THERMORESPONSIVENESS OF VENTROMEDIAL HYPOTHALAMIC (VMH) NEURONS TO PERIPHERAL (SCROTAL) THERMAL STIMULATION

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Department of Physiology
University of Saskatchewan
Saskatoon

By

Qiang Li
Fall 1996

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of the requirements for the

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ABSTRACT

The ventromedial hypothalamic nucleus (VMH) is an important central effector site involved in activating brown adipose tissue (BAT) or non-shivering thermogenesis. VMH neurons have previously been shown to be thermally responsive to changes in local temperature of the preoptic area/anterior hypothalamus (PO/AH). However, the thermoresponsiveness of VMH neurons of room temperature acclimatized and cold acclimatized rats to peripheral thermal stimulation has not been tested.

In this thesis, a series of studies was designed to determine the thermoresponsiveness of VMH neurons to peripheral (scrotal) thermal stimulation of rats. Extracellular VMH neuronal activity was recorded from urethane anaesthetized male Sprague-Dawley rats, which were acclimatized either to room temperature (21°C for 4 weeks) or to cold (4°C for 4 weeks) prior to testing, during scrotal cooling and heating with glass-micropipettes filled with 0.5 M sodium acetate containing 2% pontamine sky blue. The rats’ colonic temperatures were kept at normothermia (37°C), or hypothermia (33-35°C) during scrotal thermal stimulation. Temperatures of interscapular brown adipose tissue (T_{IBAT}, an index of IBAT activation), tail (T_{TAIL}, an index of vasomotor change) along with scrotal temperatures (T_{SCROTUM}) were continuously monitored.

In the room temperature acclimatized rats, VMH neurons were temperature responsive to scrotal heating and cooling and were classified as warm responsive (WRN), cold responsive (CRN) and temperature non-responsive neurons (TNRN), based on their thermal coefficients. The ratio of VMH WRNs and CRNs was similar to that of thermoresponsive neurons observed in other brain regions (eg, the PO/AH and thalamus). VMH WRNs and CRNs were further classified as biphasic or monophasic in nature according to their thermal responses to scrotal heating and cooling. VMH neurons sustained their thermoresponsiveness to repeated trials of scrotal thermal stimulation with colonic temperatures maintained at 37°C or when colonic temperatures were acutely lowered from 37°C to 35°C and 33°C. In addition, scrotal thermal signals specifically altered neuronal activity of VMH thermoresponsive neurons, as changes in EEG activity
did not occur with changes in VMH neuronal activity. Scrotal thermal inputs were functionally shown to be transmitted via the medial preoptic nucleus (MPO) prior to reaching the VMH nucleus because thermoresponsive VMH neuronal activity was blocked with the pretreatment of lidocaine into the MPO. Scrotal heating or cooling to 21°C-acclimatized rats did not increase IBAT temperatures, inferring that scrotal cooling had not evoked IBAT thermogenesis in this group.

In cold acclimatized (CA) rats, prolonged (over 2 hours) and transient localized scrotal cooling caused IBAT temperatures to increase, inferring that scrotal cooling activated BAT thermogenesis. Mean basal firing rates of all recorded VMH neurons of CA-groups significantly increased, compared to those of VMH neurons observed in room temperature acclimatized (RA) groups. More VMH CRNs than WRNs were recorded in the CA-group and the thermoresponsiveness (ie, thermal coefficient) of VMH CRNs significantly increased during localized scrotal cooling in the cold acclimatized group, compared to thermal coefficients of VMH CRNs of the RA-groups.

The significance of the present work is that 1) VMH neurons of rats receive scrotal thermal signals via the MPO and specifically respond to localized physiological changes in scrotal temperature. 2) VMH neurons sustain their thermoresponsiveness following repeated trials of scrotal thermal stimulation with colonic temperatures kept at normothermia (37°C) or during acute hypothermia (33-35°C). 3) Increases in basal firing rates occurred in all VMH neurons of CA-rats and may be an important step in developing neuronal adaptation to long term cold exposure. 4) Enhanced thermoresponsiveness of VMH CRNs also occurred in the CA-group with scrotal cooling and may be associated with adaptive changes of these neurons with continued cold exposure. 5) Localized scrotal cooling activated IBAT thermogenesis of CA-rats, but not RA-rats, in spite of colonic temperatures being maintained at 37°C during testing. The observed adaptive changes seen in thermoresponsive VMH neurons of the CA-group may be intricately involved in the associated enhanced capacity of BAT thermogenesis in cold acclimatized rodents during prolonged cold exposure.
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LIST OF ABBREVIATIONS

BAT  brown adipose tissue
CA   cold acclimatization
CRN  cold responsive neurons
IBAT interscapular brown adipose tissue
LPO  lateral preoptic area
MPO  medial preoptic area
NST  non-shivering thermogenesis
PO/AH preoptic area and anterior hypothalamus
RA   room temperature acclimatization
TC   thermal coefficient
TNRN temperature non-responsive neuron
VMH  ventromedial hypothalamic nucleus
WRN  warm responsive neuron

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1 INTRODUCTION AND LITERATURE REVIEW

1-1 General Purpose of This Study

The ventromedial hypothalamic nucleus (VMH) is a spherical nucleus located on either side of the third ventricle in the medial region of the hypothalamus. The VMH of mammals is thought to participate in a number of physiological functions of the brain including aggression and emotional behaviour (Barrett et al, 1987; Maeda et al, 1985), sexual behaviour (Floody et al, 1987; Cohen and Pffaf, 1992), feeding and obesity (Hetherington and Ranson, 1939; 1940; Brobeck et al, 1943), and in the regulation of endocrine hypophyseal secretions (Yoshimatu et al, 1984; Suemaru et al, 1995).

The VMH nucleus also serves as a central effector to activate thermogenesis of brown adipose tissue (BAT) and has both anatomical and functional connections to interscapular brown adipose tissue (IBAT). As activated chemically, electrically and even thermally, VMH neurons modulate the activity of sympathetic fibres innervating IBAT to activate thermogenic mechanisms. In addition, neurons within the VMH may also participate in the integration of thermal signals from other hypothalamic and extra-hypothalamic sites, including peripheral thermoreceptors.

The overall objective of the series of studies presented in this thesis was to study the thermoresponsiveness of VMH neurons to scrotal thermal stimulation. The specific objectives of this thesis were to determine:

1. if VMH neurons can respond to scrotal thermal stimulation, induced by small (2x2 cm) hot water and ice packs applied to the scrotum, in urethane-
anaesthetized, male Sprague-Dawley rats colonically maintained at normothermic (37°C) and hypothermic (< 35°C) temperatures

2. if VMH neurons of urethane-anaesthetized rats kept at the same colonic temperature could retain their thermoresponsiveness to repeated scrotal thermal stimulation; and if thermoresponsiveness of the same VMH neuron to scrotal thermal stimulation could be altered when deep body (colonic) temperature was changed

3. if responses of VMH neurons of urethane-anaesthetized rats to localized scrotal thermal stimulation is temperature specific rather than part of a general arousal response

4. if a functional medial preoptic area (MPO) is required for scrotal thermal stimulation to alter the neuronal activity of thermoresponsive VMH neurons of urethane-anaesthetized rats

5. if the thermoresponsiveness of VMH neurons to localized scrotal thermal stimulation is modulated in rats previously cold acclimatized to 4°C for 4 weeks from that of VMH neurons of room temperature (21°C for 4 weeks) acclimatized rats

6. if VMH neuronal activity changes of normothermic, acutely hypothermic and cold-acclimatized rats to scrotal thermal stimulation were associated with changes in thermal effector responses (ie, activation of IBAT or vasoactive responses of the skin (tail))

1-2 The Central Control of Thermoregulation

The maintenance of constant body temperature in humans and animals is achieved by regulating heat gain and heat loss between the body and the environment through the utilization of autonomic and behavioral mechanisms (Boulant 1980, 1996). This is an integrative process in the central nervous system (CNS) involving peripheral and central thermo-receptors or sensors, integrative neurons in the CNS, especially those located in the hypothalamus, and various thermo-effectors.
The hypothalamus in mammals has been thought to be an important thermoregulatory centre. The integrity of the hypothalamic area in the brain of mammals and other species is essential for normal control of body temperature. The hypothalamus itself is temperature sensitive and the preoptic area and anterior hypothalamus (PO/AH) are thought to be of primary importance in thermoregulation. Animals implanted with hypothalamic thermodes to change hypothalamic temperature have shown that warming of the PO/AH evoked physiological heat loss responses such as vasodilation, polypnea, panting and increases in cutaneous evaporative water loss. Warming the PO/AH also reduced cold defence or heat production responses such as vasoconstriction and shivering (Hemingway et al, 1940; Hemingway, 1963 Beaton et al, 1941). Cooling of the PO/AH evoked a series of cold defence responses, such as peripheral vasoconstriction and shivering (Hensel and Kruger, 1958, Hemingway, 1963). Lesions of the PO/AH in animals resulted in disturbances in body temperature regulation (Magoun et al 1938; Fusco et al, 1961). These results have indicated that the PO/AH area is an important thermoregulatory centre.

1-2-1 PO/AH Thermosensitive and Temperature Insensitive Neurons

With the utilization of a local thermode implanted within the PO/AH of anaesthetized cats to change hypothalamic temperature, Nakayama et al (1961; 1963) first demonstrated the existence of temperature sensitive neurons. Classically, a PO/AH neuron that increases its firing rate to local heating and/or decreases its firing rate to localized cooling is defined as a warm sensitive neuron (WSN); a PO/AH neuron that decreases its firing rate to local heating and/or increases its firing rate to localized cooling is classified as a cold sensitive neuron (CSN). Those PO/AH neurons that do not change their firing rates to localized heating and cooling are defined as a temperature insensitive neurons (TIN).

In order to determine the thermosensitivity of a neuron, criteria have been developed (Boulant, 1980). Thermosensitivity is based on the neuron’s determined thermal coefficient (TC) which is the regression coefficient (ie, slope) of the thermoresponsive
curve of the neuron’s firing rate vs. changes in hypothalamic temperature usually over a 3-5°C range in temperature. According to this methodology, neurons are classified as warm sensitive neurons (WRNs) if their thermal coefficient was at least 0.8 impulses.s⁻¹°C⁻¹ and classified as cold sensitive neurons (CSNs) if their thermal coefficient was at least -0.6 impulses.s⁻¹°C⁻¹. All other spontaneously active neurons were classified as temperature insensitive neurons (TINs) whose thermal coefficients were between the two above values. Boulant and Hardy (1974) showed, using whole animal preparations, that warm sensitive neurons in the PO/AH, with thermal coefficients above 0.8 impulses.s⁻¹°C⁻¹, and PO/AH CSNs, with thermal coefficients below -0.6 impulses.s⁻¹°C⁻¹, responded to changes in local hypothalamic temperature and to changes in peripheral temperature. These thermal coefficient values are believed to naturally separate hypothalamic thermosensitive and insensitive neurons into varying thermointegrative groups, which process local and peripheral thermal inputs differently. It has been postulated that PO/AH warm sensitive neurons are responsible for heat dissipation, while PO/AH cold sensitive neurons are thought to link with heat production mechanisms (Hammel, 1965; Boulant, 1980). In addition, Boulant and Hardy (1974) also proposed a slight change in classifying neuronal thermosensitivity of the CNS (PO/AH) neurons when peripheral thermal stimulation was being employed, i.e., thermal coefficient for PO/AH WSNs was now determined to be 0.5 impulses.s⁻¹°C⁻¹; and for PO/AH CSNs the thermal coefficient was -0.5 impulses.s⁻¹°C⁻¹.

Although the percentages of each type of thermosensitive and insensitive neurons recorded from different species varies, extensive in vivo electrophysiological single unit studies of anaesthetized and unanesthetized mammals indicate that the PO/AH contains approximately 30% WSNs, 10% CSNs and 60% TINs (Boulant, 1980, Boulant and Dean, 1986). It should be noted that the ratio of thermosensitive and insensitive neurons is also similar to the ratio found in neurons from other diencephalic structures studied in vitro (Dean and Boulant, 1989a). With intracellular recording, more recent studies of PO/AH neurons in brain tissue slice preparations indicated that some silent or very low firing neurons, which were not able to be detected by extracellular recording, are
also temperature insensitive neurons (Curra et al. 1991; Griffin and Boulant, 1995). As a result, the percentage of PO/AH temperature insensitive neurons appears to be higher than that originally proposed for PO/AH temperature insensitive neurons recorded extracellularly.

Hori et al. (1980a) first demonstrated that thermosensitivity is an inherent property of PO/AH thermosensitive neurons, as both PO/AH warm and cold-sensitive neurons within tissue slice preparations still retained their thermoresponsiveness to changes in local temperature in the presence of chemical synaptic blockade (i.e., high Mg$^{2+}$/low Ca$^{2+}$ medium). It now appears from the majority of the studies that the thermosensitivity of most hypothalamic warm sensitive neurons is not affected by synaptic blockade, i.e., it is an inherent property (Hori et al. 1980b; Baldino and Geller, 1982; Kelso and Boulant, 1982; Dean and Boulant, 1989b).

Whether cold sensitive neurons possess an inherent thermosensitivity remains controversial. In their initial study, Hori et al. (1980a) recorded only 4 cold sensitive neurons in PO/AH tissue slices of rats, and found that 3 out of 4 cold sensitive neurons did not lose their thermosensitivity during the perfusion of a high Mg$^{2+}$/Ca$^{2+}$ free (6.5 mM/0 mM) solution. In another study (Baldino and Geller 1982), the thermosensitivity of rat preoptic neurons in culture (defined on the basis of $Q_{10}$ with a $Q_{10}$ of 0.5-2.0 for temperature insensitive, higher than 2 for warm sensitive, and lower than 0.5 for cold sensitive neurons) was preserved when synaptic activity was suppressed by a medium containing high Mg$^{2+}$/low Ca$^{2+}$ (12mM/0.25mM). In contrast, Kelso and Boulant (1982), and Dean and Boulant (1989b) reported that most PO/AH cold-sensitive neurons of rats did not retain their thermosensitivity during synaptic blockade when the bath solution contained 11.4 mM Mg$^{2+}$ and 0.02 mM Ca$^{2+}$, supporting the notion that thermosensitivity of cold sensitive neurons is determined by synaptic inputs from other neurons (Hammel, 1965). As further evidence supporting the latter notion, Curra et al. (1991) demonstrated that PO/AH cold sensitive neurons did not have spontaneous pacemaker potentials, as had been observed in the PO/AH warm sensitive neurons, but appear
to receive both excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs), suggesting that thermosensitivity of the PO/AH cold sensitive neurons is synaptically mediated. The discrepancies between studies regarding the thermosensitivity of cold sensitive neurons may be due to (1) the use of different criteria for classifying these neurons, (2) neuronal recordings made from different brain tissue preparations, and (3) using different perfusion media with varied concentrations of Mg$^{2+}$/Ca$^{2+}$. Interestingly, cold sensitive neurons, as recorded from the posterior hypothalamus, the diagonal band of Broca or from the mamillary body have been found to retain their thermosensitivity during synaptic blockade (Dean and Boulant 1989b). Taken together, these apparently conflicting findings suggest that differences in the inherent cold thermosensitivity of cold sensitive neurons may also be due to the region where the neurons were recorded. It is now believed that neurons that retain their thermosensitivity during synaptic blockade are central thermoreceptors, while neurons that lose their thermosensitivity are interneurons within thermoregulatory networks.

CA1 hippocampal neurons and spinal motor neurons have membrane characteristics (e.g., duration of action potential, afterhyperpolarization potential) that are temperature sensitive and yet these neurons do not function as thermosensitive neurons (Thompson et al., 1985; Pierau et al., 1969; 1971; 1976; Klee et al., 1974). Early studies suggest that neuronal thermosensitivity may be attributed to different effects of temperature on Na$^+$ and K$^+$ permeability (Carpenter, 1970). Warming accelerates the Na$^+$/K$^+$ pump, resulting in membrane hyperpolarization and associated decreases in firing rates; while cooling slows the Na$^+$/K$^+$ pump, resulting in membrane depolarization and associated increases in firing rates. One would expect that these neurons should lose their thermosensitivity when the Na$^+$/K$^+$ pump is blocked by ouabain. However, PO/AH thermosensitive neurons do not lose their thermosensitivity in the presence of ouabain, suggesting that the Na$^+$/K$^+$ pump is not entirely responsible for neuronal thermosensitivity (Curras and Boulant 1989). Results from a more recent study (Griffin and Boulant, 1995) indicated that the membrane resting potential of PO/AH warm sensitive neurons was not affected by temperature, even though warm sensitive neurons
increased their firing rates during local warming. In addition, input resistance is an unlikely cause of PO/AH thermosensitivity since input resistance does not change in the linear portion of the current-voltage curve unless there is change in the resting membrane potential. Similarly, temperature did not change membrane potentials of PO/AH temperature insensitive neurons. Therefore, neuronal thermosensitivity may not be attributed to changes in resting membrane potential during local cooling and warming.

To date, thermosensitivity of PO/AH warm sensitive neurons was found to be associated with the rate of rise of the pre-potentials which precede each action potential (Curra et al 1991). The rate of rise of the pre-potential was temperature sensitive: warming increased the rate of rise of the pre-potential, resulting in increased firing rates and cooling had the opposite effects. However, changes in temperature did not affect the rate of rise of the pre-potentials of temperature insensitive neurons. Recently, Griffin et al, (1996), in investigating ionic mechanisms of rat PO/AH neuronal thermosensitivity, found that pre-potential depolarization of PO/AH warm sensitive neurons was associated with changes in outward potassium conductances, ie, warming increased the rate of inactivation of "A" currents of PO/AH warm sensitive neurons and this in turn increased the firing rate.

Temperature insensitive neurons in the PO/AH and other areas of the brain did not respond to local changes in brain temperature or changes in peripheral temperature. In vitro studies showed that these temperature insensitive neurons did not respond to local changes in temperature or to synaptic blockade (Dean and Boulant 1989b). One may wonder as to the role that hypothalamic temperature insensitive neurons, which fail to respond to changes in temperature, play in thermoregulation. It has been suggested that temperature insensitive neurons act as reference sources in thermoregulatory networks (Hammel, 1965; Boulant 1980). This hypothesis is partially supported by an intracellular study (Curra et al, 1991) that reported PO/AH warm sensitive neurons displayed some putative excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) and frequencies of IPSPs were not temperature dependent. That study suggested
that the IPSPs were from nearby temperature insensitive neurons. In addition, hypothalamic temperature insensitive neurons show some neuronal plasticity, since some PO/AH temperature insensitive neurons displayed thermosensitive properties when the Na\(^+\)/K\(^+\) pump was blocked by ouabain (Curras and Boulant, 1989b).

1-2-2 Extra-PO/AH Thermosensitive and Temperature Insensitive Neurons

Although the PO/AH is extremely important in regulating body temperature, there are many other neurons in the CNS which are temperature sensitive and are also involved in physiological and behavioral thermoregulatory responses (Adair, 1974; Nakayama, 1985). Electrical or chemical stimulation of many diencephalic loci evoked thermoregulatory responses (Amir, 1990, Thornhill and Halvorson, 1990). Lesions at several loci in the hypothalamus (Gilbert and Blatteis, 1977) or brain stem (Lipton et al, 1974) disrupt heat production or heat loss mechanisms.

