. The most obvious finding is the greater symmetry obtained in the nocturnal cross correlation functions. This finding implies that the ultradian cycle underlying both temperature patterns is more consistent at night; it occurs approximately every three hours, as shown by the secondary peaks around lag 18. In contrast to the cross correlation functions based on the original data (Figure 21), there does not appear to be any notable difference between day and night in the absolute size of the primary coefficient peaks, although again, the patterns of the graphs are too inconsistent from day to day to draw firm conclusions. It is unclear how much weight to put on the interpretation of these findings, but they do suggest that subtraction of the circadian cycle from BAT and core temperature patterns removes that very characteristic which serves to produce a change, from night to day, in the correlation between the two series.

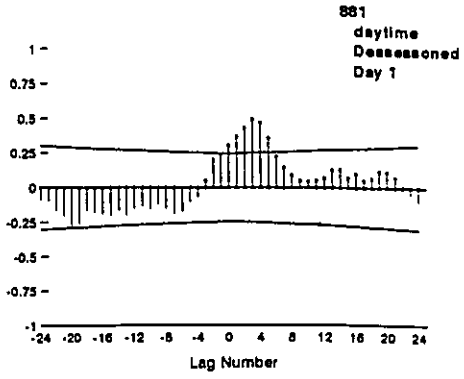
CONCLUSION

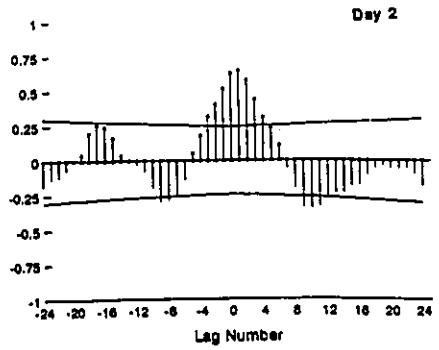
The most pervasive finding is the strong similarity in both circadian and ultradian oscillations in BAT and core temperature

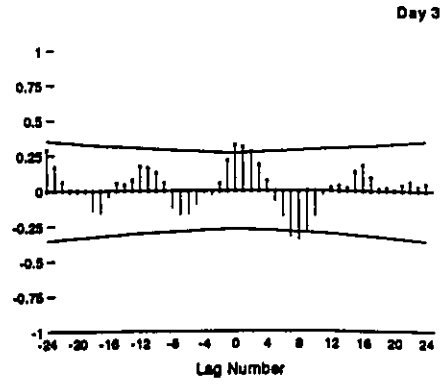
Figure Caption

Figure 23: This figure displays the six separate cross correlation functions that were derived from analyses of simultaneous BAT and core temperature data from rat 881. These cross correlation functions are based on the same data as the ones in Figure 21, but in this case the temperature profiles were deseasoned before the application of the cross correlation procedure. The functions representing data collected during the light period are shown in Column A, and the dark period in Column B. Lag numbers indicate the number of 10 min intervals by which the core and BAT profiles were offset for the calculation of each cross correlation coefficient (see the caption for Figure 20 for more detailed explanation of the abscissa units).

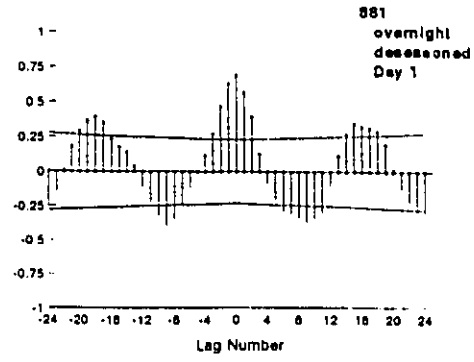
A

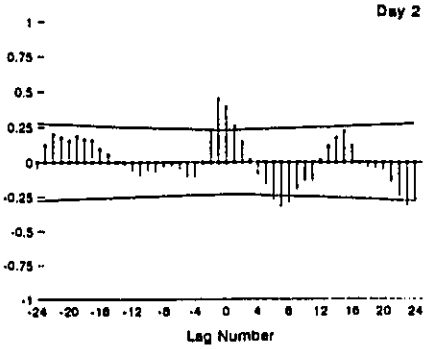

881
daytime
Deseasoned
Day 1
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number

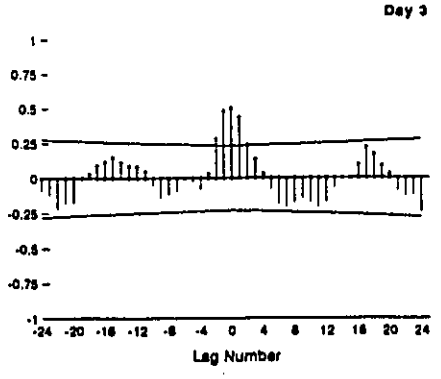

Day 2
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number


Day 3
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number

B


881
overnight
deseasoned
Day 1
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number


Day 2
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number


Day 3
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24
Lag Number

profiles; both the nocturnal elevation, and the 24h peaks and troughs appear to be in phase in the temperature patterns of both the original and deseasoned data. Spectral analysis of each profile indicates that both BAT and core rhythms share a circadian component, as expected, but additionally reveals that a distinct ultradian cycle underlies each series; core temperature appears to cycle at 3h, while the BAT temperature peak indicates a cycle of 2.7h.

Cross correlational analysis of the individual dark and light periods in two subjects highlights primarily the inconsistencies in the patterns of the ultradian cycles, both between and within rats. Thus, it is possible that the underlying ultradian cycles in both core and BAT temperatures are in fact the same, but that the day to day variability causes the peaks to appear at slightly different frequencies when examined spectrally.

However, when the cross correlation functions of the original data from 881 are examined for similarities, it appears that the BAT and core temperature profiles are more highly correlated at night than during the day, and it is this disparity which may lie behind the differing spectral peaks. Furthermore, this day night disparity may underlie the apparent inability of the Fourier analysis to tease out the hourly peaks and troughs from the 24h BAT data. It was suggested earlier (p. 155) that the inherent variability of the short cycles might render them intractable to spectral analysis; it may also be possible that if the short cycles in the night period are either different than those in the daytime, or simply more stable, then the change in the

temperature pattern could cause the spectral analysis to beecome less accurate. However, unlike the situation with the cross-correlational analysis, it is not possible to analysis the day and night data separately, because the Fourier procedure requires a continuous data set.

In the present report, the correlational analyses on both the continuous three day series and the six individual day /night series together suggest the presence of underlying ultradian cycles in the range of approximately 3 to 4 hours.

Phase Relationship of BAT and Core: All cross correlational graphs show the primary peak of coefficients at a lag of approximately 0, but a closer examination of the individual values of the coefficients within this primary peak suggests that in fact BAT may lead core by 10 to 30 minutes. Each 10 minute lag or offset yields a correlation coefficient value, and in five of seven graphs the largest coefficient appears at a lag of +1 to +3. This result, along with the BAT blood flow analysis reported by Closa et al (1993), provides the most compelling support for the idea that BAT influences core temperature. While it is true that in the graphs of the simultaneous BAT and core temperature series (Figures 17 and 18) neither profile appears to lead the other, it is also true that the presence of underlying circadian and ultradian cycles can complicate the appearance of the waveforms, and therefore simple visual inspection of the graphs cannot be used to assess the interaction of the two series (Chatfield, 1980).

Brown fat thermogenesis is known to contribute significantly to the maintenance of core temperature in neonates, cold exposed animals, during arousal from hibernation (see Arbuthnott, 1989), and during fever induction (Fyda et al, 1991; Jennings & Elia, 1987). The question remains whether there is a role for BAT heat production in the sustained elevation of core temperature at night. The results of the cross correlation analysis on the original data suggest that the relationship between core and BAT temperatures is different at night than during the day; this is not true for deseasoned data, but since it can be expected that the effect is related to the presence of the circadian rhythm, the deseasoned results are not incompatible.

This idea is also supported by the finding that removal of the BAT deposit apparently diminished the daytime ultradian cycle while leaving the nightly pattern unchanged (Figure 14). It is possible, in fact, that the underlying ultradian cycles of the BAT temperature rhythm are consistently different between dark and light periods, such that the results of the Fourier analyses represent some mathematical combination of the two contrasting patterns of cycles, rather than the true pattern. However, the spectral analysis can be applied only to a continuous series, and thus it is mathematically impossible to perform two separate Fourier analysis on night vs. day data.

It has been shown that the decrease in bodily heat loss at night is proportionately larger than the corresponding increase in heat production, which suggests that the sustained elevation of core temperature may be due primarily to changes in distal

vasoconstriction patterns. However, it is clear that metabolic heat production rises at night (Shido, 1987; Shido et al, 1989; Brown et al, 1991), and given the evidence that BAT heat production can influence core, it seems likely that brown fat thermognesis plays a role in core changes.

The related, but separate issue of whether BAT thermogenesis contributes to ultradian cycles in core temperature can also be addressed here. We report that BAT and core profiles are highly similar, in both circadian and ultradian cycles, and that the BAT pattern may be phase advanced to the core profile by 10 to 30 minutes. Along with the findings of Closa et al (1993) who demonstrated that BAT thermogenesis increases during episodes of core temperature warming, our results constitute some of the first evidence that brown fat heat production represents one factor in the ultradian pattern of core temperature. It is important to develop a method of examining the temperature patterns of peritoneal BAT deposits, given that the positions and mass of the deposits suggest a substantial role in stimulated heat production in the rat.

Finally, the persistence of the brown fat circadian rhythm in the 24h lights-on condition indicates that the BAT temperature profile is not simply a consequence of nocturnal activity levels, This fits well with the earlier finding of an anticipatory temperature rise in the BAT profile; together, the results provide strong evidence that brown fat temperature, similar to that of core, is regulated by an endogenous pacemaker.