Using the 2-deoxy-D-\([\text{\textsuperscript{14}}\text{C}]\) (\[^{14}\text{C}\text{-DG}\]) glucose autoradiographic mapping techniques, Morimoto and Murakami (1985) found that neurons in the medial preoptic area, medial forebrain bundle, ventromedial hypothalamus, dorsomedial thalamus and other areas in the brain increased metabolic activity during hypothalamic and peripheral cooling, indicating a number of intra-hypothalamic and extra-hypothalamic nuclei that were activated. Kiyohara et al (1995) functionally mapped thermoresponsive regions in the brain by examining the distribution of c-fos protein-like immunoreactivity to peripheral (cold and warm) thermal stimulation. Exposed to cold (10°C) or to warm (33°C), a significant number of c-fos positive neurons were found in a number of regions in the rat brain, which were previously confirmed to be activated in the metabolic (\[^{14}\text{C}\text{-DG}\]) mapping study. This result further suggested that neurons within different diencephalic loci including the ventromedial hypothalamic nucleus (VMH) are modulated by central or peripheral thermal stimuli, and thus may be involved in the integration of appropriate thermal effector responses.

Substantial evidence obtained from electrophysiological \textit{in vivo} and \textit{in vitro} studies
in several species (for review see Boulant and Dean, 1986; Nakayama 1985; Hori, 1991) has indicated that thermosensitivity of neurons to both local and peripheral temperature changes is a phenomenon not unique to the PO/AH. So far, thermosensitive and insensitive neurons have been consistently and frequently found in the cortex (Hellon et al., 1973; Hori et al., 1984; Shibata et al., 1988), thalamus (Hellon and Misra 1973b; Jahns 1975), septum (Eisenman and Jackson, 1967; Eisenman 1969; Disturnal et al., 1986), posterior hypothalamus (Nutik, 1973; Edinger and Eisenman, 1972), ventromedial hypothalamic nucleus (Morimoto et al., 1985a; 1985b; Hori et al., 1988), and the midbrain (Nakayama and Hardy, 1969; Hori and Harada, 1976; Cronin and Baker, 1976; Murakami et al., 1976). Mapping the distribution of thermosensitive and temperature insensitive neurons using horizontal brain tissue slices, combined with neuronal recordings, Dean and Boulant (1989a) found thermosensitive and temperature insensitive neurons in at least 17 loci within the diencephalon of rats besides the PO/AH.

Many of the neuronal properties of extra-PO/AH thermosensitive neurons recorded from in vivo and in vitro preparations of different species have been found to be similar to those properties of PO/AH thermosensitive and temperature insensitive neurons. For example, the ratio of thermosensitive and temperature insensitive neurons recorded from extra-PO/AH sites is similar to that found in the PO/AH. PO/AH and extra-PO/AH thermosensitive neurons also retain their thermosensitivity during synaptic blockade (Hori et al., 1980b) and react similarly to pyrogen administration (Wit and Wang, 1968; Eisenman, 1969). Importantly, these extra-PO/AH thermosensitive neurons, like those in the PO/AH, responded to changes in local, as well as peripheral, temperature (Nakayama et al. 1984; Imai-Matsumura and Nakayama, 1984). Thus, neurons in the extra-PO/AH sites are also capable of integrating information about localized central temperature along with afferent temperature signals arising from the periphery.

1-3 The Ventromedial Hypothalamic Nucleus (VMH) and Its Control of IBAT Thermogenesis

Brown adipose tissue (BAT) is a major thermo-effector responsible for non-shivering
thermogenesis (NST) in small animals and newborn humans. Considerable information and insight concerning the central control mechanism of BAT thermogenesis have been gained over the past ten years. Evidence derived from lesion, electrical and chemical stimulation experiments of the VMH suggests that the VMH is a predominant central site for the activation of BAT thermogenesis.

VMH lesion studies showed a suppression in interscapular brown adipose tissue (IBAT) function, as indicated by a reduction in guanosine 5'-diphosphate binding (GDPB), an index of the IBAT response to neural stimulation (Seydoux et al, 1982) along with a decreased rate of noradrenaline turnover (Vander Tuig et al, 1982). These results indicate that dysfunction of the VMH may result in impaired activation of IBAT thermogenesis by suppressing sympathetic efferent activity to the tissue.

Shimazu and Takahashi (1980) and Takahashi and Shimazu (1981) suggested that the VMH directly affects metabolism of the IBAT, since electrical stimulation of the VMH nucleus, but not other hypothalamic areas, enhanced lipogenesis in this tissue. Electrical stimulation of this nucleus in both conscious or anaesthetized rats acclimatized to room temperatures evoked increases in IBAT temperature (Perkins et al, 1981; Niijima et al, 1984; Holt et al, 1987; 1988; Thornhill and Halvorson, 1990, 1992, 1993), again indicative of activation of IBAT. Interestingly, electrical stimulation of the VMH evoked even greater increases in IBAT temperatures of urethane-anaesthetized, age matched, male Sprague-Dawley rats acclimatized to 4°C for 3 weeks prior to testing (cold acclimation) than those age-matched rats previously maintained at 21°C (Thornhill and Halvorson, 1990). The effects of electrical stimulation of the VMH on IBAT thermogenesis occurred only in the animals that had intact IBAT sympathetic fibres (Thornhill and Halvorson, 1993). The link between the VMH and IBAT has also been demonstrated in VMH-lesioned studies, in which spontaneous activity of sympathetic fibres innervating IBAT was not increased following acute cold exposure, as normally occurred in non-VMH lesioned rats (Niijima et al, 1984).
However, electrical stimulation of the VMH that previous studies showed to activate IBAT thermogenesis may be a consequence of either direct activation of neurons within the VMH or due to activation of fibres passing through the VMH. L-glutamate is often used to selectively stimulate only the cell bodies and dendrites of neurons, leaving axons unaffected (Goodchild et al, 1982). The role of the VMH neurons governing IBAT thermogenesis was further demonstrated in chemical stimulation (L-glutamate) studies. Amir (1990) found that microinjection of L-glutamate into the VMH evoked an enhanced thermogenic response of IBAT of anaesthetized rats. In a similar study, microinjection of L-glutamate into the VMH caused a significant increase in IBAT temperatures of cold acclimatized rats (Halvorson et al, 1990). Furthermore, chemical lesions (kainic acid, which destroys neuronal cell bodies, but spares fibre of passage) to the VMH nucleus resulted in a reduction in the GDP binding of IBAT mitochondria (Sakaguchi et al, 1988; Sakaguchi and Bray, 1990). The chemical studies infer that stimulation of the VMH cell bodies produces a signal that leads to activation of IBAT thermogenesis.

By monitoring spontaneous IBAT sympathetic fibre activity, microinjection of insulin (Sakaguchi and Bray, 1987; Holt and York, 1989) into the VMH nucleus depressed IBAT sympathetic activity, whereas glucose (Sakaguchi and Bray, 1987), probably acting directly on glucose receptors of VMH neurons (Ono et al, 1982; Oomura, 1983), and L-glutamate (Yoshimatsu et al, 1993) increased the sympathetic firing rate to IBAT. In addition, excitatory effects on IBAT thermogenesis evoked by microinjection of glucose into the VMH was greatly attenuated or abolished following damage to neurons in the VMH nucleus by prior microinjection of kainic acid (Sakaguchi et al, 1988; Sakaguchi and Bray, 1990).

Studies involving electrical recording of spontaneous neural activity of IBAT sympathetic fibres, after chemical and electrical stimulation or destruction of specific regions of the hypothalamus in rodents, indicated that paraventricular nucleus (PVN) (Freeman and Wellman, 1987; Amir 1990), PO/AH (Freeman and Wellman, 1987), and the supra-chiasmatic nucleus (Amir et al, 1989) also modulate IBAT thermogenesis.
These studies suggest the existence of a complex neural network modulating IBAT thermogenesis within the hypothalamus. However, it should be noted that a functional VMH nucleus is required to mediate IBAT thermogenesis as evoked by other hypothalamic (non-VMH) sites. The enhanced thermogenic response of BAT evoked by either microinjection of glutamate into the suprachiasmatic nucleus (Amir et al 1989) or electrical stimulation of the medial preoptic area (MPO) of anaesthetized rats (Thornhill et al, 1994) was greatly attenuated or abolished by prior injection of local anaesthetics into the VMH.

1-4 Brown Adipose Tissue (BAT) and Non-shivering Thermogenesis (NST)

Non-shivering thermogenesis (NST) of the BAT is activated during arousal from hibernation, during acute or sustained cold exposure, following consumption of a highly caloric meal (ie, diet induced thermogenesis, DIT), stress, pathogenic infection and injury with inflammation. In addition, non-shivering thermogenesis is also an adaptive or regulatory process that develops during acute and prolonged cold exposure and is particularly important in small animals with a large surface area-to-volume ratio, in hibernating animals, and in neonates who are often incapable of shivering.

Although IBAT is not the sole tissue that generates heat in response to reduced environmental temperatures, this tissue is the most studied depot and considered to be a dominant site of thermogenesis in small mammals (Rothwell and Stock, 1979; Thurlby and Trayhurn, 1979; Himms-Hagen, 1981; Seydoux et al, 1981; Shibata and Nagasaka, 1984). IBAT, one of several depots of BAT, consists of two adhering, symmetrical, lobular pads located on the back between the shoulders of small rodents. Each pad receives its own arterial blood supply via a thoracodorsal artery and its own innervation from a bundle of five sympathetic nerve fibres/pad that arise bilaterally as part of the intercostal muscle sheath (Smith and Horwitz, 1969). The contribution made by IBAT to non-shivering thermogenesis was well demonstrated by Foster and Frydman (1978). In that study, in vivo blood flow and oxygen extraction of IBAT by radioactive microspheres were measured and it was found that BAT could account for about 60% of
the total heat production induced by noradrenaline administration in cold adapted rats.

1-4-1 Cellular Mechanisms of BAT Thermogenesis

The thermogenic activity of IBAT is heavily affected by noradrenaline (Foster and Frydman, 1978, Thornhill and Halvorson, 1990). Other hormones, including thyroid hormones (Cottle and Carlson, 1956, Vybiral et al, 1985) and glucagon (Davison et al, 1957) can also influence BAT thermogenesis. Noradrenaline is believed to be the primary physiological activator of non-shivering thermogenesis in rats. Upon cold exposure, the sympathetic drive to brown adipose tissue is dramatically increased and noradrenaline is released. The thermogenic response to noradrenaline in brown adipocytes of mammals is mediated primarily by β-adrenergic receptors, specially β₁ and β₂ adrenergic receptors (Mohell et al, 1981; 1984; Arch, 1989). Although activation of α-adrenergic receptors on the plasma membrane of brown adipocytes will also result in a thermogenic response, it accounts for only a small percentage of the total thermogenic capability of BAT (Horwitz, 1977; Mohell et al 1984).

Cellular mechanisms of non-shivering thermogenesis induced by noradrenaline have been well described (Horwitz 1989; Himms-Hagen 1990; Jansky 1995). The initiation of non-shivering thermogenesis upon cold exposure begins with noradrenaline binding to β-adrenergic receptors. The hormone-receptor complex interacts with a guanosine triphosphate (GTP)-binding protein (Gs) which subsequently activates adenylate cyclase and stimulates the synthesis of intracellular cyclic adenosine monophosphate (c-AMP). c-AMP activates protein kinase (PK) which phosphorylates hormone-sensitive lipase. This lipase catalyses the hydrolysis of the stored triglycerides to glycerol and free fatty acids. The free fatty acids serve as the substrate for oxidative phosphorylation in the mitochondria of brown adipocytes, which produces ATP and heat.

The mechanisms whereby the BAT exhibits a high metabolic rate upon exposure to cold are well understood. Upon cold exposure, the thermogenic capacity of IBAT is enhanced mainly due to a large increase in the mobilization of substrate (free fatty acids)
and an increased rate of oxidation of this substrate inside the mitochondria. The unique feature of mitochondria of brown adipocytes is the large number of mitochondria that possess a specific polypeptide called uncoupling protein (UCP) (Nicholls 1983). The UCP acts as a membrane channel with high conductance, which permits protons to leak back into the mitochondrial matrix when it opens. As a result, a very high rate of respiration is achieved that is uncoupled from the synthesis of ATP. The increased energy generated during proton translocation via the UCP is immediately released into the blood as heat (Nicholls and Locke, 1984). The UCP has a high affinity for purine nucleotides, which inhibit the activity of the UCP in the non-stimulated state. Removal of purine nucleotides from the UCP is thought to be the underlying mechanism by which noradrenaline activates BAT thermogenesis during cold exposure.

1-5 Vegetative Functions of the VMH Nuclei

As one of the hypothalamic nuclei, the VMH nucleus takes part in a number of vegetative functions of the brain, in addition to being involved in regulating thermogenic responses of brown adipose tissue.

**Food intake**: Almost 50 years ago, Hetherington and Ranson (1939, 1940) reported that electrolytic lesions of the VMH nucleus of rats caused hyperphagia and obesity. This result indicated that this area of the hypothalamus normally exerts an inhibitory influence over the intake of food. In addition, VMH lesions usually result in secretion of insulin and free fatty acids (FFA) in the blood (Hongslo et al; 1974; Steffens, et al, 1972) and increase in gastric acid secretion (Ridely and Brooks, 1965). It is believed that VMH contains glucoreceptor neurons that sense the glucose levels in the blood which, in turn, modulate food intake behaviour (Oomura and Kita, 1984).

**Sexual behaviour**: Neurons within the VMH nucleus of female rats show a strong sensitivity to the effects of estrogen and progesterone (Barfield et al; 1983; Floody et al, 1987). Pfaff and Sakuma (1979) found that electrical stimulation of the VMH nucleus of female rats increased sexual behaviour or lordosis, while female rats with bilateral lesions
of VMH nucleus would not display lordosis behaviour. It is now known that activation of VMH neurons by estradiol and progesterone initiates a lordosis response in female rats

**Hormone secretion:** Endogenous growth hormone releasing factor (GRF) is released from the VMH and paraventricular (PVN) nucleus. Bilateral lesions of the VMH nucleus in rats cause a suppression of the pulsatile release of growth hormone (GH) (Mori et al., 1993; Tannenbaum et al., 1983), due to the impaired secretion of GRF and somatostatin.

1-6 **Thermoresponsive Neurons in the VMH**

The primary and established role of the VMH in thermoregulation has been as a central effector nucleus to activate BAT thermogenesis via increasing sympathetic activity to that tissue in response to acute temperature or dietary stimuli. Knowing that many extra-PO/AH neurons have inherent thermosensitivity properties, it is logical to ask if the VMH nucleus also contains neurons that can sense local or peripheral temperature changes, as best known for PO/AH thermosensitive neurons. To date, a few studies have been performed to explore how the neuronal activity of VMH neurons is affected by local or peripheral temperature changes.

The VMH neurons appear to be involved in thermoregulation shortly after birth, since the c-fos protein, a marker of neuronal activation (Sagar et al., 1988), can be detected in the VMH nucleus as early as 3 days after birth when young rats were exposed to an ambient temperature of 15°C for one hour. This compares to c-fos positive neurons being observed in the PVN at 9 days and in the PO/AH at 8 days of age (Joyce and Barr, 1992). These results suggest that thermoresponsiveness of the VMH neurons to changes in peripheral temperature exist at a very early post-natal age. In another study using [14C] deoxyglucose ([14C]-DG) autoradiographic methodology, Morimoto and Murakami (1985) reported that local cooling of the PO/AH of adult rats or acute cold exposure of the whole body to -3°C for about 50 minutes, significantly changed the neuronal activity of VMH neurons, as shown by significant increases in [14C]-DG incorporation (ie, metabolic activity) in VMH neurons. This result suggested that neurons within the VMH, like
those found in the preoptic area, play an important integrative role in processing thermal information arising from cooling.

Anand et al (1966) originally reported that VMH neuronal activity was not influenced by local temperature changes, suggesting these neurons were not temperature sensitive. In contrast, Nakayama et al (1981) demonstrated that VMH neurons responded to local preoptic thermal stimulation. In their study, most VMH neurons facilitated by glucose were also facilitated by preoptic warming, but only a few VMH neurons were facilitated by preoptic cooling. In a similar study, Nakayama and Imai-Matsumura (1984) further demonstrated that VMH neurons which modulate their neuronal activity to preoptic warming, also responded to scrotal warming, and that the direction of VMH neuronal activity to preoptic and scrotal thermal stimulation was the same (ie, the VMH neurons that were excited by local preoptic warming were also facilitated by scrotal warming). These results indicate that VMH neurons are temperature responsive neurons and respond to thermal signals originating from the central region (ie, preoptic area) and from the periphery (ie, scrotal area).

A few in vitro studies (Imai-Matsumura, et al, 1988; Dean and Boulant, 1989a, 1989b) have indicated that neurons within the VMH are thermosensitive to local hypothalamic temperature changes. These studies suggest a greater ratio of warm-sensitive VMH neurons than that found in thermosensitive PO/AH neurons. However, Morimoto et al (1988) found the ratio of VMH warm, cold sensitive and temperature insensitive neurons to be similar to the ratio found for PO/AH thermosensitive and temperature insensitive neurons. That study also reported that warm sensitive VMH neurons had a greater thermosensitivity (ie, higher thermal coefficient) than PO/AH warm sensitive neurons to changes in local temperature. Warm-sensitivity of VMH neurons appeared to be inherent like that of PO/AH warm sensitive neurons, as VMH warm sensitive neurons did not lose their thermosensitivity during synaptic blockade (Dean and Boulant, 1989b; Morimoto et al, 1988).
The Scrotum and Thermoregulation

Keeping scrotal temperature within an optimal range is one of the critical factors for normal spermatogenesis in male animals. Abnormal increases in testicular temperature is detrimental to spermatogenesis (Blackshaw, 1977). The maintenance of scrotal temperature 2 or 3°C lower than core temperature is essential. This core to scrotal temperature differential is pivotal to normal reproductive function, thus the scrotum is highly sensitive to changes in ambient temperature and has been used as a test model for studying thermoresponsiveness of CNS neurons to induced, controlled changes in peripheral scrotal temperature.

Scrotal Temperature Changes Evoke Thermoeffector Responses

A series of experiments performed in the ram (Waites 1961; 1962; 1970) found that elevation of scrotal skin temperature from 32°C (normal) to above 35°C evoked a dramatic increase in respiratory frequency with a considerable increase in evaporative heat loss (EHL). Core temperature was reduced about 2°C due to panting when the scrotum had prolonged exposure to temperatures between 40-42°C. However, heating equivalent skin areas of the trunk of the ram evoked no thermoeffector responses. Scrotal thermal stimulation in rats also evokes autonomic responses such as increases in blood pressure (Neya and Pierau, 1976) and respiratory frequency (Schingnitz and Werner, 1980b). This data indicates that scrotal warming evokes reflex thermoregulatory responses in rams and rats and associated effector mechanisms, including panting, peripheral vasodilation, and increased discharge in the scrotal sweat glands in these animals (Waiters and Voglmayr, 1963).

Generally, thermoregulatory effector responses evoked by scrotal heating and cooling appear to be achieved at two levels. Local thermoregulatory responses of the scrotum, e.g. sweating and vasomotor changes may be caused by the direct effects of warm or cold on scrotal skin. Other more generalized thermoregulatory responses, such as changes in respiratory frequency, panting, shivering, and non-shivering thermogenesis, are probably initiated by the activation of scrotal skin thermoreceptors affecting
thermointegrative neurons within the central nervous system to evoke the appropriate effector response (Boulant, 1980; 1996). The skin of the scrotum of animals contains sensitive thermoreceptors for detecting changes in applied temperature which transmit this sensory afferent information to the CNS.

Recording from single fibres in the scrotal nerve of the rats, Iggo (1969) demonstrated the presence of scrotal warm and cold receptors. The number of scrotal warm and cold receptors was greater than in other skin areas. Warm thermoreceptors were excited when scrotal temperatures were in the range of 35°C to 45°C, while cold thermoreceptors were facilitated by scrotal temperatures between 33°C - 13°C. Like thermoreceptors in other areas of the body, scrotal warm and cold receptors displayed both dynamic and static responses to changes in applied temperatures to the scrotum (Hellon et al, 1975).

1-7-2 Neuronal Activity Changes of CNS Neurons to Scrotal Thermal Stimulation

In male urethane-anaesthetized rats (Hollon and Misra, 1973a), 47% of recorded neurons in the lumbar dorsal horn of the spinal cord responded to changes in scrotal temperature between 13-43°C. Two types of dorsal horn neurons were classified: warm units were excited by increases in scrotal temperature above 30°C, while the cold units were excited by decreases in scrotal temperature generally below 30°C. Both warm and cold-units in the dorsal horn featured dynamic or static firing patterns, or a combination of dynamic and static responses. Most of the thermoresponsive neurons did not respond to scrotal tactile stimulation.

Hellon and Misra (1973b) first demonstrated that most neurons recorded in the ventrobasal complex in the thalamus of rats were excited or suppressed with scrotal temperature changes between 30-41°C. However, neuronal activity of thalamic neurons was not affected when scrotal temperatures were below or above this range. Most thalamic neurons also did not respond to mechanical stimuli (ie., brushing) applied to scrotal skin. Neuronal activity changes of thalamic neurons to scrotal heating were
abolished after lidocaine was applied into the scrotum (Schingnitz 1981), indicating thalamic neurons receive thermal signals arising from the scrotum.

A significant feature of thalamic warm-responsive neurons to scrotal heating is called the "switching response" or "ON-OFF response" (Hellon and Misra, 1973b; Jahns, 1975; 1976; Schingnitz and Werner, 1980a; Schingnitz 1981). This response occurred as the average firing rate of thalamic neurons abruptly increased when scrotal temperatures reached a threshold value, whereas thermal stimuli below or above that threshold temperature had no further effect. The threshold scrotal temperature for eliciting the switching response ranged from 31-40°C. The role of neuronal switching responses in temperature regulation is unknown, but the sudden change in neuronal activity to a rise in scrotal temperature suggests that neuronal response generates a warning signal for protective thermoeffecter mechanisms to be activated. If overheating of the tissue went unchecked, detrimental effects on spermatogenesis would potentially occur (Werner et al, 1986).

Hypothalamic thermosensitive neurons have been shown to be modulated by thermoafferent information arising from scrotal thermal stimulation. Nakayama et al (1979) indicated that PO/AH thermosensitive neurons, which initially responded to changes in local temperature, also responded to scrotal warming and cooling. That study suggested that scrotal thermal afferent signals project to preoptic thermosensitive neurons in rats. Interestingly, the prominent switching responses, often encountered in thermoresponsive neurons of the dorsal horn and the thalamus, appear to be less common in thermoresponsive hypothalamic neurons. Incremented changes in scrotal temperature produced a high percentage of static responses in hypothalamic neurons, but few switching responses (Nakayama et al, 1979; Nakayama et al, 1983). In addition, Shibata et al (1988) reported that neurons in the sensory cortex of rats also responded to scrotal thermal stimulation, inferring that scrotal thermal information is projected to the cortical level.
1-7-3 Thermoafferent Pathways from the Scrotum

As mentioned above, neuronal responses to scrotal stimulation have been extensively investigated in various regions of the central nervous system from the dorsal horn of the spinal cord to the sensory cortex (Hellon and Misra, 1973a; Hellon et al, 1973; Shibata et al, 1988). Figure-1 is a schematic representation of proposed thermal afferent pathways that carry warm and cold inputs from the scrotum to different loci within the central nervous system.

Scrotal thermoreceptors (warm and cold) first synapse with neurons in the dorsal horn (Hellon and Misra, 1973a). This afferent thermal information is probably carried in the anterolateral tract (eg, spinothalamic tract) of the spinal cord, as demonstrated by the cessation of thalamic neuronal activity evoked by scrotal warming of rats after electrolytic lesioning of the ipsilateral anterolateral tract (Taylor, 1979). In addition, Taylor (1979; 1982) found that extensive lesions of the brainstem medial lemniscal pathway caused no changes in neuronal responses of thalamic and hypothalamic neurons to scrotal heating and indicated that the medial lemniscal tract is probably not used for the transmission of scrotal temperature stimuli.

In the midbrain, the nuclei raphe magnus (NRM) (Hellon and Taylor, 1982; Jahns, 1976; Werner et al, 1981) appears to serve as an important thermoafferent relay centre of signals arising from peripheral thermoreceptor activation. Gottschlich and Werner (1985) found that lesioning the midbrain raphe nuclei, centralis or dorsalis, or parts of the central grey matter of rats was followed by a loss of thermoresponsiveness of neurons of the ventrobasal complex of thalamus to scrotal thermal stimulation. That study suggested that scrotal thermoafferent signals are projected to the thalamus from the nucleus raphe. Electrolytic lesions in rats of the midbrain raphe nuclei (nucleus raphe dorsalis and centralis) also immediately abolished the activity of thermoresponsive preoptic hypothalamic neurons to scrotal thermal stimulation (Werner and Bienek, 1990). These lesion studies, combined with single unit recordings, confirmed the notion that the midbrain nuclei raphe forms an essential relay centre of thermoafferent pathway for
Figure-1  Schematic diagram showing the thermoafferent pathways carrying warm
and cold thermoreceptor inputs from the scrotum to the diencephalon
and thermoeffeferent pathways carrying thermoeffeferent signals from the
hypothalamus (the VMH nuclei) to activate brown adipose tissue
(BAT).

BAT  = Brown adipose tissue
CR   = Cold receptor
NRM  = Nuclei raphe magnus
NST  = Non-shivering thermogenesis
PO/AH = Preoptic anterior hypothalamus
SC   = Spinal cord
VMH  = Ventromedial hypothalamic nuclei
Hollon and Taylor, 1982; Werner and Bienek, 1985; 1990; Kanosue et al, 1985; Werner thermal signals arising from the periphery. It is thought that parallel signals project away from the nucleus raphe magnus (NRM), one to the hypothalamus, the other to the thalamus (Hollon and Misra, 1973b; Schingnitz and Werner, 1980a; Taylor, 1982; et al, 1986), which in turn, would project to the somatosensory cortex (Hollon, et al, 1973; Shibata, et al, 1988).

Figure-1 also schematically shows a proposed thermal efferent pathway from the integrative neurons within the hypothalamus, including the VMH, which activates thermoeffectors, especially IBAT. Using horseradish peroxidase (HRP) tracing studies, Kita and Oomura (1982a, 1982b) indicated that there are anatomical and functional connections between the PO/AH and the VMH in rats. The increased IBAT thermogenesis induced by either local cooling of the PO/AH, or microinjection of prostaglandin E₂ (PGE₂) into the PO/AH of rats was attenuated or abolished by microinjection of a local anaesthetic into the VMH (Imai-Matsumura, et al, 1984; Amir and Schiavetto, 1990). These works suggested that the VMH receives thermoafferent signals from the PO/AH. On the other hand, VMH neurons send efferent projections to the periaqueductal grey matter of the medulla (Ter Host and Luiten, 1986; 1987) and onto the intermediolateral cell column of the spinal cord. Postganglionic sympathetic efferent fibres (five branches) innervate the IBAT (Ter Host and Luiten, 1986; Yoshimatsu et al, 1987).

1-8 Temperature Acclimatization
1-8-1 Acclimatization to Cold

When mammals are acutely placed in a cold environment they lose a greater amount of heat due to the increased difference between body and ambient temperature. When animals are chronically placed in colder environments they develop mechanisms to maintain body temperature during long-term exposure to cold by decreasing heat loss and by increasing metabolic heat production. These thermoregulatory adaptive changes are coordinated by the central nervous system and include changes in shivering and non-
shivering thermogenesis.

1-8-2 Shivering and Non-shivering Thermogenesis of Cold Acclimatized Animals

Shivering, initially defined by Davis and Mayer (1955) as a "reflex muscle tremor directly attributed to cold", and later modified by Kleinebeckel and Klussmann (1990) as an "increase in reflex monolocomotor muscle tone attributed to exposure to cold, with or without visible tremor", is a defence reaction to acute cold exposure in homeothermic animals. During the initial stages of intense cold, non-thermally acclimatized animals employ shivering. A CNS loci, which is proposed to coordinate and/or initiate shivering response is the posterior hypothalamus (PH) (Stuart et al 1961; Halvorson and Thornhill, 1993). Shivering responses involve muscle tremor and are best determined by measuring electromyographic (EMG) activity of the muscle. Typical shivering responses induced by cold in humans often consist of three phases: initial phase, which lasts up to 20 minutes, is featured by slight increases in muscle tone which can be seen as changes in electromyograph (EMG) activity. An adaptive phase following the initial phase shows a decline of overall muscle activity even though the cold stimulus is still present, and lasts about 15 minutes. However, during the final phase of shivering response, visible muscle tremors are seen with various rhythmical patterns (ie, single twitching, bursting, etc). More muscles will be recruited as the cold exposure is continued (Kleinebeckel and Klussmann 1990). It should be noted that cold-acclimatized animals do not lose the ability to shiver as a means to produce heat in response to cold stimulus; however, a more intense cold stimulus was found to be required to initiate shivering responses, since the threshold of shivering was shifted downward in these cold-acclimatized animals (Bruck et al, 1970; Bruck, 1981).

If cold exposure is prolonged, brown adipose tissue (BAT) plays a more significant role in heat production in small animals (e.g., rodents) to increase non-shivering thermogenesis due to the enhanced thermogenic capacity of small cold-acclimatized animals to sustained cold (Bruck 1981; Heldmaier and Buchberger, 1985). For example, a non-shivering thermogenic response replaces shivering activity in rats previously
acclimatized to 5 to 10°C for 3-4 weeks (Jansky 1973; Griggio 1982). Therefore, the conversion from shivering thermogenesis to non-shivering thermogenesis during cold acclimation is a common adaptive thermoregulatory response in small animals.

The enhanced thermogenic capacity of BAT of cold acclimatized animals is associated with BAT hypertrophy (Girardier et al, 1983; Cannon and Nedergaard, 1983; Nicholls and Locke, 1984). In mice, even a brief daily cold exposure is sufficient to induce hypertrophy of BAT (Heldmaier 1975; Wunder 1981). The hypertrophied BAT is characterized by increases in the content of uncoupling protein (UCP) in each mitochondria, as shown in the increased expression of mRNA for UCP in rats after birth (Freeman et al, 1989). As well, there are increases in the total number of mitochondria per adipocyte and increases in the total number of adipocytes (Himms-Hagen, 1990). Lastly, hypertrophied BAT evoked an increased turnover of free fatty acids, the substrate for energy (heat) production (Lafrance et al 1980).

Spontaneous activity of sympathetic nerves innervating the IBAT is increased during acute and chronic exposure to cold, and is associated with an enhanced thermogenic activity of IBAT (Niijima et al 1984). A large increase in blood flow to IBAT was also found in rats exposed to cold (Foster and Fydman, 1979). As the cold exposure is prolonged, the hypertrophied BAT in rats and other species manifests a great thermogenic response to a subsequent cold stimulation (Kuroshima et al, 1985). As well, intravenous administration of noradrenaline causes a greater thermogenic response of brown adipose tissue in cold-acclimatized rats than that in room-temperature or warm-acclimatized rats (Doi and Kuroshima, 1982; Thornhill and Halvorson, 1990).
2 MATERIALS AND GENERAL METHODS

2-1 Animals and Maintenance

Male, Sprague-Dawley rats weighing 350-500 g at the time of experiment were obtained from Charles River Laboratories, Montreal Quebec. All experimental procedures were approved by the Animal Care Committee at the University of Saskatchewan (ACCUS) and were also in accordance with the principles of the Canadian Council of Animal Care (CCAC).

Rats were randomly divided into two groups as detailed below, according to different experimental protocols. Rats in the room temperature acclimatization (RA) group were kept in an environmental chamber at 21 ± 1 °C, relative humidity = 30% and a 12 hr on-off lighting schedule. Rats had continued access to food and water. All rats in the cold acclimatization (CA) group were kept in an environmental chamber at 4 °C for at least four weeks before performing the experiments. The relative humidity of the environmental chamber was about 30% and the lighting schedule (on-off) was 12 hours. Rats had free access to food and water.

2-2 Surgical Preparation of Rats

2-2-1 Surgical Procedures

Rats in both groups were anaesthetized with ip urethane (Ethyl Carbamate, Sigma Chemical Co. St Louis, MO. 1.2 g/kg). An additional supplementary dosage of 0.1-0.2 g/kg was given if necessary. Urethane was chosen as the anesthetic in this series of studies because it is long lasting and has minimal effects on reflex responses (Malkinson
et al., 1988). Urethane shows less depressant effects on neuronal activity as diencephalic single units of rats were not affected by urethane anaesthesia (1.3 g/Kg i.p.) (Cross and Dyer, 1971; Dyball and Mc Phail, 1974).

The head of each rat was mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). An incision of ~ 2 cm was made along the midline of the skull. A burr hole of 0.5 x 0.5 cm was made for penetration of a glass micro-pipette. During surgical preparation, care was taken to avoid damaging the sagittal sinus. The coordinates used for VMH extracellular recording sites were as follows: anterior to posterior (AP) = -2.30 to -2.80 mm (from bregma), lateral = 0.5 - 0.7 mm (from midline) and ventral = 9.0 mm below dura according to the Paxinos and Watson (1986) coordinate system for the rat brain.

2-2-2 Cannulation of the Femoral Artery

In the experiments referred to in Section 3-2-3-1 (mean changes in arterial pH, pCO₂ and pO₂), the right femoral artery of rats was cannulated with non-toxic Polyethylene tubing(PE-50, I.D. = 0.58 mm, O.D. = 0.965 mm. Clay Adams, Division of Becton Dickinson and Company, Parsippany, NJ) filled with heparinized saline (100 units/ml, Hepalean*, Organon Teknika Inc, Toronto, ON). The other end of this tubing was connected to a 1.0 cc syringe.

2-2-3 Implantation of Cannula into the Medial Preoptic Area (MPO) and VMH

In the experiments referred to in Section 3-2-5-1 MPO lidocaine study, a stainless steel microinjection guide cannula (O.D. = 0.8 mm. Small Parts Inc, Miami, Fl) was implanted into the MPO, ipsilateral to the VMH extracellular recording site, using the following coordinates (AP=-0.8 mm, lateral=0.5 mm and vertical= 8.5 mm). The implanted guide cannula was secured to the skull with dental cement. The microinjection cannula, which protruded 0.5 mm beyond the tip of the guide cannula, was connected to PE-10 tubing and a 1.0 μL Hamilton syringe (Chromatographic Specialties Inc, Brockville, ON). A total of 150-200ng of 2% buffered lidocaine (pH=7.4) in a total
volume of 300 nl was microinjected into the MPO over a period of 60 seconds.

In the experiments referred to in Section 3-2-4 (tail vasomotor responses to scrotal thermal stimulation), two stainless steel microinjection guide cannulae (O.D. = 0.8 mm. Small Parts Inc, Miami, Fl) were implanted bilaterally into both VMH nuclei, using the following coordinates (AP=-2.30 mm, lateral (left and right) = ± 0.5 mm and vertical= 9.0 mm). The implanted guide cannulas were secured to the skull with dental cement. The inner injecting cannula, which protruded 0.5 mm beyond the tip of the guide cannula, was connected to PE-10 tubing and a 1.0 μL Hamilton syringe (Chromatographic Specialties Inc, Brockville, ON). 2% buffered lidocaine (200ng/300nl), previously shown to block the neuronal activity of VMH thermoresponsive neurons to scrotal heating and cooling after administration into the the medial preoptic area, was administrated into the VMH bilaterally over a period of 60 seconds.

Lidocaine was chosen in these studies because its anesthetic effects on neurons and axons are reversible (Covino and Vassallo, 1976), which allowed subsequent testing of recovery responses of the VMH neurons and vasomotor effectors to scrotal thermal stimulation.

2-3 Neurophysiological Recording of VMH Neurons

2-3-1 Preparation and Filling of Glass Micro-pipettes

Single glass micro-pipettes were pulled from glass capillaries with an inner microfilament for fast filling (O.D. = 2.0 mm, World Precision Instruments, Inc., New Haven, CT). Micro-pipettes were pulled on a vertical pipette puller (Model 700 C, David Kopf Instruments, Tujunga, CA). Glass micro-pipettes were usually made the day before experiments began and the tip of the micro-pipette was 1-2 μm with a DC resistance of 5 - 10 MΩ. Glass micro-pipettes were filled with 0.5 M sodium acetate (The McArthur Chemical Co, Ltd, Montreal, QC) containing 2% Pontamine sky blue (BDH Chemicals Ltd, Poole, England) and were kept in an electrode storage jar (World Precision
Instruments, Inc., New Haven, CT).

2-3-2 VMH Extracellular Recording

The glass micro-pipette was advanced into the VMH by a hydraulic manipulator (Model 607W, David Kopf Instruments, Tujunga, CA) through the small burr hole on the skull. The extracellular activity of neurons in the VMH was isolated and amplified by an extracellular amplifier (Dagan-2400, Dagan Corporation, Minneapolis, MN) and monitored with an oscilloscope (Tektronix-5110, Tektronix Inc. Portland, OR) and an audiometer. In addition, photographs from the oscilloscope tracings of changes in VMH single-unit neuronal activity were taken in some experiments.

2-3-3 Discrimination of Single Units

The amplified VMH action potential was fed into a Signal Processing System (SPS-8701B) Real Time Waveform discriminator (Single Processing Systems, South Australia, Australia). The SPS-8701B discriminator employed a template-matching algorithm designed to recognize the occurrence of recurrent, distinctively-shaped waveforms of neuronal activity of single units. The input signals were continuously compared with (up to) three templates (previously selected and stored) which were classified on the basis of the nearest match of waveform shape. The input signals to be matched were selected on the basis of crossing a voltage threshold (i.e. the DC voltage level in which the input signal triggered the SPS-8701B discriminator). Only spikes which had a signal:noise ratio of at least 3:1 and exceeded the trigger threshold were processed for matching and recorded in these studies. In addition, the SPS-8701B discriminator had template adaptation features that allowed automatic tracking and processing of waveform signals of action potentials of any neuron which slowly changed in amplitude over time, yet this system reliably separated action potentials of different neurons that had similar amplitudes but differed in waveform shape. In these experiments, all VMH neurons reported in the Section of Results were from single units.

The firing rate of each unit was displayed on-line on the video-graphic adaptor (VGA)
monitor of an IBM - AT computer and the outputs of firing rate were recorded on a polygraph (Grass Model 7D, Grass Instrument Co., Quincy, MA). All extracellular data were stored onto floppy diskettes and then transferred onto a spreadsheet for further analysis.