GENERAL CONCLUSIONS

The three studies described in this thesis comprise an investigation into both the central regulation and the circadian expression of brown fat temperature. The role of central structures in regulating brown fat activity was evaluated by mapping studies of the VMH and the VLT area for sites that elicit stimulation-induced changes in brown fat temperature. The physiological context for brown fat heat production was investigated through a detailed description of the daily temperature pattern of interscapular brown fat and the relationship between BAT and core temperature rhythms.

It was the discovery of sites that induce rapid drops in BAT temperature which led to speculation on the functioning of the tissue from a circadian perspective: if the radiant heat in the interscapular area can be abruptly turned off, whether by changes in circulation or in parenchymal stimulation, just as it can be rapidly turned on, then these opposing responses might influence the shape of the daily temperature rhythm of BAT by appearing as a series of oscillations. These cycles could in turn contribute to the diurnal pattern of core temperature.

There is as yet little research on the circadian temperature pattern of brown fat. Closa and coworkers (1993) presented a typical 24 h waveform but they used blood flow rather than spectral analysis to link BAT activity to the warming cycles present in core temperature profiles. Thus, one intent of the third study was to determine the specific cyclic components underlying the BAT temperature pattern.

Both the temperature profiles of brown fat and the spectral periodgrams based on these are dominated by the 24h circadian cycle. It remains unclear why the apparent hourly peaks and troughs in the BAT temperature pattern, which are mirrored in such tissues as the liver, do not appear as underlying cycles in the results of the Fourier analysis; rather, the 4h cycle is the strongest candidate for a consistent ultradian rhythm in brown fat. However, both the extent of variability observed here in the ultradian oscillations, and the frequencies of reported cycles are in agreement with the results of analyses from many other metabolic systems (Stupfel & Pavely, 1991; Hildebrandt, 1988). To our knowledge, this is the first Fourier analysis of brown fat temperature patterns.

The finding of a robust circadian component in brown fat temperature patterns provokes the question of whether the rhythm is endogenously controlled; to address this, the correlation between simultaneous BAT and core temperature were examined. The ultradian peaks and troughs, and the characteristic nocturnal rise all appear to be in phase, observations that are corroborated by the various cross correlation analyses. However, the most powerful evidence for the idea of endogenous control is the finding that BAT temperature patterns show an anticipatory rise in temperature prior to light offset, similar to core temperature.

The results of the cross correlation analyses can further be applied to investigate whether BAT heat production contributes to core or merely reflects it; the profiles point to a possible 10

to 30 min offset between BAT and core temperature, with BAT leading. This suggests that BAT heat production may not only promote core temperature rises (Closa et al, 1993), but may indeed play a role in the initiation of the rise.

Importantly, a series of methodological manipulations were performed in this study to examine the nature of the recorded temperature oscillations; the findings confirm that the transmitter readings are sensitive to BAT heat production, and that the pattern of values accurately reflects the readings of the thermoprobe. Attempts to further examine the input to the transmitter readings through surgical alteration of the BAT deposit were limited by small sample sizes, although the results do suggest that the large deposit of white fat in the interscapular area must contribute to the circadian temperature changes; sympathetic innervation of the white fat may therefore be important.

The complexity of BAT innervation, and of the vascular components must also influence the temperature readings. For example, the results of the second experiment reported here suggest that abrupt drops in BAT temperature may occur due to an α-induced vasoconstriction, while Woods and Stock (1994) speculate that AVA dilation may also be responsible for restriction of blood flow. The significance of BAT temperature changes due to blood flow, rather than direct activation of thermogenesis, is not yet clear; Closa et al (1993) reported that blood flow to the BAT deposit diminished to essentially zero during the cooling portion of core temperature cycles, which fits

with the idea that redistribution of blood volume to distal sites represents a primary means of cooling.

One of the fundamental research issues to emerge from these findings is the question of what drives the BAT temperature cycles, in light of evidence that they are endogenously controlled - an examination of BAT temperature rhythms after SCN destruction certainly represents one compelling future study. But interestingly, the VMH nucleus itself has been shown to participate in the regulation of circadian patterns. Lesions of the VMH result in disturbed food intake patterns and the loss of diurnal rhythm in blood levels of glucocorticoids (see Inoue, 1992 for review); it has been suggested that the lesions, which interfere with BAT heat production by interrupting SNS stimulation, might also interrupt neural output from the VMH thought to cyclically inhibit the nocturnal autonomic patterns which includes increased vagal tone and higher insulin levels, and responsiveness (Dallman, 1984). More recently, this cyclic inhibition has been attributed to SCN efferents coursing through the VMH (Stoynev & Ikonomov, 1987)

It has been further demonstrated that sustained high levels of VMH neural output can also disrupt circadian rhythms (Mitsushimi et al, 1994). The authors speculate that this output interferes with SCN regulation of the circadian activity patterns. Thus, both destruction and hyperstimulation of the VMH can disrupt circadian rhythms, although in neither group of studies has the effect on core temperature rhythms been examined.

The BAT response to SCN stimulation appears to be dependent

on activation of the VMH (Amir et al, 1989), which is consistent with findings from brain slice studies that an excitatory pathway is present from the SCN to the VMH (Kita et al, 1982). There is also evidence for direct information flow from the VMH to the SCN (Lutien et al, 1987), and for indirect pathways, possibly through the paraventricular thalamic nucleus. The latter is based on the specific combination of cuts around the VMH that are effective in preventing the disruptions due to hyperstimulation of the VMH nucleus (Mitsushumi et al, 1994). We found in our first study that a complex pattern of effective and ineffective sites is present within the nucleus; this hints at the redundancy and heterogeneity of those neuronal elements in the VMH that participate in either the acute BAT temperature changes, or the regulation of the cyclic pattern of temperature changes.

These studies suggest the presence of both direct and indirect routes in regulating BAT heat production; thus, it appears that the nucleus might modify only the output of the circadian pacemaker, or alternatively, modulate the original timing signals through direct input to the SCN. Both types of influence are intrinsic to the complex neural interactions underlying circadian rhythms, as is information from the environment, as well as feedback from other metabolic system such as sleep/wake, thermoregulation, and food intake; all of these elements are integrated to produce the ultimate pattern of an endogenous rhythm (Rietveld et al, 1991). If the numerous neuroendocrine factors that regulate BAT activity are also considered, it is clear that achieving a systematic portrait of

the physiological role of BAT heat production represents a challenging goal.

REFERENCES

AMIR, S., 1990. Intra-ventromedial hypothalamic injection of glutamate stimulates brown adipose tissue thermogenesis. **Brain Res. 511**: 341-344.

AMIR, S., SHIZGAL, P. and ROMPRE, P. 1989. Glutamate injection into the suprachiasmatic nucleus stimulates brown fat thermogenesis in the rat. **Brain Res. 498**: 140-144.

AMIR, S., SCHIAVETTO, A. 1990. Injection of prostaglandin E_2 into the anterior hypothalamic preoptic area activates brown adipose tissue thermogenesis. **Brain Res. 528**: 138-142.

ANAND, B. K., CHHINA, G. S., SHARMA, K. N., DUA, S. and SINGH, B. 1964. Activity of single neurons in hypothalamic feeding centers: effect of glucose. **Am. J. Physiol. 207**: 1146-1154.

ANGELI, A. & CARANDENTE, F. 1988. An update on clinical chronoendocrinology. In **Trends in Chronobiology 73**. Edited by W. Hekkens, G. Kerkhof and W. Rietveld. Pergamon, Oxford, pp.319-333.

ARASE, K., YORK, D. A. and BRAY, G. A. 1987. Corticosterone inhibition of the intracerebroventricular effect of 2-deoxy-D-glucose on brown adipose tissue thermogenesis. **Physiol. Behav. 40**: 489-495.

ARBUTHNOTT, E. 1989. Brown adipose tissue: structure and function. **Proc. Nutr. Soc. 48**: 177-182.

ARCH, J.S.R., AINSWORTH, A.T., CAWTHORNE, M.A., PIERCY, V., SENNITT,M.V., THODY, V.E., WILSON, C. AND WILSON, S. 1984. A typical β-adrenoceptor on brown adipocytes as a target for anti-obesity drugs. **Nature 309**: 163-165.

ARMITAGE, G., HERVEY, G. R., ROLLS, B. J., ROWE, E. A. and TOBIN, G. 1983. The effects of supplementation of the diet with highly palatable foods upon energy balance of the rat. **J. Physiol. 342**: 229-251.

BAZIN, R., ETEVE, D. and LAVAU, M. 1984. Evidence for decreased GDP-binding to brown adipose tissue mitochondria of obese Zucker (fa/fa) rats in the very first days of life. **Biochem J. 221**: 241-245.

BENZI, R. H. and GIRARDIER, 1986. The response of brown adipose tissue blood flow to insulin-induced hypoglycemia in conscious dogs and rats. **Pflugers Arch. 406**: 37-44.

BENZI, R. H., SHIBATA, M., SEYDOUX, J. and GIRARDIER, L. 1988. Prepontine knife cut-induced hyperthermia in the rat. **Pflügers Arch. 411:** 593-599.

BILLINGTON, C.J., BRIGGS, M.G. AND LEVINE, S. 1991. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. **Am. J. Physiol. 260:** R321-R327.

BLOOMFIELD, P. 1976. **Fourier analysis of time series: an introduction.** John Wiley & Sons, Inc. New York.

BLOUQUIT, M.F., KOUBI, H.. GELOËN, A. and GRIPOIS, D. 1991. Norepinephrine content in the brown adipose tissue of the developing obese Zucker rat. **Horm. Metab. Res. 23:** 239.

BOUILLAUD, F., RICQUIER, D., THIBAULT, J. and WEISSENBACH, J. 1985. Molecular approach to thermogenesis in brown adipose tissue: cDNA cloning of the mitochondrial uncoupling protein. **Proc. Natl. Acad. Sci. USA. 82:**445-448.