2-4 Measurement of Electroencephalographic Activity (EEG)

In the experiment referred to in Section 3-2-3-3, surface electroencephalogram (EEG) signals were recorded by a bipolar electrode placed on the scalp above the parietal region, amplified via EEG pre-amplifier (Model 7P5A, Grass Instrument Co., Quincy, MA) and displayed on a polygraph (Grass Model 7D, Grass Instrument Co., Quincy, MA). These signals were split such that the EEG signals were also displayed on the VGA monitor of a computer in order to digitize the raw EEG signal for the determination of frequency and amplitude. All EEG data were stored onto floppy diskettes and then transferred onto a spreadsheet for further analysis.

Changes in EEG activity were also determined by applying nociceptive or mechanical stimuli to the rats. A pair of forcep was used to pinch the tail and a hair brush was used to conduct mechanical stimuli, respectively.

2-5 Measurements of Arterial pH, pO₂ and pCO₂

In the experiments referred to in Section 3-2-3-1, the femoral arterial blood of urethane-anaesthetized rats was collected before and after VMH extracellular recording from a cannula inserted into the right femoral artery. A total of 0.5 ml of arterial blood was withdrawn and heparinized. pH, pO₂ and pCO₂ of arterial blood were determined using a Blood-Gas analyzer (Stat Profile 5 Plus, NOVA Biomedical, Waltham, MA).

2-6 Scrotal Thermal Stimulation and Scrotal Temperature Measurements

The hair on the scrotum was shaved and scrotal temperature changes were induced by two methods:

(a) initial experiments referred to in Sections 3-2-1 and 3-2-2 were performed using
a small hot water pack (2.0 x 2.0 cm sealed packs filled with 40°C tap water) and another sealed pack of the same size filled with ice. The induced scrotal temperature changes were recorded by placing a thermistor probe (YSI 409A, Yellow Springs Instruments, Yellow Springs, OH) between the surface of the scrotal skin and the applied hot or ice pack.

(b) in later experiments referred to in all remaining sections, scrotal temperatures were altered via a localized thermode (tip area 3 mm²) placed on the surface of the scrotum. The thermode was made of two stainless steel tubes and was connected to a water bath (Model 1268-70, temperature range -20 to 100°C, accuracy ± 0.02°C. Cole-Parmer, Chicago, Ill). Controlled, incremental cooling and heating (or heating, then cooling) of the scrotum, from 5 to 45°C, in steps of 5 to 10°C, was achieved by changing the temperature of the circulating water of the thermode (flow rate = 1 - 2ml/s). Scrotal temperature changes were continuously monitored with a thermistor probe (YSI 524, Yellow Springs Instruments, Yellow Springs, OH), glued to the tip of scrotal thermode.

2-7 Temperature Measurements

Tail temperatures (Tₜₛ) were measured using a surface thermistor probe (YSI 409A, Yellow Springs Instruments, Yellow Springs, OH) which was taped to the base of the rat’s tail, which indirectly assessed vasomotor reflex changes of the animal to scrotal heating and cooling. Interscapular brown adipose tissue temperatures (TᵢBATₛ) were measured (Perkins et al, 1981) by placing a small thermistor probe (YSI 511, Yellow Springs Instruments, Yellow Springs, OH) under the IBAT pad through a small surgical incision on the animal’s back to assess BAT thermogenic activation.

All temperatures were continuously recorded via a multi-channel temperature monitor (Acadia Clinical Corporation, Saskatoon, SK.), and displayed in real time on the VGA monitor of a IBM-AT computer. All temperature data were also stored onto floppy diskettes and transferred onto a spreadsheet for off-line analysis.
Figure-2 shows a block diagram of equipment used for the induction of scrotal temperature changes and monitoring VMH extracellular activity and changes in temperature or EEG.

2-8 Maintenance of Colonic (Core) Temperature

Colonic temperatures were servo-controlled in most experiments at 37°C via a self-regulating heating blanket (Acadia Clinical Corporation, Saskatoon, SK). In another experiment (Section 3-2-2), repeated thermal stimulations were applied to the scrotum when colonic (core) temperatures were maintained at one of three different temperatures (ie 37°C, then 35°C, and finally 33°C) by adjusting the temperature of the controller for the heating blanket.

2-9 Determination of Thermoresponsiveness of VMH Neurons

Thermoresponsiveness of each recorded VMH neuron was determined by its thermal coefficient (TC, impulses.s⁻¹.°C⁻¹), which was the slope of the frequency-temperature response curve, based on the Boulant and Hardy (1974) classification of hypothalamic thermoresponsive neurons to peripheral (skin) thermal stimulation. Thermoresponsiveness of recorded VMH neurons of these studies was determined by calculating the TCs based on the changes in firing rates vs. scrotal temperature (usually over the most sensitive 10 °C range). The VMH neuron was considered a warm responsive neuron (WRN) if its thermal coefficient (TC) was at least 0.5 impulses.s⁻¹.°C⁻¹ and a cold responsive neuron (CRN) if its thermal coefficient was at least -0.5 impulses.s⁻¹.°C⁻¹. Other VMH neurons which had thermal coefficients between -0.5 and +0.5 impulses s⁻¹.°C⁻¹ were classified as temperature non-responsive neurons (TNRNs).

2-10 Histological Verification of VMH Recording Sites and MPO Or VMH Cannulae Sites

2-10-1 Verification of VMH Recording Sites

At the end of each recording session, the site of micro-pipette penetration for
Figure-2  Block diagram showing experimental set-up to (1) record VMH extracellular activity, (2) induce scrotal temperature changes, (3) record IBAT, tail and scrotal temperatures, and (4) monitor EEG changes (some experiments).

It should be noted that 1) scrotal thermal stimulation induced by hot water or ice pack used in the first two studies is not shown in this diagram; 2) detailed descriptions of the set-ups for each study are in corresponding sections in this chapter.

AMP = Amplifier
EEG = Electroencephalography
FR = Firing rate
ME = Micropipette
OSC = Oscilloscope
$T_{IBAT}$ = Interscapular brown adipose tissue temperature
$T_{TAIL}$ = Tail temperature
$T_{SCROTUM}$ = Scrotal temperature
recording within the VMH was marked by the iontophoretic deposition of 2% Pontamine sky blue from the tip of the glass micropipette using a 5 μA cathodal current for ~ 5 minutes (Kaneko and Hashimoto, 1967). The brain of the rat was fixed by an intracardiac perfusion with physiological saline followed by 10% buffered formalin. Frozen serial coronal sections of the brain were cut at 20 μm slices using a vibrotome to confirm the position of the recording micro-pipette. Figure-3 shows a photomicrograph of dye deposition located within the VMH obtained from one of the serial coronal sections (Paxinos and Watson, 1986). It should be noted that only data from those experiments in which the dye (blue) spot of the recording micro-pipette was confirmed histologically to be within the VMH nucleus were presented in this thesis.

2-10-2 Verification of the Locations of MPO Cannulae

At the end of experiments referred to in Section 3-2-5-1, in addition to the dye deposition into the VMH recording site, 0.5μl of 2% Pontamine sky blue was injected into the MPO nucleus through the implanted cannulae. The brain of the rat was fixed, sectioned and verified following the same procedures as mentioned above.

2-10-3 Verification of the Locations of VMH Cannulae

At the end of experiments referred to in Section 3-2-4, 0.5μl of 2% Pontamine sky blue was injected bilaterally into both VMH nuclei through implanted cannulae. The brain of the rat was fixed, sectioned and verified following the same procedures as mentioned above.

2-11 Statistical Analysis of Data

Statistical analyses were performed with computer statistical packages (Instat2, GraphPad Software, Inc. San Diego, CA). Data were presented as the mean value ± S.E.M. where appropriate.

Chi-squared analysis followed by Fisher's exact test was used to determine significant differences in the ratio or percentages of classified VMH WRN, CRN and TNRNs
Figure-3  Photomicrograph showing dye deposition located in the VMH, from the tip of the glass micro-pipette following current injection of 5 μA for about 5 minutes. Site of dye deposition within the VMH is taken from a serial coronal section -2.56 mm posterior to bregma.
recorded from different groups of rats used in these studies (eg, normothermic and hypothermic rats or room temperature and cold-acclimatized rats).

The paired Student t test was used to determine significant differences in arterial pH, pCO₂ and pO₂ of urethane-anesthetized rats before and after VMH extracellular recording.

A one-way analysis of variance (ANOVA) with or without repeated measures was employed to determine significant differences in T_{IBAT}, T_{TAIL} and EEG activity (amplitude and frequency) during scrotal heating and cooling. A one-way ANOVA was also used to determine significant changes in thermal coefficients and/or basal firing rates of the VMH thermoresponsive and non-responsive neurons of normothermic, hypothermic, room temperature and cold acclimatized rats during scrotal heating and cooling. When a significant F-value was determined from the initial ANOVA, a post test (Student-Newman-Keuls comparison) was subsequently performed to determine significant differences among these groups.

Significant differences were considered to have a p value of <0.05.
3 RESULTS

3-1 General Descriptions
The experimental results presented in this thesis consist of two parts: data obtained from male urethane-anaesthetized rats which were acclimatized to room temperature (21 ± 1°C for 4 weeks)(Section 3-2) and data obtained from male, urethane-anaesthetized rats which were acclimatized to cold (4°C for 4 weeks)(Section 3-3).

3-2 Room Temperature (21°C) Acclimatization Groups

3-2-1 Neuronal Activity of VMH Neurons is Altered by Scrotal Heating and Cooling Induced by Small Hot or Ice-packs
This initial study was to determine (1) if the extracellular neuronal activity changes of VMH neurons of anaesthetized normothermic (37°C) or acutely hypothermic rats (33-35°C) were modified by cold and/or warm local thermal stimulation of the scrotum and (2) to determine if changes in scrotal temperature evoked changes in core (colonic), surface (tail) or IBAT temperatures, indicative that thermoregulatory effector mechanisms have been activated. Hypothermic animals were tested in this study since cooling has been shown previously to increase sympathetic efferent activity of interscapular brown adipose tissue nerves (Niijima et al, 1984), thus the thermoresponsiveness of neurons within the VMH nucleus that modulates IBAT nerve activity may also be altered in the hypothermic group.

3-2-1-1 Ratio of VMH Warm-responsive (WRNs), Cold-responsive (CRNs) and Temperature Non-responsive Neurons (TNRNs) in Normothermic and
Hypothemic Rats

A total of 150 neurons within the VMH were recorded in this initial study, 85 (57%) of these were recorded from hypothemic rats (n=66) and 65 (43%) neurons were from the normothermic rats (n=50). Of the 150 neurons, 76 (50%) VMH neurons were thermoresponsive (i.e., warm or cold-responsive) to either scrotal cooling and warming as determined by their thermal coefficients. The ratio of WRNs:CRNs:TNRNs was 23(35%):11(17%):31(48%) for the normothermic group and 19(22%):23(27%):43(51%) for the hypothemic group. The WRNs composed 35% of the VMH neurons recorded under normothermic conditions (colonic temperature kept at 37°C), but only 22% of VMH neurons were WRNs in hypothemic animals (colonic temperatures were between 33-35°C). The percentage of CRNs in normothermic and hypothemic groups was 17% and 27%, respectively. Chi-squared analysis of the number of WRNs and CRNs between these groups indicated that these differences were not significant (P > 0.05). It should be noted that in those rats (n=74) in which the surface of the scrotum was stimulated with a small hair brush after scrotal thermal stimulation no VMH neurons changed their extracellular neuronal activity.

3.2.1.2 Examples of VMH WRNs, CRNs and TNRNs in the Normothermic Rats Kept at 21°C Following Scrotal Thermal Stimulation with Sealed Ice or Hot Water Packs

Figure 4 shows extracellular neuronal activity changes of three VMH neurons (warm, cold responsive and temperature non-responsive) recorded from three normothermic rats (colonic temperatures maintained at 37°C) and their associated IBAT and tail temperature changes following scrotal cooling (a) and warming (b). The VMH neuron in panel A, classified as a WRN(TC = 0.62 impulses.s⁻¹.°C⁻¹), decreased its firing rate as scrotal temperature decreased as the ice pack was applied and gradually increased its firing frequency as scrotal temperature rose with scrotal warming. The neuron in panel B, classified as CRN(TC = -0.67 impulses.s⁻¹.°C⁻¹), increased its firing rate following scrotal cooling. The example in panel C is a TNRN(TC = 0.05 impulses.s⁻¹.°C⁻¹) and
Changes in extracellular neuronal activity (impulses/sec) of three VMH neurons classified as (A) warm responsive neuron (WRN, TC = 0.62 impulses.s\(^{-1}\).°C\(^{-1}\)), (B) cold responsive neuron (CRN, TC = -0.67 impulses.s\(^{-1}\).°C\(^{-1}\)) and (C) temperature non-responsive neuron (TNRN, TC = 0.05 impulses.s\(^{-1}\).°C\(^{-1}\)) of normothermic, male urethane-anaesthetized rats with colonic temperature maintained at 37°C via a servo-controlled heating blanket and their associated temperature changes (colonic, closed squares; interscapular brown adipose tissue, open squares; tail, triangles and scrotum, asterisks) are shown before and during scrotal cooling (a, ice pack 2x2 cm) and warming (b, another pack containing 40°C hot water). Thermal coefficient was calculated over sensitive 10°C range of scrotal temperature.
did not change its firing rate over the 80 minute period of scrotal cooling and heating.

Figure-4 also shows that $T_{IBAT}$s (open squares) of normothermic rats, colonically maintained at 37°C (open squares), slightly, but did not significantly decrease with scrotal cooling, compared to respective prestimulation control temperatures. $T_{IBAT}$s rose back to control values with scrotal warming. However, tail surface temperatures (solid triangles) did significantly decrease from prestimulation control temperatures with scrotal cooling and rose back towards control values with scrotal warming.

Out of 65 VMH neurons recorded from the normothermic group, 23 VMH neurons (35%) were classified as WRNs. Further sub-classification of these 23 VMH WRNs indicated that 12 out of 23 VMH neurons responded with increases in firing frequency with warm scrotal stimulation and also with decreases in firing rates when cold was applied to the scrotum (biphasic response). Another 7 VMH neurons increased their firing frequency with warm stimulation to the scrotum, but were non-responsive to cold (monophasic response) and another 4 VMH WRNs only decreased their firing rate to cold scrotal stimulation (also monophasic responses).

11 out of 65 VMH neurons (17%) were classified as CRNs, as they responded to scrotal cooling with increased firing rates (and/or decreased their firing frequencies with scrotal warming). 3 CRNs of this group increased their firing frequency to cold application to the scrotum and decreased their firing frequency with warm stimulation (ie, biphasic CRN). The remaining 8 CRNs only responded with increased firing frequencies with cold stimulation to the scrotum (monophasic responses).

Figure-5 shows a histogram of VMH thermo responsive neurons, which were classified as monophasic and biphasic neurons, from the normothermic group. There were no significant changes in number of biphasic and monophasic VMH WRNs and CRNs between the 2 groups.
Figure-5  Histogram shows the number of VMH neurons classified as monophasic and biphasic warm and cold responsive neurons of normothermic (colonic temperature kept at 37°C) and hypothermic (colonic temperature maintained between 33-35°C) male rats. A biphasic VMH WRN was defined as a neuron that increased its firing rate with scrotal heating and decreased its firing rate with scrotal cooling. A monophasic VMH WRN was classified as a neuron that increased its firing rate with scrotal heating or decreased its firing rate with scrotal cooling only. A biphasic VMH CRN was defined as a neuron that increased its firing rate with scrotal cooling and decreased its firing rate with scrotal heating. A monophasic VMH CRN was classified as a neuron that increased its firing rate with scrotal cooling or decreased its firing rate with scrotal heating only.
Thirty-one of 65 VMH neurons (48%) of this group were TNRNs, (ie thermal coefficients were between -0.5 and +0.5 impulses.s^{-1}.°C^{-1}) as their neuronal activity was not affected by scrotal heating and cooling.

3-2-1-3 VMH WRNs, CRNs and TNRNs in Acutely Hypothermic Rats Following Scrotal Thermal Stimulation with Sealed Ice or Hot Water Packs

Figure-6 shows extracellular neuronal activity changes of warm and cold responsive and temperature non-responsive VMH neurons of three acutely hypothermic rats whose colonic temperatures were maintained between 33°C-35°C during scrotal cooling (a, ice pack) and warming (b, 40°C hot water pack). The VMH neuron in panel A, classified as a WRN TC=0.67 impulses.s^{-1}.°C^{-1}), decreased its firing rate as scrotal temperature decreased when the ice pack was applied and gradually increased its firing rate as scrotal temperature rose following scrotal warming. The neuron in panel B, classified as a CRN TC= -0.71 impulses.s^{-1}.°C^{-1}), increased its firing rate following scrotal cooling. The example in panel C is a TNRN TC= 0.03 impulses.s^{-1}.°C^{-1}), which did not change its firing rate during the 80 minute period of scrotal cooling and heating.

Figure-6 also shows that T_{IBAT} (open squares) of hypothermic rats, colonically maintained at 33°C-35°C (open squares) did not significantly increase with scrotal cooling(a) in panels A and B even though colonic temperatures (solid squares) were significantly decreased as the ice pack was applied, compared to respective prestimulation control temperatures. Like normothermic rats, IBAT temperatures did not significantly decrease from prestimulation control temperatures with scrotal cooling(a) and rose back towards control values with scrotal warming(b).

Out of 85 VMH neurons recorded from the hypothermic group, 19 (22%) were classified as WRNs. Further sub-classification of these WRNs indicated that 11 out of 19 WRNs increased their firing rates with scrotal warming and also decreased their firing rates with cooling of the scrotum (classified as biphasic WRN neurons). Another 5 VMH neurons increased their firing frequency with warm stimulation to the scrotum, but were
Figure-6  Changes in extracellular neuronal activity (impulses/sec) of three VMH neurons classified as (A) WRN (TC=0.67 impulses.s⁻¹.C⁻¹), (B) CRN (TC=-0.71 impulses.s⁻¹.C⁻¹) and (C) TNRN (TC=0.03 impulses.s⁻¹.C⁻¹) of hypothermic male, urethane-anaesthetized rats with colonic temperature maintained between 33-35°C and their associated temperature changes (colonic, closed squares; interscapular brown adipose tissue, open squares; tail, triangles and scrotum, asterisks) are shown before, during scrotal cooling (a, ice pack 2x2 cm) and warming (b, another pack containing 40°C hot water). Thermal coefficient was calculated over sensitive 10°C range of scrotal temperature.
non-responsive to cold and another 3 VMH WRNs only decreased their firing rate to cold scrotal stimulation (monophasic responses).

From the hypothermic group, 23 (27%) were classified as CRNs and 10 out of 23 CRNs increased their firing rates to cooling of the scrotum and also decreased their firing frequencies to warming of the scrotum (biphasic responses). 8 CRNs increased their firing frequency to scrotal cooling only and 5 decreased their firing frequency with warm stimulation only (monophasic responses). A histogram of monophasic and biphasic VMH neurons of this group is shown in the Figure-5.

Again, the majority of VMH neurons (43/85 or 51%) of the hypothermic group did not respond to warming or cooling of the scrotum and were classified as TNRNs.

3-2-1-4 Mean Changes in T_{IBAT}s, and Tail Temperatures Following Scrotal Heating and Cooling of Normothermic and Hypothermic Rats Using Scrotal Hot or Ice Packs

Figure-7 shows mean changes in colonic, IBAT and tail temperatures in the normothermic and hypothermic groups. In the normothermic group (left panel), IBAT temperature of normothermic rats did not significantly change with scrotal cooling(a) as the ice pack was applied to the scrotum or with scrotal heating(b), compared to respective prestimulation control temperatures. Colonic temperatures showed a delayed decrease to a maximum of -0.2°C following scrotal cooling. In the hypothermic group (right panel), colonic and IBAT temperatures did decrease, with the maximum fall being 2 and 1°C, respectively, from prestimulation control temperatures with scrotal cooling(a) and both temperatures rose back towards control values with scrotal warming(b). Scrotal cooling in both groups rapidly decreased scrotal temperatures to below 20°C by the end of the 30 minute cooling period. With scrotal heating both groups increased scrotal temperatures to above respective pre-stimulation control scrotal temperatures.
Figure-7  Mean changes (Mean± S.E.M) in colonic, interscapular brown adipose tissue and tail temperatures (°C) to changes in scrotal temperatures are shown for the two groups of normothermic(37°C) (left panel, n=50) and hypothermic(33-35°C) (right panel, n=66), male, urethane-anaesthetized rats before, during scrotal cooling with ice packs(a) and scrotal warming with sealed packs containing 40°C water(b).
Tail temperatures decreased gradually to a peak of 6°C and 5°C by the end of scrotal cooling period in the normothermic and hypothermic groups, respectively. Scrotal heating increased tail temperatures back to respective pre-stimulation control surface temperatures in the normothermic and hypothermic groups.

3-2-2 Thermoresponsiveness of VMH Neurons is Retained to Repeated Scrotal Thermal Stimulation

Results presented in the previous section indicated that VMH neurons of normothermic and acutely hypothermic rats respond to a single trial of scrotal heating and cooling using ice and hot water packs. However, the thermoresponsiveness to repeated trials of peripheral (scrotal) thermal stimulation of the same VMH WRN, CRN and TNRN may change if colonic temperatures of the animals were altered between trials. The first experiment (Section 3-2-2-1) was designed to determine if the same VMH thermoresponsive neuron is able to retain its thermoresponsiveness to repeated warm (40°C hot water) and cold (ice pack) trials of scrotal thermal stimulation when the rat’s colonic temperature was maintained at 37°C during two trials of thermal stimulation. The second study (Section 3-2-2-2) was designed to determine if the same VMH thermoresponsive neuron is able to retain its thermoresponsiveness to repeated warm (40°C hot water) and cold (ice pack) trials of scrotal thermal stimulation when the rat’s colonic temperature was kept at 37°C, 35°C and 33°C, respectively during the three trials of scrotal thermal stimulation.

3-2-2-1 Thermoresponsiveness of VMH Neurons Following Repeated (2 Trials) of Scrotal Thermal Stimulation of Rats Maintained at 37°C

In this study, a total of 42 VMH neurons were isolated from 36 male, urethane-anaesthetized rats maintained colonic at 37°C during 2 trials of scrotal thermal stimulation. Based on their thermal coefficients to the first trial of scrotal warming and cooling with colonic temperature kept at 37°C, 12 were classified as WRNs, 10 were CRNs and 20 were TNRNs. 9 out of 12 WRNs, 7 out of 10 CRNs, and 18 out of 20 TNRN VMH neurons were recorded long enough to test their thermoresponsiveness to
a second trial of scrotal thermal stimulation with colonic temperatures kept at 37°C.

Figure-8 shows changes in firing rate (impulses/sec) of three individual VMH neurons classified as (A) WRN, (B) CRN and (C) TNRN of three male, urethane-anesthetized rats with colonic temperatures kept at 37°C. Figure-8A shows a VMH WRN fired at about 2 impulses/sec (solid circles) when scrotal temperature was maintained at an initial control temperature of 20°C. This neuron steadily increased its firing rate to 14 impulses/sec as scrotal temperature was elevated to about 40°C. A thermal coefficient of 0.85 impulses.s⁻¹°C⁻¹ for this VMH WRN was determined from the first trial of scrotal thermal stimulation. With the scrotal temperature returned to the initial control temperature of 20°C, the firing rate of this VMH WRN returned to about 2 impulses/sec (open circles). With the second trial of scrotal warming with core temperatures kept at 37°C, this VMH WRN again increased its firing rate (open circles), the thermal coefficient for the second trial of scrotal thermal stimulation was 0.83 impulses.s⁻¹°C⁻¹. This VMH WRN retained its thermoresponsiveness to the second trial of scrotal thermal stimulation.

Figure-8B shows changes in neuronal activity of VMH CRN to two trials of scrotal thermal stimulation with core temperature kept at 37°C throughout both trials. The firing rate of this VMH was about 2 impulses/sec (solid circles) when scrotal temperature was initially at 20°C. This CRN neuron steadily increased its firing rate to 12 impulses/sec as scrotal temperature decreased to 10°C. Thermal coefficient of -0.80 impulses.s⁻¹°C⁻¹ of this VMH CRN was determined for the first trial of scrotal stimulation. With the scrotal temperature returned to the initial control temperature of 20°C, the firing rate of this VMH CRN also returned to ~ 2 impulses/sec. With the second trial of scrotal thermal stimulation with core temperature kept at 37°C, this VMH CRN again increased its firing rate (open circles) to scrotal cooling. The thermal coefficient of -0.79 impulses.s⁻¹°C⁻¹ was calculated and indicated that this VMH CRN retained its thermoresponsiveness to the second trial of scrotal thermal stimulation with colonic temperatures kept at 37°C.
Changes in neuronal activity (impulses/sec) of three individual VMH neurons classified as (A) WRN, (B) CRN and (C) TNRN of three male, urethane-anaesthetized rats with colonic temperature maintained at 37°C are shown during two trials of scrotal heating (pack sealed with 40°C hot water) and cooling (ice pack).

Thermal coefficients calculated for these three VMH neurons after 1st and 2nd trials of scrotal thermal stimulation were:

\[ TC_{1st} = 0.85 \text{ impulses.s}^{-1} \cdot \text{°C}^{-1} \text{ and } TC_{2nd} = 0.83 \text{ impulses.s}^{-1} \cdot \text{°C}^{-1} \] for VMH WRN(A)

\[ TC_{1st} = -0.80 \text{ impulses.s}^{-1} \cdot \text{°C}^{-1} \text{ and } TC_{2nd} = -0.79 \text{ impulses.s}^{-1} \cdot \text{°C}^{-1} \] for VMH CRN(B), and

\[ TC_{1st} = 0.03 \text{ impulses.s}^{-1} \cdot \text{°C}^{-1} \text{ and } TC_{2nd} = 0.02 \text{ impulses.s}^{-1} \cdot \text{°C}^{-1} \] for VMH TNRN(C).
Comparison of mean thermal coefficients of all VMH WRNs(A, n=9), CRNs(B, n=7) and TNRNs(C, n=18) following the two trials of scrotal heating and cooling are shown of male, urethane-anaesthetized rats with colonic temperature maintained at 37°C during both trials. One-way ANOVA did not show significant changes in thermoresponsiveness of these VMH thermoresponsive and temperature non-responsive neurons to repeated scrotal thermal stimulation.
Mean TCs of VMH WRNs, CRNs and TNRNs after 2 trials of thermal stimulation with Tcolonic kept at 37°C
Unlike these VMH thermoresponsive neurons, VMH TNRNs did not respond to either trial of scrotal thermal stimulation. Figure-8C shows from an individual TNRN that no change in thermoresponsiveness occurred with either of 2 trials of scrotal thermal stimulation when colonic temperature was kept at 37°C.

Figure-9 shows the mean changes in thermoresponsiveness of all 9 VMH WRNs(A), 7 CRNs(B) and 18 TNRNs(C) to two trials of scrotal thermal stimulation of urethane-anaesthetized rats with colonic temperatures maintained at 37°C during both trials. There was no significant difference in the mean thermal coefficients for VMH WRNs, CRNs or TNRNs between the first or second trial of scrotal thermal stimulation (ie their thermoresponsiveness remained the same).

3-2-2-2 Thermoresponsiveness of VMH Neurons Following 3 Trials of Scrotal Thermal Stimulation of Rats with Colonic Temperature Kept at 37°C, 35°C and 33°C

Results in the previous section indicated that VMH thermoresponsive and temperature non-responsive neurons retained their thermoresponsiveness to two trials of scrotal thermal stimulation when colonic temperatures were maintained at 37°C. This study was designed to determine if VMH thermoresponsive or temperature non-responsive neurons initially recorded when colonic temperatures were maintained at 37°C still retained their same thermoresponsiveness to a second or third trial of scrotal thermal stimulation when colonic temperatures was servo-controlled at 35°C and 33°C.

In this study, a total of 65 VMH neurons were isolated from 48 urethane-anaesthetized, male rats. Thermoresponsiveness to the first trial of scrotal warming (40°C hot water) and cooling (ice pack) of 65 VMH neurons were determined as colonic temperatures were kept at 37°C. Of these 65 VMH neurons, 22 VMH neurons were classified as WRNs, 13 were CRNs and 30 were TNRNs. 19 out of 22 WRNs, 11 of 13 CRNs and 21 out of 30 TNRNs were recorded long enough to test their thermoresponsiveness to a second trial of scrotal thermal stimulation with colonic
temperature maintained at 35°C.

Figure-10 shows changes in the firing rate (impulses/sec) of three individual VMH neurons classified as (A) WRN, (B) CRN and (C) TNRN of three male, urethane-anesthetized rats when colonic temperatures were kept at 37°C for the first trial, then at 35°C for the second trial of scrotal thermal stimulation. Figure-10A shows a VMH WRN fired at about 2 impulses/sec (solid circles) when scrotal temperature was maintained at an initial control temperature of 20°C. This neuron steadily increased its firing rate to 13 impulses/sec as scrotal temperature was elevated to about 40°C. A thermal coefficient of 0.84 impulses.s⁻¹.°C⁻¹ for this VMH WRN was determined from the first trial of scrotal thermal stimulation. With the scrotal temperature returned to the initial control temperature of 20°C, the firing rate of this VMH WRN returned to about 4 impulses/sec (open circles). With the second trial of scrotal warming with colonic temperatures now kept at 35°C, this VMH WRN again increased its firing rate (open circles). The thermal coefficient for the second trial of scrotal thermal stimulation was 0.86 impulses.s⁻¹.°C⁻¹.

Similarly, VMH CRNs also retained their thermoresponsiveness to two trials of scrotal thermal stimulation as colonic temperatures were initially kept at 37°C for the first trial, then at 35°C for second trial of scrotal thermal stimulation, as shown in Figure-10B. The firing rate of this VMH neuron was about 2 impulses/sec (solid circles) when the scrotal temperature was initially at 20°C. This CRN neuron steadily increased its firing rate to 12 impulses/sec as scrotal temperature decreased to 10°C. A thermal coefficient of -0.81 impulses.s⁻¹.°C⁻¹ for this VMH CRN was determined for the first trial of scrotal thermal stimulation. When scrotal temperature was returned to the initial control temperature, the firing rate of this VMH CRN also returned to about ~2 impulses/sec. With the second trial of scrotal thermal stimulation with colonic temperatures now kept at 35°C, this VMH CRN again increased its firing rate (open circles) to scrotal cooling. The thermal coefficient of -0.80 impulses.s⁻¹.°C⁻¹ was calculated and indicated that this VMH CRN retained its thermoresponsiveness to both
Changes in neuronal activity (impulses/sec) of three individual VMH neurons classified as (A) WRN, (B) CRN and (C) TNRN are shown of three male, urethane-anaesthetized rats with colonic temperature maintained initially at 37°C, then at 35°C during the two trials of scrotal heating (40°C hot water) and cooling (ice pack).

Thermal coefficients calculated for these three VMH neurons after 1st and 2nd trials of scrotal thermal stimulation were:

\[ TC_{1st} = 0.84 \text{ impulses.s}^{-1}.\text{oC}^{-1} \text{ and } TC_{2nd} = 0.86 \text{ impulses.s}^{-1}.\text{oC}^{-1} \] for VMH WRN(A)

\[ TC_{1st} = -0.81 \text{ impulses.s}^{-1}.\text{oC}^{-1} \text{ and } TC_{2nd} = -0.80 \text{ impulses.s}^{-1}.\text{oC}^{-1} \] for VMH CRN(B), and

\[ TC_{1st} = 0.02 \text{ impulses.s}^{-1}.\text{oC}^{-1} \text{ and } TC_{2nd} = 0.02 \text{ impulses.s}^{-1}.\text{oC}^{-1} \] for VMH TNRN(C).
Comparison of mean thermal coefficients of all VMH WRNs (A, n=19), CRNs (B, n=11) and TNRNs (C, n=21) following two trials of scrotal heating (hot pack) and cooling (ice pack) are shown of male, urethane-anaesthetized rats with colonic temperature maintained initially at 37°C for the first trial, then at 35°C for the second trial of thermal stimulation. One-way ANOVA did not show significant changes in thermoresponsiveness of these VMH thermoresponsive and temperature non-responsive neurons between trials of scrotal thermal stimulation.
Mean TCs of VMH WRNs, CRNs and TNRNs after 2 trials of thermal stimulation with Tcolonic kept at 37°C and 35°C
trials of scrotal thermal stimulation. VMH TNRNs did not respond to either trial of scrotal thermal stimulation. Figure-10C shows from an individual TNRN that no change in thermoresponsiveness occurred with either of 2 trials of scrotal thermal stimulation when colonic temperature was kept at 37°C or 35°C.

Figure-11 shows the mean changes in thermoresponsiveness of all 19 VMH WRNs(A), 11 CRNs(B) and 21 TNRNs(C) to two trials of scrotal thermal stimulation of urethane-anaesthetized rats with core temperatures maintained first at 37°C, then at 35°C for the two trials. There was no significant difference (p > 0.05) in the mean thermal coefficients of VMH WRNs, CRNs and TNRNs between the first and second trial of scrotal thermal stimulation (ie their thermoresponsiveness remained the same).

In continuing this protocol, 11 out of 19 WRNs, 7 out of 11 CRNs and 15 out of 21 TNRNs, were recorded long enough to test their thermoresponsiveness to a third trial of scrotal thermal stimulation with colonic temperature maintained at 33°C. Figure-12 shows the changes in firing rates (impulses/sec) of three VMH neurons classified as (A) WRN, (B) CRN and (C) TNRN of three urethane-anaesthetized rats with colonic temperatures maintained at 37°C, 35°C and 33°C respectively during the three trials of scrotal thermal stimulation. Regardless of their thermoresponsiveness as determined from the first trial of thermal stimulation with colonic temperature kept at 37°C, these VMH thermoresponsive and temperature non-responsive neurons retained their initial thermoresponsiveness over three trials of scrotal thermal stimulation with colonic temperature maintained at 37°C, 35°C and finally 33°C. The mean thermal coefficients of VMH WRNs(A), CRNs(B) and TNRNs(C) did not significantly change between the three trials of scrotal thermal stimulation (Figure-13).

Figure-14 shows changes in basal firing rate of VMH neurons of rats when colonic temperatures were lowered from 37°C to 35°C and finally 33°C. Regardless of their thermoresponsiveness, recorded VMH neurons increased their basal firing rates as colonic temperatures decreased from 37° to 33°C (Figure-14A). Further analysis
Changes in neuronal activity (impulses/sec) of three individual VMH neurons classified as (A) WRN, (B) CRN and (C) TNRN of three male, urethane-anaesthetized rats with colonic temperature maintained initially at 37°C, then 35°C and finally 33°C are shown during three trials of scrotal heating and cooling.

Thermal coefficients calculated for these three VMH neurons after 1st, 2nd and 3rd trials of scrotal thermal stimulation were:

\[ \text{TC}_{1\text{st}} = 0.84 \text{ impulses.s}^{-1}.\text{°C}^{-1}, \quad \text{TC}_{2\text{nd}} = 0.81 \text{ impulses.s}^{-1}.\text{°C}^{-1} \text{ and} \]
\[ \text{TC}_{3\text{rd}} = 0.83 \text{ impulses.s}^{-1}.\text{°C}^{-1} \text{ for VMH WRN(A)} \]

\[ \text{TC}_{1\text{st}} = -0.83 \text{ impulses.s}^{-1}.\text{°C}^{-1}, \quad \text{TC}_{2\text{nd}} = -0.84 \text{ impulses.s}^{-1}.\text{°C}^{-1} \text{ and} \]
\[ \text{TC}_{3\text{rd}} = -0.86 \text{ impulses.s}^{-1}.\text{°C}^{-1} \text{ for VMH CRN(B), and} \]

\[ \text{TC}_{1\text{st}} = 0.03 \text{ impulses.s}^{-1}.\text{°C}^{-1}, \quad \text{TC}_{2\text{nd}} = 0.02 \text{ impulses.s}^{-1}.\text{°C}^{-1} \text{ and} \]
\[ \text{TC}_{3\text{rd}} = 0.02 \text{ impulses.s}^{-1}.\text{°C}^{-1} \text{ for VMH TNRN(C)}. \]
Comparison of mean thermal coefficients of all VMH WRNs (A, n = 11), CRNs (B, n = 7) and TNRNs (C, n = 15) following three trials of scrotal heating (hot pack) and cooling (ice pack) are shown of male, urethane-anaesthetized rats with colonic temperatures maintained initially at 37°C for the first trial, 35°C for the second trial and 33°C for the third trial of thermal stimulation. One-way ANOVA did not show significant changes in thermoresponsiveness of these VMH thermoresponsive and temperature non-responsive neurons to the 3 trials of scrotal thermal stimulation.
Mean TCs of VMH WRNs, CRNs and TNRNs after 3 trials of thermal stimulation with Tcolonic kept at 37°C, 35°C and 33°C
Graph shows changes in the mean basal firing rates (impulses/sec) of (A) all recorded VMH neurons, (B) VMH WRNs, (C) VMH CRNs and (D) VMH TNRNs for the three trials of scrotal thermal stimulation when colonic temperatures were maintained at 37°C, 35°C and 33°C, respectively. Firing rates were determined just prior to start of each trial of scrotal thermal stimulation. There was a tendency for each classification of VMH neurons to increase their basal firing rates as colonic temperatures were lowered, but these values were not statistically significant.
Comparison of basal firing rates of VMH neurons prior to the 3 trials of scrotal thermal stimulation of rats with colonic temperatures maintained at 37°C, 35°C and 33°C
indicated that all VMH WRNs (Figure-14B), CRNs (Figure-14C) and TNRNs (Figure-14D) showed a tendency to increase basal firing rates as colonic temperature decreased to 33°C from 37°C initially though their thermoresponsiveness did not change.

3-2-3 VMH Thermoresponsive Neurons Specifically Respond to Scrotal Thermal Stimulation

Data from first two studies indicated that thermal afferent signals caused by scrotal cooling (ice pack) and heating (same size pack containing 40°C hot water) of rats modified the neuronal activity of a population of thermoresponsive VMH neurons. However, it is not known if VMH thermoresponsive neurons responded specifically only to thermal stimulation or changed their activity as part of a general arousal phenomenon. This study was designed to determine the neuronal activity changes of VMH neurons in response to better controlled, incremental heating and cooling (within physiological limits) of a more localized area (3.0 mm²) of the scrotum of anaesthetized rats. Secondly, associated EEG activity was measured simultaneously along with VMH neuronal activity following scrotal heating and cooling, or after the induction of mechanoreceptors (scrotal brushing) or nociceptors (tail pinch) at each of the various scrotal temperatures tested to determine the potential specificity of VMH cold and warm responsive neurons.