BORER, K. and CLOVER, K. 1994. Control by light of the temperature rhythm in food-restricted hamsters. **Physiol. Behav. 56:** 385-391.

BRAY, G. 1990. Obesity- a state of reduced sympathetic activity and normal or high adrenal activity (the autonomic and adrenal hypothesis revisited). **Int. J. Obesity 14** (suppl. 3): 77-92.

BRAY, G., YORK, D. A. and SWERDLOFF, R. 1973. Genetic obesity in rats. I. the effects of food restriction on body composition and hypothalamic function. **Metabolism 22:** 435-442.

BROCKWELL, P.J. and DAVIS, R. A. 1987. **Time series: theory and methods.** Springer-Verlag, New York.

BROOKS, S. L., ROTHWELL, N. J., STOCK, M. J. 1980. Increased proton conductance pathway in brown adipose tissue mitochondria of rats exhibiting diet-induced thermogeneisis. **Nature 286:** 274-276.

BROWN, D., LIVESEY, G. AND DAUNCEY, M. J. 1991. Influence of mild cold on the components of 24 hour thermogenesis in rats. **Journal of Physiology 441:** 137-154.

CALASSO, M., ZANTEDESCHI, E. and PARMEGGIANI, P.L. 1993. Cold-defense function of brown adipose tissue during sleep. **Am. J. Physiol. 265:** R1060-R1064.

CALINGASAN, N. and RITTER, S. 1992. Hypothalamic paravnetricular nucleus lesions do not abolish glucoprivic or lipoprivic feeding. **Br. Res. 595:** 25-31.

CANNON, W. B., MCIVER, M. A. and BLISS, S. W. 1924. Studies on the conditions of activity in endocrine glands. XIII. A sympathetic and adrenal mechanism for mobilizing sugar in hypoglycemia. **Am. J. Physiol. 69**: 44-66.

CANNON, B., HEDIN, A. and NEDERGAARD, J. 1982. Exclusive occurrence of thermogenin antigen in brown adipose tissue. **FEBS Lett. 150**:129-132.

CANNON, B., NEDERGAARD, J. LUNDBERG, J.M. HÖKFELT, T. TERENIUS, L. and GOLDSTEIN,M. 1986. Neuropeptide tyrosine (NPY) is co-stored with noradrenaline in vascular but not in parenchymal sympathetic nerves of brown adipose tissue. **Exp. Cell. Res. 164**: 546-550.

CARNEHEIM, C., NEDERGAARD, J. and CANNON. B. 1984. β-stimulation of lipoprotein lipase in rat brown adipose tissue druing acclimation to cold. **Am. J. Physiol. 246**: E327-E33.

CHAMPIGNY, O and RICQUIER, D. 1990. Effects of fasting and refeeding on the level of uncoupling protein mRNA in rat brown adipose tissue: evidence for diet-induced and cold-induced responses. **J. Nutr. 120**: 1730-1736.

CHATFIELD, C. 1980. **Analysis of time series: an introduction.** 2nd Edition. Chapman & Hall, New York.

CHENG, S., GRAHAM. B., YAKUBU, F., LIN, D.,PETERS, J.C., HILL, J.O. 1990. Metabolic differences between obesity-prone and obesity resistant. **Am. J. Physiol. 259**: R1103-R1110.

CHRISTENSON, R. C. 1991. **Linear models for multivariate, time series, and spatial data.** Springe-Verlag, New York.

CLOSA, D., ALEMANY, M. AND REMESAR, X. 1992. Effect of cold exposure on organ temperatures in wistar and zucker fa/fa rats. **J. Therm. Biol. 17**: 83-88.

CLOSA, D., GÓMEZ-SIERRA, J., LATRES, E., ALEMANY, M. and RAMESAR, X. 1993. Short term oscillations of aortic core temperature and thermogenic organ blood flow in the rat. **Exp. Physiol. 78**: 243-253.

COHN, C. and JOSEPH, D. 1967. Feeding frequency and lipogenesis in undernutrition. **Can. J. Physiol. Pharmacol. 45**: 609-612.

CORRELL, J. W. 1963. Adipose tissue: ability to respond to nerve stimulation in vitro. **Science 140**: 387-388.

COTTLE, W. H., NASH, C. W., VERESS, A. T. and FERGUSON, B. A. 1967. Release of noradrenaline from brown fat of cold-acclimated rats. **Life Sci. 6**: 2267-2271.

DALLMAN, M.F. 1984. Viewing the ventromedial hypothalamus from the adrenal gland. **Am. J. Physiol.** 246: R1-R12.

DAVIDOVICH, V. and PETROVIC, V. M. 1981. Diurnal variations in the catecholamine content of noradrenaline in the rat heart. **Int. Physiol. Biochem. 89**: 457-460.

DE CASTRO, J.M. 1978. Diurnal rhythms of behavioral effects on core temperature. **Physiol. Behav. 21**: 883-886.

DESAUTELS, M. 1985. Mitochondrial thermogenin content is unchanged during atrophy of BAT fasting mice. **Am. J. Physiol. 249**: E99-E106.

DESAUTELS, M., ZAHROR-BEHRENS, G. and HIMMS-HAGEN, J. 1978. Increase purine binding, altered polypepetide composition, and thermogenesis in brown adipose tissue mitochondria of cold-acclimated rats. **Can. J. Physiol. Pharmac. 56**:378-383.

DESAUTELS, M. and HIMMS-HAGEN, J. 1979. Roles of noradrenaline and protein synthesis in the cold-induced increase in purine nucletotide binding by rat brown adipose mitochondria. **Can. J. Biochem. 57**: 968-976.

DESAUTELS, M. DULOS, R. A. and MOZAFFARI, B. 1986. Selective loss of uncoupling protein from mitochondira of surgically denervated brown adipose tissue of cold-acclimated mice. **Biochem. Cell. Biol. 64**: 1125-1134.

DESAUTELS, M. MICHALSKA, E. and MOZAFFARI, B. 1990. Role of acid proteases in brown adipose tissue atrophy caused by fasting in mice. **Biochem. Cell Biol. 68**: 441-447.

DESAUTELS, M. and DULOS, R. A. 1991. Effects of neo-natal sympathectomy in brown fat development and susceptibility to high fat diet induced obesity in mice. **Can. J. Physiol. Pharmacol. 69**: 1868-1874.

DESAUTELS, M., WOLLIN, A., HALVORSON, I., MURALIDHARA, D.V. and THORNHILL, J. 1994. Role of mast cell histamine in brown adipose tissue thermogenic response. **Am. J. Physiol. 266**: R831-R837.

DIB, B., ROMPRE, P.P., AMIR, S. and SHIZGAL, P. 1994. Thermogenesis in brown adipose tissue is activated by electrical stimulation of the rat dorsal raphe nucleus. **Brain Res. 650**: 149-152.

DIETL, H., PRAST, H. and PHILIPPU, A. 1993. Pulsatile release of catecholamines in the hypothalamus of conscious rats. **Naunyn-Schmeid. Arch. Pharmacol. 347**: 28-33

DIGGLE, P.J. 1990. **Time series**. Clarendon Press, Oxford.

DILSAVER, S., OVERSTREET, D. and PECK, J. 1992. Measurement of temperature in the rat by rectal probe and telemetry yields compatible results. **Pharmacol. Biochem. Behav. 42**: 549-552.

DOWSE, H.B. and RINGO, J.M. 1989. The search for hidden periodicities in biological time series revisited. **J. Theor. Biol. 139**: 487-415.

DOWSE, H.B. and RINGO, J.M. 1991. Comparisons between periodgram and spectral analysis: apples are apples after all. **J. Theor. Biol. 148**: 139-144.

DULLOO. A., YOUNG, J. and LANDISBERG, L. 1988. Sympathetic nervous system response to cold exposure and diet in rat skeletal muscle. **Am. J. Physiol. 255**: E180-E188.

EASTMAN, C. and RECHTSCHAFFEN, A. 1983. Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. **Physiol. Behav. 31**: 417-427.

EASTMAN, C., MISTLBERGER, R. and RECHTSCHAFFEN, A. 1984. Suprachiasmatic nuclei lesions eliminate circadian temperature and sleep rhythms in the rat. **Physiol. Behav. 32**: 357-368.

EGAWA, M., YOSHIMATSU, H. AND BRAY, G.A. 1991. NPY suppresses sympathetic activity to interscapular brown adipose tissue in rats. **Am. J. Physiol. 260**: R328-R334.

ENGEL, B. T., SATO, A. and SATO, Y. 1992. Responses of sympathetic nerves innervating blood vessels in intrascapular brown adipose tissue and skin during cold stimulation in anesthetized C57BL/6J mice. **Japan. J. Physiol. 42**: 549-559.

ENRIGHT, J. T. 1990. Comparisons between periodgram and spectral analysis -don't expect apples to taste like oranges- Letter. **J. Theor. Biol. 143**: 425-430.

FAIN, J. N. and GARCÍA-SAINZ, J. A. 1983. Adrenergic regulation of adipocyte metabolism. **J. Lipid. Res. 24**: 945-966.

FAIN, J. N., MOHELL, N., WALLACE, M. A. and MILLS, I. 1984. Metabolic effects of **β**, **α_1**, and **α_2** adrenoceptor activation on brown adipocytes isolated from the perirenal adipose tissue of fetal lambs. **Metabolism 33**: 289-294.,

FILLENZ, M. 1990. **Noradrenergic neurons**. Cambridge University Press. New York.

FLAIM, K., HOROWITZ, J. M. and Horwitz, B. A. 1976. Functional and anatomical characteristics of the nerve-brown adipose interaction in the rat. **Pflugers Arch. 365**: 9-14.