3-2-3-1 Metabolic Changes of Urethane-anaesthetized Rats over the Duration of Recording Neuronal Activity Changes to Localized Scrotal Heating and Cooling

Cortical activity of urethane-anaesthetized rats is affected by the depth of urethane anaesthesia (Euler and Soderberg, 1957). EEG desynchronization often occurs when rats are deeply anaesthetized with urethane with doses of 2.5 g/kg (Kanosue et al, 1985). It is, therefore, important to determine the possible metabolic effects that urethane anesthesia may have on the animals over the course of the experiment. In the present study, measuring arterial blood gas changes (pO₂, pCO₂ and pH) at the start and end of the scrotal thermal stimulation experiments performed in urethane anesthetized rats would
provide an indirect index of changes in the metabolic state of the animals over the hours of VMH neuronal recording.

In this study, the first blood sample was collected about 40 minutes after the rats were anesthetized with urethane just prior to VMH extracellular recording. However, the time when the second blood sample was taken was variable and dependent upon when the neuronal recording session was completed. In most cases, the second blood sample was collected about 3 hours after the first blood sample was taken, but occasionally the second sample was collected as long as 6 hours after the first sample.

Figure-15 shows changes in femoral arterial pCO₂ (A), pO₂(B) and pH (C) of 33 urethane(1.2g/kg)-anaesthetized rats before and after the completion of VMH neuronal recording. It should be noted that although there were slight but significant changes in pO₂ and pCO₂ of femoral arterial blood before and after VMH extracellular recording, the changes in pO₂ and pCO₂ were still in the normal physiological range (Miura et al 1994). In addition, no significant differences in pH of femoral arterial blood were seen before and after VMH extracellular recording and indicated that the buffering system of the blood was functioning properly to offset the slight changes in pO₂ and pCO₂ over the duration of VMH extracellular recording experiments.

3-2-3-2 Thermoresponsiveness of VMH Neurons to Localized, Incremental Scrotal Thermal Stimulation

Figure-16 shows neuronal activity changes of 5 different VMH neurons to localized, controlled, incremental scrotal cooling and heating. The first example (A), classified as a TNRN, did not change its firing rate over the 2 hour period of scrotal cooling and heating. Another VMH neuron (B), classified as a VMH biphasic WRN, decreased its firing rate below the initial baseline firing rate when scrotal temperature was 20°C as scrotal temperatures fell below 20°C and increased its firing rate above the initial baseline control firing rate (when scrotal temperature= 20°C) as scrotal temperatures increased above 30°C (i.e. firing rate changes of this WRN were bidirectional, above
Figure-15 Changes in pCO₂, pO₂ and pH of femoral arterial blood before and after VMH extracellular recording of male, urethane-anaesthetized (1.2g/kg) rats (n=33) with colonic temperature maintained at 37°C. The first blood samples were collected about 40 minutes after the rats were anaesthetized with urethane, prior to the start of VMH extracellular recording. The second blood samples were usually taken 3 hours (range = 3-6 hours) after the first blood sample (ie, at the end of VMH extracellular recording session). The paired student-t test indicated there were significant changes in arterial blood pCO₂ and pO₂ (P<0.05), but not in pH (P>0.05) between the 2 sampling times. Symbol α denotes a significant change (P<0.05).
Mean changes in arterial Pco2, Po2 and pH of urethane-anaesthetized rats prior to and at the end of VMH extracellular recording session.
Changes in neuronal activity (impulses/sec) of five individual VMH neurons classified as (A) TNRN (TC = 0.07 impulses⁻¹ °C⁻¹), (B) biphasic WRN (TC = 0.68 impulses⁻¹ °C⁻¹), (C) monophasic WRN (TC = 0.73 impulses⁻¹ °C⁻¹), (D) biphasic CRN (TC = -0.71 impulses⁻¹ °C⁻¹) and (E) monophasic CRN (TC = -0.67 impulses⁻¹ °C⁻¹), are shown during localized, incremental cooling and heating of the scrotum (bottom graph) for ≈ 2 hours of five male, anaesthetized rats with colonic temperatures maintained at 37°C.
and below the baseline control firing rate as scrotal temperatures were increased and decreased respectively. Trace (C) is an example of a VMH neuron, classified as a monophasic WRN. Its firing rate changed (increased) above the control firing rate when scrotal temperature rose above 30°C; however, this neuron did not fall below the control firing rate when scrotal temperature was cooled below 20°C. In (D) a VMH neuron, classified as a biphasic CRN, increased its firing rate above the control firing rate when scrotal temperatures were cooled below 20°C and also decreased its firing rate to zero when scrotal temperatures were elevated above 30°C. The final example (E) shows the neuronal activity changes of a VMH neuron, classified as a monophasic CRN, as its firing rate decreased below the baseline firing rate when scrotal temperatures were elevated above 30°C; however decreasing the scrotal temperatures below 20°C did not change its firing rate from that seen during the initial baseline (scrotal temperature = 20°C) control period.

A total of 125 VMH neurons were recorded in this study. Sixty-two VMH neurons (50%) were classified as TNRNs. The number of VMH biphasic and monophasic neurons were also determined. Of all the WRNs recorded (40 of 125, or 32%) of VMH neurons, 24 (60%) of these WRNs were classified as biphasic. Of all the CRNs recorded (23 of 125, or 18%), 14 (60%) of these CRNs were classified as biphasic. Forty percent of WRNs or CRNs were classified as monophasic. Histogram of number of monophasic and biphasic VMH WRNs and CRNs is shown in Figure-17.

Figure-18 shows a histogram of the number of VMH thermoresponsive WRNs or CRNs that began to increase their firing rates above the initial baseline control (scrotal temperature = 20°C) firing rates in response to an elevation or fall in scrotal temperature. Most WRNs first increased their firing rates above control firing rates when scrotal temperature ranged between 30 to 35°C, while most CRNs increased their firing rates when scrotal temperatures were between 15 to 20°C.

3-2-3-3 VMH Neuronal Responses and Associated EEG Activity Changes with
Figure-17 Histogram shows the number of biphasic and monophasic VMH WRNs and CRNs. A VMH biphasic WRN was defined as a neuron that decreased its firing rate with scrotal warming above 20°C and decreased its firing rate with scrotal cooling below 20°C (i.e. firing rate changes of this WRN were bidirectional, above and below the baseline control firing rate as scrotal temperatures were increased and decreased, respectively). A VMH monophasic WRN was classified as a VMH neuron whose firing rate increased above the control firing rate when scrotal temperature rose above 30°C or decreased its firing rate when scrotal temperature was cooled below 20°C. A biphasic VMH CRN increased its firing rate when scrotal temperatures were cooled below 20°C and also decreased its firing rate when scrotal temperatures were elevated above 30°C (firing rates were bidirectional above and below the baseline scrotal firing rates when scrotal temperatures were at 20°C, with scrotal cooling and scrotal warming, respectively). A monophasic VMH CRN increased its firing rate with scrotal cooling below 20°C or decreased its firing rate to scrotal warming above 20°C.
Figure-18  Histogram shows the number of VMH WRNs and CRNs that began to increase their firing rates above the initial baseline control firing rates (scrotal temperature at 20°C) in response to incremental elevations or falls in scrotal heating or cooling. Most WRNs changed their firing rates when scrotal temperatures were between 30 - 35°C, while most CRNs changed their firing rate when scrotal temperature was between 15 - 20°C.
Scrotal Thermal (5-45°C) or Mechanical (Brushing) or Nociceptive (Tail Pinch) Stimulation

Figure-19 shows the neuronal activity of a VMH neuron, classified as a biphasic WRN during incremental scrotal heating and cooling. This WRN neuron (TC = 0.82 impulses/sec/°C, bottom graph) increased its firing rate above baseline control firing rate when scrotal temperatures were above 30°C, which again is observed from the photographs taken from oscilloscope tracings during scrotal warming of this single neuron (labelled a-c). This biphasic WRN decreased its firing rate below the initial control firing rate when scrotal temperatures fell below 15°C. The upper left graph also shows that neuronal activity (firing rate) of this WRN transiently increased with each tail pinch regardless of the scrotal temperature, but scrotal brushing had no effect on firing rate.

Figure-20 (A-C) shows the simultaneous EEG activity of the same animal whose VMH neuronal activity is shown in Figure-19. EEG activity did not change when scrotal temperatures were maintained at 20°C, 10°C or 30°C, (i.e. scrotal temperatures at which VMH neuronal activity was previously shown in Figure-19 to be changed). Mechanical stimuli (scrotal brushing) when scrotal temperatures were 20, 10 or 30°C did not evoke significant EEG changes from those seen before brushing occurred; however, tail pinching caused significant EEG changes (frequency increased whereas amplitude decreased) from respective EEG patterns seen before tail pinching was performed when scrotal temperatures were 20, 10 or 30°C. EEG desynchronization (EEG frequency ~doubled, and amplitude decreased) also occurred in this animal when scrotal temperatures were elevated to 45°C (Figure-20 D), but VMH neuronal activity (cf Figure-19) did not abruptly change even with this high scrotal temperature.

The VMH neuronal activity of a biphasic CRN (TC = -0.62 impulses/sec/°C, bottom graph) is shown in Figure-21 as its firing rate increased above the initial baseline firing rate as scrotal temperatures fell below 15°C. The increase in firing rate of this CRN with scrotal cooling is also seen in the oscilloscope tracings of this neuron (labelled a-c). Again, each tail pinch but not scrotal brushing, regardless of scrotal temperature at which
Figure-19 Neuronal activity (impulses/sec) of a VMH WRN is shown in response to localized incremental scrotal heating and cooling or after scrotal brushing (B) or tail pinch (P) of a male, urethane-anaesthetized rat maintained at 37°C colonically. (a,b,c,) show photographs of oscilloscope tracings of this WRN during changes in scrotal temperature. Bottom graph shows the firing rate of this neuron with changes in scrotal temperature over 10°C range for determination of its thermal coefficient (TC=0.82 impulses^-1.C^-1). Calibration bar: 0.1 mV, 0.1s.
Figure-20  Associated electroencephalographic (EEG) activity changes (raw, middle graphs and 1000 msec digitized traces of each) are shown for the same animal whose VMH neuronal activity is shown in Figure-19 when scrotal temperatures were (A) 20°C (B) 10°C (C) 30°C (lines) or after scrotal brushing (plus signs) or tail pinching (solid squares) at each of those scrotal temperatures. In Graph D, EEG changes are shown when scrotal temperatures were at 45°C (solid triangles) and compared to EEG changes when scrotal temperature was 20°C (line, same data as from Graph A). Calibration bar: 30μV, 1 min. P = tail pinch; B = scrotal brushing.
Figure-21 Neuronal activity (impulses/sec) of a VMH CRN neuron is shown in response to localized incremental scrotal heating and cooling and after scrotal brushing (B) or tail pinching (P) of a male, urethane-anaesthetized rat maintained at 37°C. (a,b,c,) show photograph tracings of single cell recordings taken of this CRN during changes in scrotal temperature. Bottom graph shows the firing rate of this neuron with changes in scrotal temperature over 10 °C range for determination of its thermal coefficient (TC = -0.62 impulses⁻¹.°C⁻¹). Calibration bar: 0.1 mV, 0.1 s.
Associated electroencephalographic (EEG) activity changes (raw, middle graphs and 1000 msec digitized traces of each) are shown for the same animal whose VMH neuronal activity is shown in Figure-21 when scrotal temperatures were (A) 20°C (B) 10°C (C) 30°C (lines) or after scrotal brushing (plus signs) or tail pinching (solid squares) at each of those scrotal temperatures. In Graph D, EEG changes are shown when scrotal temperatures were at 45°C (solid triangles) and compared to EEG changes when scrotal temperature was 20°C (line, same data as from Graph A). Calibration bar: 30 μV, 1 min. P = tail pinch; B = scrotal brushing.
these were applied, transiently increased neuronal activity of this neuron. Figure-22 (A-C) shows that EEG patterns (frequency and amplitude) of this animal did not change when scrotal temperatures were held at 20°C, 10°C or 30°C or when scrotal brushing was tested. EEG desynchronization (↑ EEG frequency and ↓ amplitude) did occur with tail pinching (Graphs A-C) or when the scrotal temperature was elevated to 45°C (Graph D).

Figure-23 shows the neuronal activity of a VMH neuron, classified as a TNRN, (TC = 0.09 impulses/sec/°C) which did not change its firing rate as scrotal temperatures were changed over the 2 hour period. These insignificant changes in firing rate during scrotal thermal stimulation are also evident from the oscilloscope tracings of this neuron (labelled a,b). Each tail pinch episode increased the firing rate transiently of this VMH TNRN, as it did with the WRN and CRN in Figures-19 and Figure-21, respectively. Scrotal brushing caused no changes in neuronal activity to this TNRN. Figure-24 indicates that EEG patterns for this animal whose VMH neuronal activity is shown in Figure-23 did not change when scrotal temperatures were 20°, 10° or 30°C (A-C) or when scrotal brushing was tested at those same scrotal temperatures. Again EEG frequency increased (doubled), amplitude decreased with tail pinching when scrotal temperatures were maintained at 20, 10 or 30°C and when scrotal temperature was elevated to 45 °C.

Figure-25 shows the mean changes in EEG amplitude and frequency (±S.E.M.) of all animals of this study following scrotal thermal stimulation alone (5-45°C), or when scrotal brushing (mechanical stimuli) or tail pinching (noxious stimuli) were tested at each scrotal temperature. Data from all animals were pooled together as no statistical differences in the mean EEG amplitude or frequency changes occurred among groups of rats whose VMH neurons were classified as warm or cold thermoresponsive or temperature non-responsive when their associated EEG changes to the same applied scrotal temperature, mechanical stimuli or noxious tail stimuli were compared. The graph shows that scrotal brushing did not significantly change the EEG pattern (either
Figure-23  Neuronal activity (impulses/sec) of a TNRN neuron is shown in response to localized incremental scrotal heating and cooling and after scrotal brushing(B) or tail pinching(P) of a male, urethane-anaesthetized rat maintained at 37°C. (a,b,c) show photographs of oscilloscope tracings of this TNRN during changes in scrotal temperature. Bottom graph shows the firing rate of this neuron with changes in scrotal temperature over 10°C range for determination of its thermal coefficient (TC = 0.09 impulses.s⁻¹.°C⁻¹). Calibration bar: 0.1 mV, 0.1 s.
Figure-24  Associated electroencephalographic (EEG) activity changes (raw, middle graphs and 1000 msec digitized traces of each) are shown for the animal whose VMH neuronal activity is shown in Figure-23 when scrotal temperatures were (A) 20°C (B) 10°C (C) 30°C (lines) or after scrotal brushing (plus signs) or tail pinching (solid squares) at each of those scrotal temperatures. In Graph D, EEG changes are shown when scrotal temperatures were at 45°C (solid triangles) and compared to EEG changes when scrotal temperature was 20°C (lines, same data as from Graph A). Calibration bar: 30 μV, 1 min. P = tail pinch; B = scrotal brushing.
Mean EEG amplitude and frequency changes (±S.E.M.) of all male, urethane-anaesthetized rats, regardless of their classifications of VMH neuronal activity, are shown when scrotal temperatures were maintained at 20°C, 10°C, 30°C, 5°C or 45°C, or after scrotal brushing (B) or tail pinching (P) at each of those scrotal temperatures. Data from all neurons were pooled as EEG changes among groups of rats whose VMH neurons were classified as WRNs, CRNs and TNRNs were not different at each scrotal temperature or with scrotal brushing or tail pinching. Symbols (α, β) denote significant changes (p < 0.05) in EEG amplitude and frequency, respectively, with tail pinching at each scrotal temperature tested (20°C, 10°C or 30°C), compared to respective EEG patterns before tail pinching was evoked at that scrotal temperature. Symbols (σ and δ) denote significant changes (p < 0.05) in EEG amplitude and frequency, respectively when scrotal temperatures were elevated to 45°C, compared to respective EEG amplitude and frequency values when scrotal temperatures were 20°C.
mean frequency or mean amplitude) from those respective EEG patterns seen before each brushing when scrotal temperatures were maintained at 20°C, 10°C or 30°C. Mean EEG frequency significantly increased and mean EEG amplitude significantly decreased with tail pinching, compared to respective EEG patterns before each tail pinching was evoked when scrotal temperatures were kept at 20°C, 10°C or 30°C. In addition, the mean EEG frequency significantly increased and mean amplitude decreased when scrotal temperatures were increased to 45°C, compared to respective mean EEG frequency and amplitude values observed when scrotal temperatures were kept at 20°C.

3-2-3-4  IBAT and Surface (Tail) Temperature Effector Responses of Rats to Localized Incremental Heating and Cooling of the Scrotum

The results indicated that localized scrotal thermal stimulation can evoke specific neuronal activity changes of VMH neurons. In order to determine if thermoeffector responses can also be activated by localized thermal stimulation to the scrotum, with applied temperatures in the physiological range, IBAT and tail temperatures were also recorded along with monitoring VMH extracellular activity.

Figure-26 shows the effects of (A) scrotal heating, then cooling or (B) scrotal cooling, then heating on the IBAT and tail temperatures of male, urethane-anaesthetized rats kept colonically at 37°C. The order in which thermal stimuli was applied to the scrotum in 10°C increments was randomly performed with each rat. In Graph A, (n=31) in which the scrotum was first warmed and later cooled, no significant changes in IBAT temperatures occurred over the 2 hour period of scrotal thermal stimulation. Changes in tail temperature revealed that tail (surface) temperatures increased ~25 min after scrotal temperatures were elevated above 20°C. As the scrotum was heated toward 45°C, tail temperatures rose above the initial tail temperature when scrotal temperatures were maintained at 20°C, with the maximum rise being 2.6°C. Tail temperatures then decreased back to prestimulation control temperatures when the scrotum was cooled. In Group B, mean change in IBAT temperatures of 25 rats was not altered with either scrotal cooling or heating when compared to respective baseline IBAT temperatures;
Figure-26  Mean changes (±S.E.M.) in IBAT (upper graphs) and tail (middle graphs) temperatures, from respective control readings at 20°C are shown following (A) controlled incremental (10°C steps), localized (3 mm²) scrotal heating first, then cooling or (B) controlled incremental, localized scrotal cooling first, then heating for 2 groups of male, urethane-anaesthetized rats while maintained colonically at 37°C. Scrotal heating or cooling did not evoke significant changes in IBAT temperatures (i.e. no BAT thermogenic activation occurred with scrotal temperature stimuli) whereas tail temperatures in both groups slowly but significantly rose or fell from respective baseline tail surface temperatures (when scrotal temperature was 20°C) in response to scrotal heating or cooling (i.e. vasoactive changes occurred).
however, surface tail temperatures decreased slowly in response to the initial period of scrotal cooling. Then, tail temperatures increased when scrotal temperatures were raised toward the initial control temperature of 20°C and increased above control tail temperatures when scrotal temperatures were above 40°C (maximum rise = 2.3°C above the initial baseline tail temperature when scrotal temperature was 20°C).

3-2-4 VMH is not Involved in the Mediation of The Evoked Tail Temperature (Vasomotor) Responses to Scrotal Thermal Stimulation

Previous results indicated that surface (tail) temperatures changed following scrotal heating and cooling, suggesting that neurally-mediated vasomotor effector responses may have been activated by scrotal thermal stimulation. This study was undertaken to determine if a functional VMH is required for the tail temperature (vasomotor effector) changes to occur following scrotal thermal stimulation.

Figure-27 shows changes in surface (tail) temperatures of rats, with colonic temperature maintained at 37°C, following (A) scrotal heating first and then cooling or (B) scrotal cooling first and then heating. In group A, after bilateral microinjection of saline (first arrow) into both VMH nuclei, tail temperatures rose to a maximum of 1 ± 0.5°C (mean ± S.E.M) above initial control temperature when scrotal temperatures were kept at 20°C following first trial of scrotal heating and fell to a maximum of -0.6 ± 0.4°C during the first trial of scrotal cooling from the initial control scrotal temperatures. Before the second trial of scrotal thermal stimulation began, 2% buffered lidocaine (200 ng/300nl, second arrow) was microinjected bilaterally into the VMH. Tail temperatures still increased to a peak of 1.0 ± 0.4 and decreased to a maximum of 0.5 ± 0.3 following scrotal heating and cooling from control tail temperatures. Similarly, In graph B (scrotal cooling first, then scrotal heating), bilateral microinjection of either saline (first arrow) or lidocaine (second arrow) into the VMH nuclei did not block the delayed tail temperature responses to the second trial of scrotal thermal stimulation (peak tail temperature changes were the same following the injection of saline or lidocaine into the VMH).
Figure-27  Effects of bilateral microinjection of lidocaine into the VMH of male, urethane-anaesthetized rats, maintained colonically at 37°C, on the changes in tail temperature ($T_{tail}$) are shown following (A) localized, incremental scrotal heating, then cooling or (B) scrotal cooling, then heating. Bilateral microinjection of saline (300nl/site denoted by 1st arrow) and 2% buffered lidocaine (200ng/300nl/site, denoted by 2nd arrow) into the VMH failed to block the tail temperature responses to scrotal cooling and heating.
3-2-5 Thermal Signals from the Scrotum to the VMH is via the Medial Preoptic Nucleus (MPO)

Anatomical studies (Conrad and Pfaff, 1976a; 1976b) and central cooling studies (Imai-Matsumura et al, 1984) indicate the presence of intrahypothalamic projections from the preoptic area to the VMH nucleus. This study was designed to determine if the thermal signals from the scrotum that selectively alter the neuronal activity of VMH thermoresponsive neurons are first relayed to the medial preoptic area before reaching the VMH.

3-2-5-1 Microinjection of Lidocaine into the Medial Preoptic Area (MPO) Blocked the Thermoresponsiveness of VMH Neurons to Localized Scrotal Thermal Stimulation

In this study, a total of 32 VMH neurons (WRN: CRN: TNRN = 6:6:20) were recorded. The thermoresponsiveness of these VMH neurons to scrotal thermal stimulation was determined following saline or 2% buffered lidocaine administration into the MPO.

Figure-28 shows the changes in neuronal activity of a VMH neuron, classified as WRN, based on a TC of 0.63 impulses.s⁻¹.°C⁻¹ during the first cycle of thermal heating and cooling of the scrotum. When lidocaine was injected into the MPO, neuronal activity was not elevated with the second cycle of scrotal warming as was seen with the first cycle of scrotal warming (TC = +0.03 impulses.s⁻¹.°C⁻¹). When the third cycle of scrotal thermal stimulation began following MPO injection of sterile saline (C), the neuronal activity increased with scrotal warming (TC = 0.58 impulses.s⁻¹.°C⁻¹) as occurred during the first control cycle of scrotal warming when saline was also injected into the MPO (A).

Figure-29 indicates that the increase in neuronal activity with scrotal cooling of a VMH neuron classified as CRN from the first control cycle of scrotal stimulation (TC = -0.52 impulses.s⁻¹.°C⁻¹) was later blocked upon the second cycle of scrotal cooling when lidocaine was previously injected into the MPO (TC = 0.04 impulses.s⁻¹.°C⁻¹).
Figure-28 Neuronal activity (impulses/sec) of a VMH WRN (top graph) of a male, urethane-anaesthetized rat with colonic temperature maintained at 37°C is shown following 3 cycles of localized scrotal heating and cooling, as indicated by changes in scrotal temperature (lower graph). Neuronal activity changes of this WRN are shown following (A) medial preoptic (MPO) injection of sterile saline (B) MPO injection of 200 ng of 2% buffered lidocaine and (C) a second MPO injection of sterile saline. Thermal coefficients (TC, impulses·s⁻¹·°C⁻¹) of the neuron are shown for the 3 cycles of scrotal heating and cooling.
$TC = +0.63 \quad TC = +0.03 \quad TC = +0.58$

$T_{scrotum} (°C)$

A  B  C

10 min
Figure-29  Neuronal activity (impulses/sec) of a VMH CRN (top graph) of a male, urethane-anaesthetized rat with colonic temperature maintained at 37°C is shown following 3 cycles of localized scrotal heating and cooling, as indicated by changes in scrotal temperature (lower graph). Neuronal activity changes of the CRN are shown following (A) MPO injection of sterile saline (B) MPO injection of 200 ng of 2% buffered lidocaine and (C) a second MPO injection of sterile saline. Thermal coefficients (TC, impulses.s⁻¹.°C⁻¹) are shown for the 3 cycles of scrotal heating and cooling.
$TC = -0.52 \quad TC = +0.04 \quad TC = -0.54$

$T_{SCROTUM}$ (°C)

A

B

C

10min

IMP/S

0

2

4

6

8

10

20

30

40

50

60

70

80

90

100
When scrotal cooling was applied during the third cycle of scrotal thermal stimulation \(\sim 60\) min later, the thermoresponsiveness of this VMH neuron again was restored (TC = -0.54 impulses.s\(^{-1}\).°C\(^{-1}\)).

Figure-30 shows the neuronal activity changes of a VMH TNRN (TC = -0.04 impulses.s\(^{-1}\).°C\(^{-1}\)) after the first cycle of scrotal thermal stimulation when sterile saline was injected into MPO (A). Lidocaine administration into the MPO (B) before the second cycle of scrotal heating and cooling did not significantly alter the neuronal activity (TC was -0.03 impulses.s\(^{-1}\).°C\(^{-1}\)) from that after the first cycle of thermal stimulation. Approximately 60 minutes later, the third cycle of scrotal thermal stimulation after sterile saline administration into MPO again caused no change in the thermal coefficient (TC = -0.05 impulses.s\(^{-1}\).°C\(^{-1}\)).

In addition to the recorded neuronal activity changes of 32 VMH neurons before and after lidocaine administration into the ipsilateral MPO, another 4 VMH (2 WRNs and 2 TNRNs) neurons had their neuronal activity changes recorded before and after saline and lidocaine were administrated into the ipsilateral lateral preoptic area (LPO). Figure-31 shows the changes in neuronal activity of a VMH WRN (TC = 0.59 impulses.s\(^{-1}\).°C\(^{-1}\)) during the first cycle of thermal heating and cooling of the scrotum. When lidocaine (B) was injected into the lateral preoptic area (LPO), not in the MPO, this VMH WRN still responded to the second cycle of scrotal warming with increases in firing rate, thus giving it a TC of 0.56 impulses.s\(^{-1}\).°C\(^{-1}\). When the third cycle of scrotal thermal stimulation began following MPO injection of sterile saline (C), the neuronal activity again increased with scrotal warming (TC = 0.57 impulses.s\(^{-1}\).°C\(^{-1}\)) as occurred with the first 2 trials of scrotal thermal stimulation when either saline or lidocaine was injected into the LPO.

Table-1 shows the mean thermal coefficients for warm, cold and temperature non-responsive VMH neurons, computed for the three cycles of scrotal heating and cooling, when sterile saline or 2% lidocaine was injected into the MPO prior to the start of a
Figure-30  Neuronal activity (impulses/sec) of a VMH TNRN (top graph) of a male, urethane-anaesthetized rat with colonic temperature maintained at 37°C is shown following 3 cycles of localized scrotal heating and cooling, as indicated by changes in scrotal temperature (lower graph). Neuronal activity changes of this TNRN are shown following (A) MPO injection of sterile saline (B) MPO injection of 200 ng of 2% buffered lidocaine and (C) a second MPO injection of sterile saline. Thermal coefficients (TC, impulses.s⁻¹.°C⁻¹) for this neuron are shown for the 3 cycles of scrotal heating and cooling.
TC = -0.04  TC = -0.03  TC = -0.05

IMP/S
0 2 4 6

TSCROTUM (°C)
0 10 20 30 40

A  B  C

10min

111
Figure-31  Neuronal activity (impulses/sec) of a VMH WRN (top graph) of a male, urethane-anaesthetized rat with colonic temperature maintained at 37°C is shown following 3 cycles of localized scrotal heating and cooling, as indicated by changes in scrotal temperature (lower graph). Neuronal activity changes of this WRN are shown following (A) lateral preoptic (LPO) injection of sterile saline (B) LPO injection of 200 ng of 2% buffered lidocaine and (C) a second LPO injection of sterile saline. Thermal coefficients (TC, impulses.s⁻¹.°C⁻¹) for this neuron are shown for the 3 cycles of scrotal heating and cooling. Microinjection of lidocaine into the LPO, unlike MPO lidocaine injection, did not block thermal response of this VMH WRN to scrotal thermal stimulation.
TABLE-1: Mean thermal coefficients (TCs) for VMH neurons to three trials of scrotal thermal stimulation.

<table>
<thead>
<tr>
<th></th>
<th>(A) 1st MPO</th>
<th>(B) MPO</th>
<th>(C) 2nd MPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>+0.65±0.03</td>
<td>+0.06±0.02&quot;</td>
<td>+0.58±0.04</td>
</tr>
<tr>
<td>Injection</td>
<td>2% buffered Lidoae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WRN (n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRN (n=6)</td>
<td>-0.62±0.03</td>
<td>+0.02±0.02&quot;</td>
<td>-0.64±0.03</td>
</tr>
<tr>
<td>TNRN (n=20)</td>
<td>+0.05±0.03</td>
<td>+0.03±0.02</td>
<td>+0.02±0.02</td>
</tr>
</tbody>
</table>

* significant change (p < 0.01) in TC after MPO lidocaine administration in that group, compared to respective TCs after 1st (control) or 2nd (recovery) saline injection into the MPO of that group.
cycle of thermal stimulation. For all VMH WRNs and CRNs, the mean thermal coefficients computed for scrotal thermal stimulation after MPO lidocaine administration were significantly decreased (p < 0.01) from respective mean thermal coefficients found after the first or third cycle of scrotal heating and cooling when sterile saline was injected into the MPO. However, the mean thermal coefficients of VMH TNRNs were not significantly changed (p > 0.05) from any of the three trials of scrotal thermal stimulation, regardless of whether saline or lidocaine had been previously injected into the MPO.

Table-2 shows the number of VMH warm- and cold-responsive neurons whose neuronal activity to scrotal thermal stimulation was significantly blocked by lidocaine administration into the MPO. MPO lidocaine pretreatment before scrotal thermal stimulation significantly blocked the neuronal activity of only VMH thermoresponsive neurons (either warm or cold) but not that of the VMH temperature non-responsive neurons.

Figure-32 schematically shows, following histological preparation, the medial preoptic (MPO) cannula sites (upper diagrams) and the associated ventromedial hypothalamic (VMH) extracellular recording sites (lower diagrams) from brains of anaesthetized rats whose VMH neurons were classified as (A) warm responsive (B) cold responsive or (C) temperature non-responsive neurons. The cannulae sites from the LPO are not shown in Figure-32.

3-3 Neuronal Activity Changes of VMH Neurons and Resultant Thermo-effector Responses to Scrotal Thermal Stimulation of Rats Previously Acclimatized to the Cold

All previous results were obtained from rats kept at room temperature (21° ± 1°C) and indicated that VMH neurons can specifically respond to localized scrotal thermal stimulation under those conditions. However, IBAT temperature did not change in response to scrotal thermal (cooling) stimulation, indicating that localized scrotal cooling
**TABLE 2**: Number of VMH neurons whose neuronal activity to scrotal thermal stimulation was blocked by MPO pretreatment with lidocaine.

<table>
<thead>
<tr>
<th>Type of VMH Neuron</th>
<th>Neurons Blocked by MPO Lidocaine</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRN (n=6)</td>
<td></td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>CRN (n=6)</td>
<td></td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>TNRN (n=20)</td>
<td></td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>
Medial preoptic (MPO) cannula sites (upper diagrams) and associated recording sites within the VMH (lower diagrams) are shown schematically following histological verifications of brains of male, urethane-anaesthetized rats whose VMH neurons were classified as (A) WRNs (B) CRNs and (C) TNRNs. The cannula sites for LPO injection are not shown in the upper diagram. Numbers in the middle indicate the distance(mm) posterior to bregma for each section. Dots represent the centre of dye injected at the end of each experiments.
was not able to evoke IBAT thermogenesis.

However, it is well established that when the animals are chronically exposed to cold, a series of physiological thermoregulatory adaptations will occur in order for the organism to cope with the new environment. Physiological thermoregulatory changes include increases in basal metabolic rate, increased capacity of BAT thermogenesis and a lower shivering threshold (Bruck et al., 1970). Experimentally, rats were housed individually in an artificial environmental chamber of 4°C for at least 4 weeks, so that they would acclimatize to the cold. The present study was designed to determine (1) if cold acclimatized rats would activate BAT thermogenesis upon localized scrotal cooling when colonic temperatures are maintained at 37°C as before (2) if so, what would be the associated changes, if any, in the thermoresponsiveness of VMH neurons to localized scrotal heating and cooling and (3) how would the thermoresponsiveness and ratio of thermoresponsive VMH neurons to localized scrotal thermal stimulation of the cold acclimatized rats compare to that previously seen in room acclimatized (21°C) rats.

3-3-1 IBAT Thermogenesis of Cold Acclimatized(CA) Rats is Activated by Localized Scrotal Cooling

In this study, 10 male rats were kept in an artificial environmental chamber of 4°C for at least 4 weeks prior to testing. Another 12 rats were kept at room temperature of 21°C for 4 weeks before experiments began. Figure-33 shows mean changes in IBAT temperatures of cold-acclimatized rats (CA) and room temperature-acclimatized rats (RA) during localized scrotal cooling at 10°C for about 2 hours with colonic temperatures maintained at 37°C (B). IBAT temperatures (A, closed squares) of RA-rats did not significantly change throughout the entire scrotal cooling period, compared to respective control pre-cooling IBAT temperature when scrotal temperatures were at 20°C. However, IBAT temperatures (A, closed circles) of CA-rats gradually yet significantly increased as scrotal temperature dropped to 10°C from the initial control temperature of 20°C. IBAT temperatures had a peak rise of 0.31 ± 0.02°C (mean ± S.E.M.) about 30 minutes after scrotal temperature was lowered. IBAT temperatures were sustained about
Figure-33  Mean changes in IBAT temperatures (A) (± S.E.M.) are shown for 2 groups of male, urethane-anaesthetized rats, maintained colonically at 37 °C during localized scrotal cooling (B) at 10 °C for 2 hours. Cold-acclimatized (CA) rats (n=10, closed circles) were previously exposed to 4°C for 4 weeks before experiments began and room temperature acclimatized (RA) rats (n=12, closed squares) were kept at 21 ± 1°C for 4 weeks prior to testing. Scrotal cooling did evoke a significant increase (P<0.01) in IBAT temperatures in CA-rats, but not in RA-rats, from respective pre-scrotal cooling (control) IBAT temperatures.
0.3°C above control IBAT temperatures while scrotal temperature was maintained at 10°C over the 2 hour period.

3-3-2 Comparison of IBAT temperature changes and Thermoresponsiveness of VMH Neurons to Localized, Incremental Thermal Stimulation of the Scrotum of RA- and CA-rats

The results of the previous section indicated that a localized scrotal cooling over 2 hours evoked a significant increase in IBAT thermogenesis of cold-acclimatized rats, a response not seen in room-temperature acclimatized rats. The next study was designed to determine changes in thermoresponsiveness of VMH neurons of 21°C (n=55) and 4°C (n=45) acclimatized rats to scrotal thermal stimulation along with changes in evoked IBAT and vasomotor thermoeffector responses.

3-3-2-1 Transient Increase in IBAT Temperatures to Localized, Incremental Scrotal Cooling of CA Rats

Figure-34 shows mean changes in IBAT and tail temperatures of urethane-anaesthetized, cold acclimatized (CA) rats with colonic temperatures maintained at 37°C during a trial of scrotal heating first, then cooling(A, n=20) or scrotal cooling first, then heating(B, n=25). The order in which thermal stimuli was applied to the scrotum in 10°C increments was randomly performed in this CA group. In Graph A, IBAT temperatures did not change as scrotal temperatures were elevated from 20°C to 45°C. During the cycle of scrotal cooling, IBAT temperatures started to increase as scrotal temperature was attenuated to 10°C. IBAT temperatures rose to a peak of 0.21°C ± 0.04 above the initial IBAT control temperature as scrotal temperatures decreased to 5°C. IBAT temperatures returned to their initial control values during scrotal heating. In this group tail surface temperatures started to increase ~20 min after scrotal temperatures were elevated above the initial control temperature of 20°C. As the scrotum was heated toward 45°C, tail surface temperatures rose to a maximum of 2.8°C above baseline control values. Tail temperatures then decreased back to prestimulation control temperatures when the scrotum was cooled. In Group B (scrotal cooling first,
Mean changes (Mean ± S.E.M.) in IBAT (upper graphs) and surface (tail) (middle graphs) temperatures are shown of cold-acclimatized, urethane anaesthetized rats (previously kept at 4°C for 4 weeks before testing) that underwent the following: (A) localized, incremental scrotal heating first, then cooling (n=20) or (B) scrotal cooling, then heating (n=25). Rats were maintained colonically at 37°C during scrotal thermal stimulation. Incremental, localized scrotal cooling evoked transient, yet significant increases in IBAT temperature whereas tail temperatures in both groups slowly rose or fell significantly following each phase of scrotal heating and cooling.
then heating), IBAT temperatures again transiently but significantly increased (0.20°C ± 0.03) in response to localized scrotal cooling, compared to respective baseline IBAT temperatures. Tail temperatures decreased slowly to a peak of 1°C ± 0.5 in response to the initial period of scrotal cooling. Then, tail temperatures increased in response to scrotal temperatures being increased. Tail temperatures increased to a peak of 1.9 ± 0.5 °C above the initial baseline tail temperatures when scrotal temperatures reached 40°C.

3-3-2-2 Thermoresponsiveness of VMH Neurons In CA- and RA-rats to Localized Scrotal Thermal Stimulation

As shown in the previous sections, prolonged (over 2 hours, Figure-33) or transient localized incremental scrotal cooling (Figure-34) evoked increases in IBAT temperature in this group, indicating that IBAT thermogenic activation had occurred in CA-rats, a response not seen with the same scrotal cooling in room temperature acclimatized(RA) rats. It was deemed important to determine if the thermoresponsiveness of VMH neurons was also modified in the CA-rats during scrotal thermal stimulation.