FLAIM, K. E.., HORWITZ, B. and HOROWITZ, J. M. 1977. Coupling of signals to brown fat: **α**- and **β**- adrenergic signals in intact rats. **Am. J. Physiol. 232**: R101-109.

FLATMARK, T. and PEDERSEN, J. I. 1975. Brown adipose tissue mitochondria. **Biochem. Biophys. Acta. 416**: 53-103.

FOSTER, D. O. 1985. Participation of alpha-adrenoreceptors in brown adipose tissue thermogenesis in vivo. **Int. J. Obesity 9** (Suppl. 2): 25-29.

FOSTER, D. O. and FRYDMAN, M. L. 1979. Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by non-shivering thermogenesis. **Can. J. Physiol. Pharmacol. 57**: 257-270.

FOSTER, D. O. and DEPOCAS, F. 1980. Evidence against noradrenergic regulation of vasodilation in rat brown adipose tissue. **Can. J. Physiol. Pharmacol. 58**: 1418-1425.

FOSTER, D. O., DEPOCAS, F. and FRYDMAN, M. L. 1980. Noradrenaline-induced calorigenesis in warm- and in cold-acclimated rats: relations between concentrations of noradrenaline in arterial plasma, blood flow to differently located masses of brown adipose tissue, and calorigenic response. **Can. J. Physiol. Pharmacol. 58**: 915-924.

FOSTER, D. O., DEPOCAS, F. ZAROR-BEHRENS, G. 1982a. Unilaterality of the sympathetic innervation of each pad of rat interscapular brown adipose tissue. **Can. J. Physiol. Pharmacol. 60**: 107-113.

FOSTER, D. O., DEPOCAS, F. and ZUCKER, M. 1982b. Heterogeneity of the sympathetic innervation of rat interscapular brown adipose tissue via intercostal nerves. **Can. J. Physiol. Pharmacol. 60**: 747-754.

FREEMAN, P. H. and WELLMAN, P. 1987. Brown adipose tissue thermogenesis induced by low level electrical stimulation of hypothalamus in rats. **Brain Res. Bull. 18**: 7-11.

FROHMAN, L. 1980. Hypothalamic control of metabolism. In **Handbook of Physiology** (Vol. 2). Edited by P. J. Morgane and J. Panksepp. Marcel Delcker Inc. New York.

FROHMAN, L., and BERNARDIS, LEE L. 1971. Effect of hypothalamic stimulation on plasma glucose, insulin, and glucagon levels. **Am. J. Physiol. 221:** 1596-1603.

FUKUSHIMA, M., TOKUNAGA, K., LUPIEN, J. KEMNITZ, J. W. and BRAY, G.A. 1987. Dynamic and static phases of obesity following lesions in PVN and VMH. **Am. J. Physiol. 253:** R523-R529.

FULLER, C., LYDIC, R., SULZMAN, F., ALBERS, H.E., TEPPER, B. and MOORE-EDE, M.C. 1981. Circadian rhythm of body temperature persists after suprachiasmatic lesions in the squirrel monkey. **Am. J. Physiol. 241:** R385-R391.

FULLER, C., SULZMAN, F. AND MOORE-EDE, M. 1985. Role of heat loss and heat production in generation of the circadian temperature rhythm of the squirrel monkey. **Physiol. Behav. 34:** 543-546.

FULLER, W. 1976. **Introduction to statistical time series.** John Wiley & Sons: New York.

FYDA, D., COOPER, K. E., and VEALE, W. 1991. Contribution of brown adipose tissue to central PGE_1-evoked hyperthermia in rats. **Am. J. Physiol. 29:** R59-R66.

GAIKWAD, A. RAMASARMA, T. and KURUP, C. 1990. Regulation of activity of brown adipose tissue mitochondria through changes in cytochromes. **Biotechnol. Appl. Biochem. 12:** 562-566.

GALLISTEL, C.R., SHIZGAL, P. and YEOMANS, J.S. 1981. A portrait of the substrate for self-stimulation. **Psychol. Rev. 88:** 228-273.

GLICK, Z. 1984. Meal intake and brown adipose tissue in the rat. **Nutr. Behav. 2:** 65-75.

GLICK, Z. and TEAGUE, R. J. and BRAY, G. A. 1981. Brown adipose tissue thermic response is increased by a single low protein, high carbohydrate meal. **Science. 213:** 1125-1126.

GLICK, Z., TEAGUE, R. J., BRAY, G. A. and LEE, M. 1983. Compositional and metabolic changes following single test meal. **Metabolism 32:** 1146-1150.

GLICK, Z. WICKLER, S. J., STERN, J. S. and HORWITZ, B. A. 1984. Regional blood flow in rats following a single low protein, high carbohydrate test meal. **Am. J. Physiol.** 247: R160-R166.

GÓMEZ-SIERRA, J. M., CANELA, E., ESTEVE, M., RAFECAS, I., CLOSA, D., REMESAR, X. and ALEMANY, M. 1993. Analysis of ultradian heat production and aortic core temperature rhythms in the rat. **Arch. Int. Physiol. Biochem. Biophys. 101:** 117-122.

GONG, T. W., STERN, J. S., and HOROWITZ, B. H. 1990. High fat feeding increases brown fat GDP binding in lean but not obese rats. **J. Nutr. 120**: 786-792.

GORDON, C. J. 1983. A review of terms for regulated vs. forced neurochemical-induced changes in body temperature. **Life Sciences 32**: 1285-1295

GORDON, C. J. 1991. Thermal biology of the rat. **Physiol. Behav. 47**: 227-233.

GOUBERN, M., CHAPEY, M., PORTET, R. 1991. Time-course variations of effective proton conductance and GDP-binding in brown adipose tissue mitochondira of rats during prolonged cold exposure. **Comp. Biochem. Physiol. 100B**: 727-732.

GUILLAUME-GENTIL, C., ROHNER-JEANRENAUD, F., ABRAMO, F., BESTETTI, G., ROSSI, G. and JEANRENAUD, J. 1990. Abnormal regulation of the hypothalamo-pituitary-adrenal axis in the genetically obese fa/fa rat. **Endocrinol. 126**: 1873-1879.

GUYTON, A. 1991. **Textbook of medical physiology**. 8th Edition. W. B. Saunders Co., Toronto.

HALVORSON, I., GREGOR, L. and THORNHILL, J. A. 1990. Brown adipose tissue thermogenesis is activated by electrical and chemical (L-glutamate) stimulation of the ventromedial hypothalamic nucleus in cold-acclimated rats. **Brain Res. 552**: 76-82.

HENNINGFIELD, M. F. and SWICK, R. 1987. Immunochemical detection and quantitation of brown adipose tissue uncoupling protein. **Biochem. Cell. Biol. 65**: 245-251.

HERVEY,G. R. and TOBIN, G. 1983. Luxosconsumption, diet induced thermogenesis and brown fat: a critical review. **Clin Sci. 64**: 7-18.

HILDEBRANDT, G. 1988. Temporal order of ultradian rhythms im man. In **Trends in chronobiology 73**. Edited by Hekkens, W., Kerkof, G. and Rietveld, W., Pergamon Press, London. pp. 107-122.

HIMMS-HAGEN, J. 1965. Lipid metabolism in warm acclimated and cold-acclimated rats exposed to cold. **Can. J. Physiol. Pharmacol. 43**:379-403.

HIMMS-HAGEN, J. 1976. Cellular thermogenesis. **Ann. Rev. Physiol. 38**: 315-351

HIMMS-HAGEN, J. 1979. Obesity may be due to a malfunctioning of brown fat. **Can. Med. Assoc. J. 121**: 1361-1364

HIMMS-HAGEN, J. 1985. Brown adipose tissue metabolism and thermogenesis. **J. Ann. Rev. Nutr. 5**: 69-94.

HIMMS-HAGEN, J. 1989a. Role of thermogenesis in the regulation of energy balance in relation to obesity. **Can. J. Physiol. Pharmacol. 67**: 394-401.

HIMMS-HAGEN, J. 1989b. Brown adipose tissue thermogenesis and obesity. **Prog. Lipid Res. 28**: 67-115.

HIMMS-HAGEN, J. 1990. Brown adipose tissue thermogenesis: role in thermoregulation, energy regulation and obesity. In **Thermoregulation: physiology and biochemistry**. Edited by E. Schönbaum and P. Lomax. Pergamon Press, Inc., New York, pp 327-414.

HIMMS-HAGEN, J. 1991. Neural control of brown adipose tissue thermogenesis, hypertrophy, and atrophy. **Front. Neuroendocrin. 12**: 38-93.

HIMMS-HAGEN, J. 1995. Role of brown adipose tissue thermogeneis in control of thermoregulatory feeding in rats: a new hypothesis that links thermostatic and glucostatic hypothesis for control of food intake. **Proc. Soc. Exp. Biol. Med. 208**: 159-169.

HIMMS-HAGEN, J. and DESAUTELS, M. 1978. Mitochondrial defect in brown adipose tissue of obese (ob-ob) mouse - reduced binding of purine nucleotides and a failure to respond to cold by an increase in binding. **Biochem. Biophys. Res. Commun. 83**: 628-634.

HIMMS-HAGEN, J., CUI, J. and SIGURDSON, S.L. 1990. Sympathetic and sensory nerves in control of growth of brown adipose tissue: effects of denervation and capsaicin. **Neurochem Int. 17**: 271-279.

HINCKEL, P., CRISTANTE, L. and BRÜCK,K. 1983. Inhibitory effects of the lower brain stem on shivering. **J. Therm. Biol. 8**: 129-131.

HIROSHIGE, T., HONMA, K. and HONMA, S. 1991. SCN-independent circadian oscillators in the rat. **Brain Res. Bull. 27**: 441-445.

HOGAN, S., COSCINA, D. and HIMMS-HAGEN, J. 1982. Brown adipose tissue of rats with obesity-inducing ventromedial hypothalamic lesions. **Am. J. Physiol. 243**: E338-E344.

HOLLOWAY, B., HOWE, R., RAO, B., STRIBLING, D., MAYERS, R., BRISCOE, M. and JACKSON, J. 1991. ICI D7114 a novel selective β-adrenoceptor agonist selectively stimulates brown fat and increases whole-body oxygen consumption. **Br. J. Pharmacol. 104**: 97-104.

HOLT, S. J., WHEAL, H. V. and YORK, D. A. 1987. Hypothalamic control of brown adipose tissue in zucker lean and obese rats. Effect of electrical stimulation of the ventromedial hypothalamic nucleus and other hypothalamic areas. **Brain Res. 405**: 227-233.

HOLT, S. J. and YORK, D. A. 1989. Interaction of intracerebroventricular insulin and glucose in the regulation of the activity of sympathetic efferent nerves to brown adipose tissue in lean and obese Zucker rats. **Brain Res. 500**: 384-388.

HONMA, K. AND HIROSHIGE, T. 1978. Simultaneous determination of circadian rhythms of locomotor and body temperature in the rat. **Japan. J. Physiol. 28**: 159-169.

HONMA, S., HONMA, K., SHIRAKAWA, T. and HIROSHIGA, T. 1988. Rhythms in behaviors, body temperature and plasma corticosterone in SCN lesioned rats given metamphetamine. **Physiol. Behav. 44**: 247-255.

HOPPENBRUOWERS, T. 1986. Ontogenesis of ultradian respiratory rhythms. **J. Interdisc. Cycle Res. 17**: 140-141.

HOROWITZ, B. A. and HAMILTON, J. 1984. Alpha-adrenergic-induced changes in hamster (Mesocricetus) brown adipocyte respiration and membrane potential. **Comp. Biochem. Physiol. 78C**:99-104.

HUGIE, T., HALVORSON, I. and THORNHILL, J. 1992. Brown adipose tissue temperature responses following electrical stimulation of ventromedial hypothalamic and lateral preoptic areas or after norepinephrine infusion to Long Evans or Sprague Dawley rats. **Brain Res. 575**: 57-62.

HULL, D. and SEGALL, M. M. 1965. Sympathetic nervous control of brown adipose tissue heat production in the newborn rabbit. **J. Physiol. 181**: 458-467.

IMAI-MATSUMURA, K., NAKAYAMA, T. 1986. The central efferent mechanism of brown adipose tissue thermogenesis induced by preoptic cooling. **Can. J. Physiol. Pharmacol. 65**: 1299-1303.

INOUE, S. 1992. Animal models of obesity: hypothalamic lesions In **Obesity**. Edited by P. Björntrop and B. Brodoff. J.B Lippincott Co., Philadelphia. pp 266-277.

INTERNATIONAL UNION OF PHYSIOLOGICAL SCIENCES 1987. Glossary of terms for thermal physiology. **Pflugers. Arch. 410**: 567-587.

IWAI, K., HELL, N. and SHIMAZU, T. 1987. Effect of ventromedial hypothalamic stimulation on blood flow of brown adipose tissue in rats. **Pflugers Arch 410**:44-47.

JECQUIER, E., MUNGER, R. and FELBER, J. 1992. Thermogenic effects of various β-adrenoceptor agonists in humans: their potential usefulness in the treatment of obesity. **Am. J. Clin. Nutr. 55**: 249S-251S.

JENNINGS, G. and ELIA, M. 1987. Effect of E. coli endotoxin on temperature, oxygen consumption, and brown adipose tissue thermogenesis in rats and mice. **Biosci. Rep. 7**: 517-523.

JILGE, B., FRIESS, L. and STÄHLE, H. 1986. Internal coupling of five functions of rabbits exhibiting a bimodal circadian rhythm. **J. Interdisc. Cycle Res.: 7-28.**

JOHANSSON, B. 1958. Brown fat: a review. **Metab. Clin. Exptl. 8**: 221-239.

JOHNSON, C. 1992. Nutrient intake as a time signal for circadian rhythm. **J. Nutr. 122**: 1753-1759.

KAUL, R., HELDMAIER, G. and SCHMIDT, I. 1990. Defective thermoregulatory thermogenesis does not cause onset of obesity in zucker rats. **Am. J. Physiol. 259**: E11-E18.

KENDALL, M. 1976. **Time series.** 2nd edition. Charles Griffen and Company Ltd, London.

KELLY, L. and BIELAJEW, C. 1991. Ventromedial hypothalamic regulation of brown adipose tissue. **NeuroRep. 2**: 41-44.

KENT, S., HURD, M. and SATINOFF, E. 1991. Phantolomine and thermoregulation in rats. **Pharm. Biochem. Behav. 40**: 709-716.

KIKUCHI, K., OKANO, S., NOZU, T., YAHATA, T. and KUROSHIMA, A. 1992. Effects of chronic administration of noradrenaline and glucagon on in vitro brown adipose tissue thermogenesis. **Japan. J. Physiol. 42**: 165-170.

KITA, M., SHIBATA, S., OOMURA, Y. and OHKI, K. 1982. Excitatory effects of the suprachiasmatic nucleus in the rat hypothalamic slice. **Brain Res. 235**: 227-233.

KLAUS, S. CASTEILLA, F. BOUILLAUD, F. and RICQUIER, D. 1990. The uncoupling protein UCP: a membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. **Int. J. Biochem. 23**: 791-801.

KOOPMANS, L.H. 1974. **The spectral analysis of time series.** Academic Press, New York.

KOOPMANS, L.H. 1983. A spectral analysis primer. In **Time series in the frequency domain**. Edited by D.R. Brillinger and P.R. Krishnaiah. North-Holland Publishing Co., Amsterdam. pp 169-183.

KOPECKY, J., SIGURDSDON, L., PARK, I. R. A. and Himms-Hagen, j. 1986. Thyroxime 5'-deiodinase in hamster and rat brown adipose tissue: effect of cold and diet. **Am J. Physiol. 251**: E1-E7.

KRÄUCHI, K. AND WIRZ-JUSTICE, A. 1994. Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditons in men. **Am. J. Physiol. Behav. 267**: R819-R829.

KRIEGER, D.T. and HAUSER, H. and KREY L. 1977. Suprachiasmatic nuclear lesions do not abolish food-shifted circadian adrenal and temperature rhythmicity. **Science 197**: 398-399.

KUMON, A., TAKAHASHI, A., HARA, T. and SHIMAZU, T. 1976. Mechanism of lipolysis induced by electrical stimulation of the hypothalamus in the rabbit. **J. Lipid Res. 17**: 551-558.

KUROSAWA, M. 1991. Reflex changes in thermogenesis in the interscapular brown adipose tissue response to thermal stimulation of the skin via sympathetic efferent nerves in anaesthetized rats. **J. Autonom. Nerv. Syst. 33**: 15-24.

LAFONTAN, M. and BERLAN, M. 1992. Fat cell adrenergic receptors and the control of white and brown fat cell function. **J. Lipid Res. 34**: 1057-1091.

LAURY, M. C., CHAPEY, M. F. and PORTET, R. 1987. Involvement of the sympathetic nervous system in lipolytic activity in brown adipose tissue of cold acclimated rats. **Comp. Biochem. Physiol. 87A**: 197-203.

LE MAGNEN, J. 1985. **Hunger**. Cambridge University Press. New York.

LEAN, M. E. and JAMES, W. P. T. 1983. Uncoupling protein in human brown adipose tisue mitochondria- isolation and detection by specific anti-sera. 1983. **FEBS Lett.** 163:235-240.

LEAN, M. E. J. 1989. Brown adipose tissue in humans. **Proc. Nutr. Soc. 48**: 243-256.

LEBLANC, J. and VILLEMARIE, A. 1970. Thyroxine and noradrenaline on noradrenaline sensitivity, cold resistance, and brown fat. **Am. J. Physiol. 218**: 1742-1745.

LEVIN, B., GOLDSTEIN, A. and NATELSON, B.H. 1978. Ultradian rhythm of plasma noradrenaline in the rheusus monkey. **Nature 272**: 164

LEVIN, B., TRISCARI, J. and SULLIVAN, A. 1983. Studies of the origin of abnormal sympathetic function in obese Zucker rats. **Am. J. Physiol. 245**: E87-E93.

LEVIN, B. E., FINNEGAN, M., MARGUET, E. and SULLIVAN, A. 1984. Defective brown adipose tissue oxygen consumption in obese zucker rats. **Am. J. Physiol. 247**: E94-E100.

LEVIN, B. E., HOGAN, S. and SULLIVAN, A. 1989. Initiation and perpetuation of obesity and obesity resistance in rats. **Am. J. Physiol. 256**:R766-R771.

LIN, C. and KLINGENBERG, M. 1980. Isolation of the uncoupling protein from brown adipose tissue mitochondria. **FEBS Lett. 113**:299-303.

LISNEY, S.J.W. and BHARALI, L.A.M. 1989. The axon reflex: an outdated idea or a valid hypothesis. **NIPS 4**:45-48.

LOWELL, B. B., S-SUSULIC, V. HAMANN, A., LAWITTS, J., HIMMS-HAGEN, J., BOYER, B. B., KOZAK, L. P. and FLIER, J. S. 1993. Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. **Nature 366**:740-742.

LUBOSHITZKY, R., BERNARDIS, L. L., GOLDMAN, J. K. and KODIS, M. 1983. Brown adipose tissue metabolism in hypothalamic-obese rats. **Metabolism 32**: 108-113.

LUITEN, P.G.M., TER HORST, G.J., and STEFFENS, A.B. 1987. The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. **Prog. Neurobiol. 28**: 1-54

LUPIEN, J., GLICK, Z., BRAY, G. and LEE, M. 1984. Brown adipose tissue mitochondrial GDP-binding and NA/K-ATPase activity following a low protein, high carbohydrate meal. **Pflügers Arch. 392**: 172-177.

MA, S.W. and FOSTER, 1984. Potentiation of in vivo thermogenesis in rat brown adipose tissue by stimulation of α_1 adrenoceptors associated with increased release of cyclic AMP. **Can.J. Physiol. Pharmacol. 62**: 943-948.

MA, S.W., FOSTER, D.O., NADEAU, B.E. and TRIANDAFILLOU, J. 1988. Absence of increased oxygen consumption in brown adipose tissue of rats exhibiting "cafeteria" diet-induced thermogenesis. **Can. J. Physiol. Pharmacol. 66**: 1347-1354.

MA, S. W. and FOSTER, D. O. 1989. Brown adipose tissue, liver, and diet-induced thermogenesis in cafeteria diet-fed rats. Can. **J. Physiol. Pharmac. 67**: 376-381.

MARSHALL, N. B., BARRNETT, R. J. and MAYER, J. 1955. Hypothalamic lesions in gold-thioglucose injected mice. **Proc. Soc. Exp. Biol. Med. 90**: 240-244.

MARTIN, R. J., HARRIS, R. and JONES, D. D. 1986. Evidence for a central mechanism of obesity in the Zucker fatty rat (fa/fa). **Proc. Soc. Exp. Biol. Med. 183**: 1-10.

MARTIN, R., ATGIE, C., GINESTE, J., NIBBELINK, M., AMBRID,L. and RICQIER, D. 1990. Increased GDP-binding and thermogenic activity in brown adipose tissue mitochondria during arousal of the hibernating garden dormouse (eliomys quercinus l.) **Comp. Biochem. Physiol. 98A**: 311-316

MATTHEWS, G. 1977. Strength-duration properties of single units driven by electrical stimulation of the lateral hypothalamus in rats. **Br. Res. Bull. 3**: 171-174.

MATTHIAS, A., RICHARDS, S. M., DORA, K., MICHAEL, G. C., and COLQUHOUN, E. 1994. Characterization of perfused periaortic brown adipose tissue from the rat. **Can. J. Physiol. Pharmacol. 72**: 344-352.

MERCER, D.M.A. 1965. The limitations of detection of periodicities in random noise. In **Circadian clocks**. Edited by J. Aschoff. North-Holland Publishing Co. Amsterdam.

MILIARESSIS, E. and GRATTON, A. 1981. A chronic moveable nonrotating electrode for brain stimulation in the rat. **Physiol. Behav. 26**: 891-894.

MILNER, R. E. and TRAYHURN, P. 1989. Rapid quantiation of uncoupling protein in brown adipose tissue mitochondria by a dot imuunoblotting ("dot blotting") procedure: application to the measurement of uncoupling protein in Richardson's ground squirrel, rats, and mice. **Biochem. Cell. Biol. 68**: 973-979.

MINOKOSHI, Y. SAITO, M. and SHIMAZU, T. 1986a. Metabolic and morphological alterations of brown adipose tissue after sympathetic denervation in rats. **J. Autonom. Nerv. Sys. 15**: 197-204.

MINOKOSHI, Y. SAITO, M. and SHIMAZU, T. 1986b. Sympathetic denervation impairs responses of brown adipose tissue to VMH stimulation. **Am. J. Physiol. 251**: R1005-R1008.

MINOKOSHI, Y. SAITO, M. and SHIMAZU, T. 1988. Sympathetic activation of lipid synthesis in brown adipose tissue in the rat. **J. Physiol. Behav. 398**: 361-370.

MISTLBERGER, R.E. 1994. Circadian food-anticipatory activity: formal models and physiological mechanisms. **Neurosci. Behav. Rev. 18**: 171-195

MITSUSHIMI, D., YOKAWA, T., NISHIHARA, M. and TAKAHASHI, M. 1994. Attenuation of the expression of circadian rhythms by chronic output from the VMH in rats. **Physiol. Behav.** 56: 891-899.

MOHELL, N., CONNOLLY, E. and NEDERGAARD, J. 1987. Distinction between mechanism underlying α_1- and β-adrenergic respiratory stimulation in brown fat cells. **Am. J. Physiol. 253**: C301-C308.

MOORE, B. 1987. The cafeteria diet - an inappropriate tool for studies of thermogenesis. **J. Nutr. 117**: 227-231.

MOORE, R.Y. and EICHLER, V. B. 1972. Loss of circadian adrenal corticosterone rhythm following suprachiasmatic nucleus lesions in the rat. **Brain Res. 71**: 17-33.

MORATH, M. 1974. The four-hour feeding rhythm of the baby as a free-running endogenously regulated rhythm. **Int. J. Chronobiology 2**: 39-45.

MORIMOTO, A. MURAKAMI, N. ONO, T. WATANABE, T. and SAKATA, Y. 1986. Stimulation of ventromedial hypothalamus induces cold defence responses in conscious rabbits. **Am. J. Physiol. 250**: R560-R566.

MROSOVSKY, N. and JANIK, D. 1993. Behavioral decoupling of circadian rhythms. **J. Biol. Rhythms. 8**: 57-65.

MUNDL, W. J. 1980. A constant-current stimulator. **Physiol. Behav. 24**: 991-993.

NAKAYAMA, T., YAMAMOTO, K., ISHIKAWA, Y., IMAI, K. 1981. Effects of preoptic thermal stimulation on the ventromedial hypothalamic neurons in rats. **Neurosci. Lett. 26**: 177-181.

NEDERGAARD, J. and CANNON, B. 1984. Preferential utilization of brown adipose tissue lipids during arousal from hibernation in hamsters. **Am. J. Physiol. 247**: R506-R512.

NEDERGAARD, J., RAASMAJA, A. and CANNON, B. 1984. Parallel increases in amount of (^{3}H)GDP-binding and thermogenin antigen in brown adipose tissue mitochondria of cafeteria-fed rats. **Biochem. Biophys. Res. Commun. 122**: 1328-1336.

NEUROGRAPHICS, Inc. 1993. "The Rat Brain: Clipart for the Scientist", Kanata, Ontario, Canada.

NICHOLLS, D. 1976. Hamster brown adipose tissue mitochondria. purine nucleotides contr9ol of the ion conductance of the inner membrane, the nature of the nucleotide binding site. **Eur. J. Biochem. 62**: 223-228.

NICHOLLS, D. and LOCKE, R. 1984. Thermogenic mechanisms in brown fat. **Physiol. Rev. 64**: 1-64.

NIIJIMA, A. 1986. Effect of glucose and other hexoses on efferent discharges of brown adipose tissue nerves. **Am. J. Physiol. 251**: R240-R242.

NIIJIMA, A., ROHNER-JEANRENAUD, F., JEANRENAUD, B. 1984. Role of ventromedial hypothalamus on sympathetic efferents of brown adipose tissue. **J. Physiol. 247**: R650-R654.

NNODIM, J.O. AND LEVER, J. 1988. Neural and vascular provisions of rat interscapular brown adipose tissue. **Am. J. Anat. 182**: 283-293.

NORMAN, D., MUKHERJEE, S., SYMONS, D., JUNG, R.T. and LEVER, J.D. 1988. Neuropeptides in interscapular and perirenal brown adipose tissue in the rat: plurality of innervation. **J. Neurocytol.** 17: 305-311.

OHLSON, K. MOHELL, N., CANNON, B., LINDAHL, S. NEDERGAARD, J. 1994. Thermogensesis in brown adipocytes is inhibited by volatile anesthetic agents. **Anesth. 81**: 176-183.

ORTEGA, G.J., ROMANELLI, L. and GOLOMBEK, D.A. 1994. Statistical and dynamical analysis of circadian rhythm. **J. theor. Biol. 169**: 15-21.

PARK, I. and HIMMS-HAGEN, J. 1988a. A circadian study of the function of brown adipose tissue in the rat. **FASEB J. 2**: A1612.

PARK, I. and HIMMS-HAGEN, J. 1988b. Neural influences on trophic changes in brown adipose tissue during cold acclimation. **Am. J. Physiol. 255**: R874-881.

PAXINOS, G. and WATSON, C. 1986. **The Rat Brain in Stereotaxic Coordinates**, 2nd edn., Academic Press, New York.

PEACHEY, T., FRENCH, R. and YORK, D. 1988. Regulation of GDP-binding and uncoupling protein concentration in brown adipose tissue mitochondira. **Biochem. J. 249**: 451-457.

PERKINS, M. N., ROTHWELL, N. J., STOCK, M. J. and STONE, T. 1981. Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. **Nature 289**: 401-402.

PHILLIPS, J.L. and MIKULKA, P.J. 1979. The effects of restricted food access upon locomotor activity in rats with suprachiasmatic nucleus lesions. **Physiol. Behav. 23**: 257-262.

PRESTON, E., TRIANDAFILLOU, J. and HAAS, N. 1989. Colchicine lesions of ventromedial hypothalamcus: effects on regulatory thermogenesis in the rat. **Pharmacol. Biochem. Behav. 32**: 301-307.

PUIGSERVER, P., GIANOTTI, M. and PALOU, A. 1992. Impaired starvation-induced loss of mitochondrial protein in the brown adipose tissue of dietary obese rats. **Int. J. Obesity 16**: 255-261.

PUIGSERVER, P., HERRON, D., GIANOTTI, M., PALOU, A. CANNON, B. and NEDERGAARD, J. 1992. Induction and degradation of the uncoupling protein thermogenin in brown adipocytes in vitro and in vivo. **Biochem. J. 284**: 393-398.

RANCK, J.B. 1975. Which elements are excited in electrical stimulation of mammalian central nervous system: a review. **Brain Research 98**: 417-440.

REDLIN, U., NUESSLEIN, B. AND SCHMIDT, I. 1992. Circadian changes of brown adipose tissue themogenesis in juvenile rats. **Am. J. Physiol. 31**: R504-R508.

REFINETTI, R. 1992. Analysis of circadian rhythm of body temperature. **Behav. Red. Meth. 24**: 28-36.

REFINETTI, R. 1993. Laboratory instrumentation and computing: comparison of six methods for the determination of the period of circadian rhythms. **Physiol. Behav. 54**: 869-875.

REFINETTI, R. 1994. Contribution of locomotor activity to the generation of the daily rhythm of body temperature in golden hamsters. **Physiol. Behav. 56**: 829-831.

REFINETTI, R. and CARLISLE, H. J. 1989. Thermoregulation during pentobarbitol and ketamine anesthesia in rats. **J. Physiol. (Paris) 83**: 300-303.

REFINETTI, R. and MENAKER, M. 1991. The circadian rhythm of body temperature. **Physiol. Behav. 51**: 613-637.

RIAL, E. and NICHOLLS, D. G. 1984. The mitochondrial uncoupling protein from guinea pig brown adipose tissue mitochondria: synchronous increase in structural and functional parameters during cold-adaptation. **Biochem J. 222**: 685-693.

RICARDO, J. A. 1981. Efferent connections of the subthalamic region in the rat. II. the zona incerta. **Brain Res. 214**: 43-60.

RICHARD, D., BOILY, P., DUFRESNE, M. and LECOMPTE, M. 1988. Energy balance and facultative diet-induced thermogenesis in mice fed a high-fat diet. **Can. J. Physiol. Pharmacol. 66**: 1297-1302.

RICQUIER, D. AND KADER, J. C. 1976. Mitochondrial protein alteration in active brown fat. A sodium dodecyl sulfate-polyacrilamide gel electrophoritic study. **Biochem. Biophys. Res. Commun. 73**: 577-583.

RICQUIER, D., MORY, G., BOUILLAUD, F., THIBAULT, J. and WEISSENBACH, J. 1984. Rapid increase of mitochondrial uncoupling protein and its mRNA in stimulated brown adipose tissue. **FEBS Lett. 178**:240-244.

RICQUIER, D., BOUILLAUD, F. TOUMELIN, P., MORY, G. BAZIN, R. ARCH, J. and PENICAUD, L. 1986. Expression of uncoupling protein mRNA in thermogenic or weakly thermogenic brown adipose tissue. Evidence for a rapid β-adrenoreceptor-mediated and transcritionally regulated step during activation of thermogenesis. **J. Biol. Chem. 261**: 13905-13910.

RIETVELD, W.J., MINORS, D. and WATERHOUSE, J.M. 1993. Circadian rhythms and masking: on overview. **Chronobiol. Int. 10**: 306-312.

ROHNER-JEANRENAUD, F., SEYDOUX, J., CHINET, A., BAS, S., GIACOBINO, J., ASSIMACOPOULAS-JEANNET, F., JEANRENAUD, B. and GIRARDIER, L. 1983. Defective diet-induced but normal cold-induced brown adipose tissue adaptation in hypothalamic obesity in rats. **J. Physiol., Paris 78**:833-837.

ROTHWELL, N. J. 1989. Central control of brown adipose tissue. **Proc. Nutr. Soc. 48**: 197-206.

ROTHWELL, N. J. and STOCK, M. J. 1979. A role for brown adipose tissue thermogenesis. **Nature 281**: 31-35.

ROTHWELL, N. and STOCK, M. J. 1980. Similarities between cold- and diet-induced thermogenesis in the rat. **Can. J. Physiol. Pharmac. 58**:842-848.

ROTHWELL, N. and STOCK, M. 1981. Influence of noradrenaline on blood flow to brown adipose tissue in rats exhibiting diet-induced thermogenesis. **Pflugers Arch. 389**: 237-242.

ROTHWELL, N., STOCK, M., WARWICK, B. and WINTER, P. 1983. Diurnal variations in circulating hormone levels and brown adipose tissue activity in "cafeteria"-fed rats. **Comp. Biochem. Physiol. A Comp. Physiol. 75**: 961-965.

ROTHWELL, N. and STOCK, M. 1984. Effects of denervating brown adipose tissue on the response to cold, hyperphagia and noradrenaline treatment in the rat. **J. Physiol. 355**: 457-463.

ROTHWELL, N. and STOCK, M. 1992. Surgical removal of brown fat results in rapid and complete compensation by other depots. **Am J. Physiol. 257**: R253-R258.

RUIS, J.F., RIETVELD, W.J. and BUYS, P.J. 1987. Effects of suprachiasmatic nuclei lesions on circadian and ultradian rhythms in ocular enucleated rats. **J. Interdisc. Cycle Res. 18**: 259-273.

RUSAK, B. 1977. The role of the suprachiasmatic nuclei in the generation of circadian rhythms in the golden hamster, mesocriscetus auratus. **J. Comp. Physiol. A118**: 145-164.

SAITO, M., MINOKOSHI, Y. and SHIMAZU, T. 1985. Brown adipose tissue after ventromedial hypothalamic lesions in rats. **Am. J. Physiol. 248**: E20-E25.

SAITO, M., MINOKOSHI, Y. and SHIMAZU, T. 1989a. Accelerated norepinephrine turnover in peripheral tissues after ventromedial hypothalamic stimulation in rats. **Brain Res. 481**: 298-303.

SAITO, M., NISHIMURA, K. and KATO, H. 1989b. Modifications of circadian cortisol rhythm by cyclic and continuous total enteral nutrition. **J. Nutr. Sci. Vitaminol. 35**: 639-647.

SAKAGUCHI, T. and BRAY, G. 1987. The effect of intrahypothalamic injections of glucose on sympathetic efferent firing rate. **Brain Res. Bull. 18**:591-595.

SAKAGUCHI, T. and YAMAZAKI, M. 1988. Hepatic portal injection of glucose elevates efferent sympathetic discharges of interscapular brown adipose tissue. **Exp. Neurol. 101**: 464-469.

SAKAGUCHI, T., TAKAHASHI, M. and BRAY, G. A. 1988. Diurnal changes in sympathetic activity. **J. Clin. Invest. 82**: 282-286.

SATINOFF, E. and PROSSER, R.A. 1988. Suprachiasmatic nuclear lesions eliminate circadian rhythms of drinking and activity, but not of body temperature, in male rats. **J. Biol. Rhythms. 3**: 1-22.

SAUNDERS, D. S. 1977. **An introduction to biological rhythms.** Blackie & Son Ltd., London.

SCALES, W. and KLUGER, M. 1987. Effect of antipyrogenic drugs on circadian rhythm in body temperature of rats. **Am. J. Physiol. 253**: R306-R313.

SCHÖNBAUM, E. JOHNSON, G.E. and SELLERS, E. A. 1966. Acclimation to cold and norepinephrine; effects of immunosympathectomy. **Am J. Physiol. 211**: 647-650.

SCHÖNBAUM, E. and LOMAX, P. 1990. Temperature regulation and drugs: an introduction. In **Thermoregulation.** Edited by E. Schönbaum and P. Lomax. Pergamon Press, Inc., Elmsford, NY. pp 1-16.

SEYDOUX, J., CONSTANTINIDIS, J., TSACOPOULOS, M. and GIRARDIER, L. 1977. In vitro study of the control of the metabolic activity of brown adipose tissue by the sympathetic nervous system. **Physiol. Paris. 73**: 985-996.

SEYDOUX, J., ROHNER-JEANRENAUD, F., ASSIMACOPOULOS-JEANNET, F., JEANRENAUD, B. and GIRARDIER, L. 1981. Functional disconnection of brown adipose tissue in hypothalamic obesity in rats. **Pflugers. Arch. 390**: 1-4.

SEYDOUX, J., RICQIEUR, D. ROHNER-JEANRENAUD, F., ASSIMACOPOULOS-JEANNET, F., GIACOBINO, J. P., JEANRENAUD, B. and GIRARDIER, L. 1982. Decreased guanine nucleotide binding and reduced equivalent production by brown adipose tissue in hypothalamic obesity. Recovery after cold acclimation. **FEBS Letters 146**: 161-164.

SHIBATA, H. 1982. Baroflex suppresion of nonshivering thermogenesis in rats. **Japan. J. Physiol. 32**:937-944.

SHIBATA, M., BENZI, R. H., SEYDOUX, J. AND GIRARDIER, L. 1987. Hyperthermia induced by pre-pontine knife-cut: evidence for a tonic inhibition of non-shivering thermogenesis in anaesthetized rat. **Brain Res. 436**: 273-282.

SHIDO, O., SUGANO, Y. AND NAGASAKA, T. 1986. Circadian change of heat loss in response to change in core temperature in rats. **J. Thermal. Biol. 11**: 199-202.

SHIDO, O. 1987. Day-night variation of thermoregualtory responses to intraperitoneal electric heating in rats. **J. Thermal Biol. 12**: 273-279.

SHIDO, O., YONEDA, Y. and NAGASAKA, T. 1989. Changes in brown adipose tissue metabolism following intraventricular vasoactive peptide and other gastrointestinal peptides in rats. **Japan. J. Physiol. 39**:359-369.

SHIMAZU, T. and AMAKAWA, A. 1968. Regulation of glycogen metabolism in liver by the autonomic mervous sytem. III Differential effects of the sympathetic-nerve stimulation and of catecholamines on liver phosphorylase. **Biochem. Biophys. Acta 165**: 349-356.

SHIMAZU, T. and TAKAHASHI, A. 1980. Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose tissue. **Nature 284**:62-63.

SHIMAZU, T. 1981. Central nervous system regulation of liver and adipose tissue metabolism. **Diabetologia 20**: 343-356.

SHIMIZU, Y. and SAITO, M. 1991. Activation of brown adipose tissue thermogenesis in recovery from anesthetic hypothermia in rats. **Am. J. Physiol. 261**: R301-R304

SIDMAN,R. L., PERKINS, M and WEINER, N. 1962. Noradrenaline and adrenaline content of adipose tissues. **Nature 193**: 36-37.

SMITH, R. E. 1961. Thermogenic activity of the hibernating gland in the cold-acclimated rat. **Physiologist 4**: 113.

SMITH, R. E. and HOCK, R. J. 1963. Brown fat: thermogenic effector of arousal in hibernators. **Science 140**: 199-200.

SMITH, R. E., and ROBERTS, J. C. 1964. Thermogenesis of brown adipose tissue in cold-acclimated animals. **Am. J. Physiol. 206**: 143-148.

SMITH, R. E. 1969. Circadian variations in human thermoregulatory responses. **J. Appl. Physiol. 26**: 554-560.

SMOLANDER, J., HÄRMÄ, M., LINDQVIST, A., KOLARI, P. and LAITINEN, L. 1993. Circadian variation in peripheral blood flow in relation to core temperature at rest. **Eur. J. Physiol. 67**: 192-196.

SOKOLOVE, P.G. and BUSHELL, W. 1978. The chi-square periodgram: its utility for analysis of circadian rhythms. **J. Theor. Biol. 72**: 131-160.

SPSS, Inc. 1990. **SPSS/PC+ Trends Manual**

STEINER, G. and CAHILL, G. F. Jr. 1964. Brown and white adipose tissue metabolism in cold-exposed animals. **Am. J. Physiol. 207**: 840-844.

STEINER, G., LOVELAND, M. and SCHÖNBAUM, E. 1970. Effect of denervation on brown adipose tissue metabolism. **Am. J. Physiol. 218**: 566-570.

STEPHAN, F. K. 1986. The role of period and phase in interactions between feeding and light entrainable circadian pacemakers in the rat. **Physiol. Behav. 36**: 1515-158

STORLIEN, L., GRUNSTEIN, M. and SMYTHE, G. 1985. Guanethidine blocks the 2-deoxy-D-glucose-induced hypothalamic drive to hyperglycemia. **Brain Res. 335**: 144-147.

STOYNEV, A.G. and IKONOMOV, O.C. 1987. Circadian regulation of feeding in rats: suprachiasmatic versus ventromedial hypothalamic nuclei. **Appetite 9**: 217-229.

STUPFEL, M. AND PAVELY, A. 1990. Ultradian, circahoral and circadian structures in endothermic vertebrates and humans. **Comp. Biochem. Physiol. 96**: 1-11.

SUNDIN, U. and CANNON, B. 1980. GDP-binding to the brown fat mitochondria of developing and cold-adapted rats. **Comp. Biochem. Physiol. 65B**: 463-371.

SWICK, A. and SWICK. R. 1986. Rapid changes in number of GDP binding sites on brown adipose tissue mitochondria. **Am. J. Physiol. 251**: E192-E195.

SZILLAT, D. and BUKOWIECKI, L. J. 1983. Control of brown adipose tissue lipolysis and respiration by adenosine. **Am. J. Physiol. 245**: E555-E559.

SZREDER, Z., HORI, T. AND KAIZUKA, Y. 1994. Thermoregulatory effect of intracerebral injections of neuropeptide Y in rats at different environmental temperatures. **Gen. Pharmac. 25**: 85-91.

TAPP, W. N., LEVIN, B. E. and NATELSON, B. H. 1981. Ultradian rhythm of plasma norepinephrine in rats. **Endocrinol. 109**: 1781-1783.

TEPPER, B. J. and KANAREK, R. B. 1984. 2DG-induced glucopenia does not elicit feeding in rats with lesions of the zona incerta. **Physiol. Behav. 32**: 441-446.

THORNHILL, J. and HALVORSON, I. 1990. Brown adipose tissue thermogenic responses of rats induced by central stimulation: effect of age and cold acclimation. **J. Physiol. 426**: 317-333.

THORNHILL, J. and HALVORSON, I. 1992. Differences in brown adipose tissue thermogenic responses between Long Evans and Sprague Dawley rats. **Am. J. Physiol. 263**: R59-R69.

THORNHILL, J. and HALVORSON, I. 1993. Intrascapular brown adipose tissue (IBAT) temperature and blood flow responses following ventromedial hypothalamic stimulation to sham and IBAT-denervated rats. **Brain Res. 615**: 289-294.

THURLBY, P.L. 1985. The interaction of alpha-adrenoceptor and beta adrenoceptor stimulation in the thermogenic reponse ofthe anesthetized rat- Meeting. **Int. J. Obes. 9**: 227.

THURLBY, P.L. and ELLIS, R.D.M. 1986. Differences between the effects of noradrenaline and the β-adrenoceptor agonist BRL 28410 in brown adipose tissue and hind limb of the anaesthetized rat. **Can. J. Physiol. Pharmacol. 64**: 111-1114.

TRAYHURN. P. and JAMES. W. 1978. Thermoregulation and non-shivering thermogenesis in the genetically obese (ob/ob) mouse. **Pflugers Arch. 373**: 189-193.

TRAYHURN, P., ASHWELL, M., JENNINGS, G., RICHARD, D. and STIRLING, D. 1987. Effect of warm or cold exposure on GDP-binding and uncoupling protein in rat brown fat. **Am. J. Physiol. 252**: E237-E243.

TRAYHURN, P. and JENNINGS, G. 1988. Non-shivering thermogenesis and the thermogenic capacity of brown fat in fasted and/or refed mice. **Am. J. Physiol. 254**: R11-R16.

TRAYHURN, P. and MILNER, R. 1989. A commentary on the interpretation of in vitro biochemical measures of brown adipose tissue thermogenesis. **Can. J. Physiol. Pharmacol. 67**: 811-819.

TRAYHURN, P. 1993. Brown adipose tissue: from thermal physiology to bioenergetics. **J. Biosci. 18**: 161-173.

TRIANDAFILLOU, J. and HIMMS-HAGEN, J. 1983. Brown adipose tissue in genetically obese (fa/fa) rats: response to cold and diet. **Am. J. Physiol. 244**: E145-E150.

TULP, O. L., FRINK, R. and DANFORTH, E., Jr. 1982. Effect of cafeteria feeding on brown and white adipose tissue cellularity, thermogenesis, and body composition in rats. **J. Nutr. 112**: 2250-2260.

UCAR, D., TOCCO, R. and KLUGER, M. 1983. Circadian variation in circulating pyrogen: Possible role in resistance to infection. **Proc. Soc. Exp. Biol. Med. 173**: 319-323.

UCHIDA, Y., TSUKAHARA, F., IRIE, K., NOMOTO, T. and MURAKI, T. 1994. Possible involvement of L-arginine-nitric oxide pathway in modulating regional blood 1fow to frown adipose tissue of rats. **Arch. Pharmacol. 349**: 188-193.

VANDER TUIG, J.G., KERNER, J., CHRIST, K.A. and ROSMOS. 1985. Hypothalamic obesity, brown adipose tissue, and sympathoadrenal activity in rats. **Am. J. Physiol. 248**: E607-E617.

VIVES, F., GAYOSO, M., OSORIO, C. and MORA, F. 1983. Prefontal cortex self-stimulation: afferent pathways determined by teh horseradish peroxidase technique. **Behav. Brain Res. 8**: 23-45.

WALGREN, M. C. and POWLEY, T. L. 1985. Effects of hyperalimentation on pair-fed rats with ventromedial hypothalamic lesions. **Am. J. Physiol. 248**: R172-R180.

WALSH, L. L. and GROSSMAN, S. P. 1975. Loss of feeding in response to 2 deoxy-D-glucose but not insulin after zona incerta lesions in rat. **Physiol. Behav. 15**: 481-485.

WIRSEN, C. 1964. Adrenergic innervation of adipose tissue examined by fluorescence microscopy. **Nature 202**: 913.

WOLLNICK, F., GÄRTNER, K. and BÜTTNER, D. 1987. Genetic analysis of circadian and ultradian locomotor activity rhythms in laboratory rats. **Behav. Genet. 17**: 167-178.

WOODS, A.J. AND STOCK, M.J. 1994. Biphasic brown fat temperature responses to hypothalamic stimulation in rats. **Am. J. Physiol. 266**: R328-R337.

YAHATA, T., HABARA, Y. and KUROSHIMA, A. 1983. Effects of glucagon and noradrenaline on the blodd flow through brown adipose tissue in temperature-acclimated rats. **Japan. J. Physiol. 33**: 367-376.

YORK, D. A., MARCHINGTON, D. HOLT, S. J. and ALLARS, J. 1985. Regulation of sympathetic activity in lean and obese zucker (fa/fa) rats. **Am. J. Physiol. 249**: E299-E303.

YOSHIDA, T., FISLER, J. S., FUKUSHIMA, M., BRAY, G. and SCHEMMEL. R. A. 1987. Effects of diet, lighting and food intake on norepinephrine turnover in dietary obesity. **Am. J. Physiol. 252**: R402-R408.

YOSHIMATSU, H., EGAWA, M. and BRAY, G.A. 1993. Sympathetic nerve activity after discrete hypothalamic nerve injections of L-glutamate. **Brain Res. 601**: 121-128.

YOUNG, J. B., SAVILLE, E., ROTHWELL, N. J., STOCK, M. J. and LANDSBURG, L. 1982. Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue of the rat. **J. Clin. Invest. 69**: 1061-1071.

YOUNGSTROM, T. and BARTNESS, J. 1995. Catecholamine innervation of white adipose tissue in siberian hamsters. **Am. J. Physiol. 268:** R744-R751.

ZERATH, E., LAGARDE, D., FERNANDES, T., ROUSSELT, D. AND LALOUETTE, A. 1994. Dissociation in body temperature, drinking and feeding rhythms following a light-dark cycle inversion in the rat. **Med. Sci. Res. 22:** 53-55.