Figure-35 shows changes in firing rate (impulses/sec) of six VMH neurons following scrotal thermal stimulation from three room temperature (21°C, RA) and three cold (4°C, CA) acclimatized rats. VMH neurons in both C panels were both classified as TNRNs, as neither responded to scrotal thermal stimulation. Thermal coefficients for these two TNRNs from the RA- and CA-groups were 0.03 and 0.04 impulses.s⁻¹.°C⁻¹, respectively. VMH neurons in panel B of both groups were WRNs (TC=0.92 impulses.s⁻¹.°C⁻¹ for the RA-rat, 0.88 impulses.s⁻¹.°C⁻¹ for the CA-rat). The CRNs from rats of both groups are shown in the panel A. CRN recorded from a CA-rat showed a dramatic and prolonged increase in firing rate as scrotal temperature dropped to 10°C from the initial control temperature of 20°C (thermal coefficient of -1.68 impulses.s⁻¹.°C⁻¹), compared to changes in firing rate of CRN recorded from a RA-rat to the same scrotal cooling(TC=-0.86 impulses.s⁻¹.°C⁻¹).

In this study, a total of 155 VMH neurons were recorded from RA-and CA-rats,
respectively. 70 VMH neurons were recorded from the RA-rats as their thermoresponsiveness to localized scrotal thermal stimulation was tested. 27%(19/70) of these VMH neurons were classified as WRNs, 15%(11/70) CRNs and 58%(40/70) were TNRNs. In the CA-rats, 85 VMH neurons were isolated and the percentage(%) of each type of VMH WRN:CRN:TNRN was 12%(10/85):24%(21/85):63%(54/85), respectively. Changes in percentages of VMH thermoresponsive neurons for both WRNs and CRNs of both acclimatization groups were significantly different (P<0.05).

In addition, applying the same criteria used in Section 3-2-3-2, biphasic and monophasic VMH thermoresponsive neurons were also determined. The ratio of biphasic and monophasic VMH WRNs and CRNs was 13:6 and 8:3, respectively in the RA-group. In CA-group, the ratio of biphasic and monophasic VMH WRNs and CRNs was 7:3 and 14:7, respectively. The number of VMH biphasic and monophasic VMH WRNs and CRNs of both groups is shown in Figure-36. The number of biphasic and monophasic VMH WRNs and CRNs of both acclimatization groups was not significantly different.

Regardless of their thermoresponsiveness, all VMH neurons recorded from CA-group had significantly higher basal firing rates (5.20 ± 0.03 impulses/sec) prior to scrotal heating or cooling (scrotal temperature at 20°C) compared to the initial basal firing rate of all VMH neurons of RA-group (2.85 ± 0.13 impulses/sec), as shown in Figure-37A. Further analysis indicated that mean basal firing rates of VMH WRNs, CRNs and TNRNs of the CA-group were significantly higher than those of respective VMH WRNs, CRNs and TNRNs of the RA-group as shown in Figure-37B.

Changes in firing rates of all individual VMH thermoresponsive neurons of CA and RA-rats following localized scrotal thermal stimulation over a 10 °C range are shown in Figure-38. All VMH WRNs recorded from CA-rats (panel C) and RA-rats (panel D) increased their firing rate as scrotal temperature was elevated to 40°C from the initial control scrotal temperature of 20°C. There was no significant difference in
thermoreponsiveness of VMH WRNs to scrotal thermal stimulation between the two acclimation groups. However, compared to changes in firing rates of VMH CRNs of the RA-group (panel B), CRNs recorded from the CA-group (panel A) significantly increased their firing rates as scrotal temperature was decreased to 10°C from its initial control temperature of 20°C (ie, CRNs of CA-rats showed an increased thermoreponsiveness to scrotal cooling). Thermal coefficients of VMH WRNs, CRNs and TNRNs of RA- and CA-group were compared in Figure-39. This Figure indicates that CRNs of CA-rats increased their thermoreponsiveness to scrotal cooling as thermal coefficients were significantly increased from that of CRNs of the RA-group.
In the left panel, changes in neuronal activity (impulses/sec) are shown of three individual VMH neurons of RA-rats classified as (A) CRN (TC= -0.86 impulses$s^{-1}$.°C$^{-1}$), (B) WRN (TC=0.92 impulses$s^{-1}$.°C$^{-1}$) and (C) TNRN (TC=0.03 impulses$s^{-1}$.°C$^{-1}$) to localized, controlled incremental cooling and heating of the scrotum (bottom graph) of three male, anaesthetized rats with colonic temperatures maintained at 37°C during testing.

In the right panel, changes in neuronal activity (impulses/sec) are shown of three individual VMH neurons of CA-rats classified as (A) CRN (TC= -1.68 impulses$s^{-1}$.°C$^{-1}$), (B) WRN (TC=0.88 impulses$s^{-1}$.°C$^{-1}$) and (C) TNRN (TC=0.04 impulses$s^{-1}$.°C$^{-1}$) to localized, controlled incremental cooling and heating of the scrotum (bottom graph) of three male, anaesthetized rats with colonic temperatures maintained at 37°C during testing.
Figure-36  Histogram shows the number of biphasic and monophasic VMH WRNs and CRNs recorded from RA-group (21°C for 4 weeks, top graph) and CA-group (4°C for 4 weeks, bottom graph). Changes in ratio of biphasic and monophasic VMH WRNs and CRNs of both groups were not significant.
Figure-37  Graph (A) shows comparison of the basal firing rates (impulses/sec) of all VMH neurons, regardless of their thermoresponsiveness of RA and CA-groups, during the initial control scrotal temperature of 20°C prior to scrotal heating and/or cooling. There was a significant difference in mean basal firing rates between the RA- and CA-groups ($P<0.05$). Graph (B) shows that mean basal firing rates of VMH WRNs, CRNs and TNRNs of CA group were significant higher than those of respective VMH WRNs, CRNs and TNRNs of RA-group, when scrotal temperatures were initially controlled at (20°C). Symbols $\alpha$ denote significant changes.
Comparison of basal firing rates of VMH neurons between RA- and CA-groups

A

B

<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRN</td>
<td>2.5 ± 0.5</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>CRN</td>
<td>3.0 ± 0.5</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>TNRN</td>
<td>3.0 ± 0.5</td>
<td>4.0 ± 0.5</td>
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α indicates significant difference.
Changes in firing rates of all VMH warm and cold responsive neurons are shown of cold acclimatized (CA) or room temperature acclimatized (RA) rats to localized scrotal thermal stimulation. Changes in frequencies (impulses/sec) of all VMH thermoresponsive neurons are plotted when scrotal temperatures were changed from the initial baseline (control) of 20°C to 10°C for VMH CRNs recorded from CA-rats (A) and RA-rats (B) and when scrotal temperatures were changed from 30°C to 40 °C for VMH WRNs recorded from CA-rats(C) and RA-rats(D). Thermal coefficient (slope) of each VMH thermoresponsive neuron is shown as a continuous line.
Figure-39  Comparison of mean thermal coefficients (TC) of VMH WRNs, CRNs and TNRNs of RA and CA-rats is shown. There was a significant change in mean thermal coefficient of CRNs between RA- and CA-rats ($P < 0.05$). Symbol $\alpha$ denotes significant changes.
Comparison of TCs of VMH WRNs, CRNs and TNRNs between CA- and RA-groups

A

B

C

THERMAL COEFFICIENTS (TC)

CA

RA
4 DISCUSSION

4-1 The Usefulness of Thermal Stimulation of the Scrotum as a Model to Test the Thermoresponsiveness of VMH Neurons

In determining how central processing of thermal information arising from the periphery occurs, various skin regions, such as the tongue of cats (Schräfer, 1987) and rats (Schingnitz and Werner, 1980a), facial skin of cats (Dostrovsky and Hellon, 1978), and the trunk and legs of rats (Martin and Manning, 1971) and cats (Schingnitz and Werner, 1980b) as well as the scrotum of rats (Hellon and Misra, 1973a; 1973b) have been used as sites of thermal activation. Among these areas, thermal stimulation of the scrotum of rats and other rodents has been extensively employed to investigate the characteristics of its thermoreceptors and the properties of thermoresponsive CNS neurons as they modulate their activity to scrotal thermal stimulation.

The scrotum of rats (and other rodents) provides a good model site to study the effects of peripheral heating and cooling on the activity of thermoresponsive neurons within the central nervous system. To produce viable sperm, an optimal temperature is necessary in the scrotum for its normal reproductive capacity to be maintained. Therefore, the scrotum is particularly sensitive to changes in ambient temperature. The importance of maintaining an optimal scrotal temperature is shown by the large number of neurons in the CNS that respond to changes in scrotal temperature. For example, Schingnitz and Werner (1980a) reported that only 20% of thalamic neurons responded to thermal stimulation of the hindlimbs of rats and only 10% of thalamic neurons responded to thermal stimulation of the tongue. However, 66% of thalamic neurons responded to scrotal thermal stimulation. A similar percentage of thermoresponsive
neurons has also been demonstrated in thalamic, dorsal horn, and hypothalamic neurons when the scrotum of rats was thermally stimulated (Hellon and Misra, 1973b; Jahns, 1975, Nakayama et al, 1979). In this thesis, ~ 50% of VMH neurons responded to scrotal thermal stimulation induced by either hot water or ice packs in the initial study or by more localized incremental heating and cooling (between 5-45°C) via thermode in the latter study. These findings again indicate that the activation of scrotal thermoreceptors modulate the neuronal activity of many thomoresponsive neurons in the CNS.

Because the scrotum of rats is sensitive to changes in ambient temperature, a small area of scrotal skin only needs to be thermally stimulated to modulate the activity of thomoresponsive neurons. Changes in scrotal temperature induced by thermodes with working areas covering a relatively large receptive field (30 x 30 mm) of the scrotum modulated neuronal activity of ~ 50% of CNS neurons (Hellon and Misra, 1973b; Nakayama et al, 1979). In the present study, 50% of recorded VMH neurons were found to respond to a more localized thermode (tip area 3mm²) as used in the latter studies and this percentage was comparable to the initial study in which 50% of VMH neurons also responded to scrotal thermal stimulation induced by hot water and ice packs (20 x 20mm area) applied to the scrotum.

The test system not only provided a means for sensory thermal induction and recording neuronal activity changes from thomoresponsive neurons in the VMH, but also was found to be effective in activating thermoeffectors (peripheral (tail) vasomotor and IBAT thermogenic responses), even with a small scrotal skin area being thermally stimulated. Ishikawa et al (1980) reported that tail temperatures of rats rose about 7°C when scrotal temperatures were elevated from 23°C to 36°C by a scrotal thermode with 30 x 15mm area. In the present study, a mean rise in tail temperature was about 2°C when scrotal temperatures were increased about 10°C by a more localized thermode (3 mm² area). The present results further indicated that prolonged and transient scrotal cooling via a localized thermode evoked IBAT thermogenic responses of cold
acclimatized rats, as IBAT temperatures rose in the CA-group with localized scrotal cooling.

Lastly, because a relatively small receptive field of the scrotum was activated by the small thermode, this lessened the chance of other non-specific modalities from activating these thermoreceptors, as discussed later.

4-2 Characteristics of VMH Neurons of Room Temperature Acclimatized Rats to Scrotal Thermal Stimulation

4-2-1 Thermal Responses of VMH Neurons to Scrotal Thermal Stimulation

Neuronal characteristics of VMH thermoresponsive neurons to scrotal thermal stimulation appear to be different from those thermoresponsive neurons recorded in the ventrobasal complex of the thalamus or the nuclei raphe magnus in the midbrain. Previous studies indicated that the majority of thermoresponsive thalamic neurons were characterized by an abrupt change in neuronal activity (ie, "switching" response) when scrotal temperature was above a threshold temperature (Hellon and Taylor, 1982; Schingnitz and Werner, 1980; 1983; Werner et al, 1986). The neuronal "switching" responses to scrotal thermal stimulation were occasionally seen in some thermoresponsive hypothalamic neurons (Kanosue et al, 1984; Nakayama et al, 1979). In the present study, a few VMH thermoresponsive neurons displayed an abrupt change in neuronal activity, resembling a switching response, when scrotal temperatures were above 30°C. However, the majority of VMH thermoresponsive neurons did not show the abrupt "switching" responses. Most VMH WRNs and CRNs did not show dynamic changes in firing rate but rather gradually increased or decreased their neuronal activity following stepwise changes (ie, increases and decreases) in scrotal temperature. Shibata et al, (1988) also reported that a majority (54%) of warm and cold responsive neurons in the sulcal prefrontal cortex of rats showed no dynamic changes in neuronal activity but rather gradual increases and decreases in firing rates following scrotal heating (32-42°C) and cooling (32-25°C).
Many VMH WRNs and CRNs were classified as biphasic responsive neurons in the present study. The bidirectional nature of firing rates of these biphasic VMH thermoresponsive neurons was similar to those thermosensitive neurons observed in the PO/AH in vitro and in vivo. The changes in VMH neuronal activity induced by changes in scrotal temperature were linear over a wide range of scrotal temperatures for the VMH thermoresponsive neurons in the present study. It is not known if VMH WRN mono- and biphasic neurons or CRN mono- and biphasic neurons activate different thermoefferent mechanisms.

Previous studies reported that most supraspinal warm-responsive and cold responsive neurons are excited and inhibited only when scrotal temperatures are in the range of 30-40°C. For example, the PO/AH warm and cold responsive neurons altered their firing rate to scrotal temperature above 30°C (Nakayama et al., 1983). A similar scrotal temperature range was observed for thalamic thermoresponsive neurons (Hellon and Misra, 1973b) and prefrontal cortical neurons (Shibata et al., 1988). These results implied that neuronal activity changes of thermoresponsive neurons in these regions were due to excitation and/or suppression of scrotal warm receptors. In the present study, VMH neurons responded to scrotal temperature changes between 10-40°C. It is likely that these VMH neurons receive afferent signals from activation of both cold and warm thermoreceptors in the scrotum, as has been shown by excitation of dorsal horn neurons of the spinal cord with scrotal temperatures below 30°C.

4-2-2 Percentage of VMH Thermoresponsive and Temperature Non-responsive Neurons to Scrotal Thermal Stimulation

A total of 484 VMH neurons were recorded from room temperature (21°C) acclimatized male Sprague-Dawley rats and had their thermoresponsiveness tested. Although there was a slight variation in the percentage of VMH WRNS, CRNs and TNRNs in each individual study, the overall percentage of VMH WRNs, CRNs and TNRNs was 29% (141/484), 20% (97/484) and 51% (246/484), respectively. It should be noted that the classification of VMH neurons was based on their thermal coefficients
of +0.5 impulses.s\(^{-1}\).°C\(^{-1}\) for a VMH warm responsive neuron and -0.5 impulses.s\(^{-1}\).°C\(^{-1}\) for a VMH cold responsive neurons. The proportion of VMH warm responsive to cold responsive neurons in the room temperature acclimatized rats is comparable to the ratio of PO/AH thermosensitive neurons, as determined by changes in local hypothalamic temperature (Boulant, 1980; 1989; 1996). The present proportion of VMH warm and cold responsive neurons recorded in vivo was also comparable to previous in vitro studies that demonstrated the presence of more VMH warm responsive neurons than cold responsive neurons in rats (Imai-Matsumura et al, 1988; Morimoto et al, 1988b). The percentage of VMH thermoresponsive neurons in this study was also similar to the percentage of thermoresponsive striatal neurons (∼50%) found with scrotal thermal stimulation (Lin & Tsay, 1985). Only Imai-Matsumura et al, (1988), using an in vivo preparation, reported more cold than warm responsive neurons from the VMH nucleus of rats with preoptic heating and cooling.

4-2-3 Thermoresponsiveness of VMH Neurons was Retained Following Repeated Scrotal Thermal Stimulation

The results presented in section 3-2-2-1 showed that with colonic temperatures maintained at 37°C, the thermoresponsiveness of VMH neurons was consistent between the two trials of scrotal thermal stimulation, as thermal coefficients of VMH thermoresponsive and temperature non-responsive neurons did not change significantly.

VMH warm and cold responsive neurons also retained their thermoresponsiveness to three trials of scrotal thermal stimulation, when colonic temperature were acutely changed for each trial (from 37°C to 35°C and 33°C). Schingnitz and Werner (1983) reported that the appearance of the "switching response" of thalamic neurons induced by scrotal warming was associated with changes in rectal temperatures. They found that high scrotal temperatures were required to evoke the "switching" response of thalamic warm responsive neurons when colonic temperatures of rats were kept at 33.5°C. With colonic temperatures kept at 37.5°C, a low scrotal temperature could also evoke the switching response. That study indicated that neuronal activity of thalamic thermoresponsive
neurons can be modified by changes in thermal input from areas outside the scrotum.

The present results demonstrated that VMH neurons kept their thermoresponsiveness to scrotal thermal stimulation over time. VMH neuronal activity was recorded for more than 80 minutes with the 2 trials of scrotal thermal stimulation with colonic temperatures kept at 37°C during both trials and more than 180 minutes during the 3 trials of scrotal thermal stimulation with colonic temperatures maintained at 37°C, 35°C and 33°C, respectively. VMH neurons increased their basal firing rates when colonic temperatures were acutely lowered from 37°C to 33°C between the three trials of scrotal thermal stimulation, but insignificant changes in thermoresponsiveness of VMH neurons occurred over this same time frame.

4-2-4 Thermoresponsiveness of VMH Neurons to Scrotal Thermal Stimulation is Modality Specific

Kanosue et al (1985) reported that rats deeply anaesthetized with urethane (2.5 g/Kg, ip), the EEG did not change its pattern to scrotal warming up to 40°C or to noxious pinching. In the present study, a dose of 1.2 g/Kg of urethane was administrated i.p., which was previously found not to alter the response of single units in the frontal cortex of rats to electrical stimulation of the medial forebrain bundle (Pirch et al, 1985). Lincoln (1969) also reported that surgical anaesthesia induced by the 1.2 g/kg dose of urethane did not overly depress brain activity for 8-12 hours, as EEG patterns of anaesthetized rats resembled those found during the sleeping cycle of rats. The present study indicated that the level of anaesthesia (1.2 g/kg, i.p.) allowed EEG desynchronization to occur with tail pinching during scrotal thermal stimulation. In addition, metabolic (blood gas) changes of urethane anaesthetized rats showed that the anaesthetics did not alter arterial pH during the hours of extracellular recording of VMH neurons.

Controversy has arisen as to the specificity of the evoked changes in neuronal activity of CNS neurons (hypothalamus, striatum, brainstem) by applied thermal stimulation of
the scrotal area (Kanosue et al., 1985; Grahn and Heller, 1989a). Although the scrotum has been clearly demonstrated to possess both warm and cold thermal receptors (Hellon et al., 1975) and to be activated by temperatures (e.g. 15-40°C) physiologically relevant to the animal’s environment, often supraspinal neurons show "switching" responses at some threshold temperature at the same time as EEG desynchronization occurs, or, as occurs in some cases, with scrotal mechanoreceptor activation by tactile stimulation. These latter findings suggest scrotal thermal stimulation evokes non-specific (polymodal) induced changes in supraspinal neuronal activity, as reported for thalamic and rostral ventromedial medullary neurons (Grahn and Heller, 1989b; Kanosue et al., 1985).

Recording potential changes in EEG activity in humans or animals (indicative of a general arousal response) in association with any type of evoked noxious vs innocuous stimuli has been extensively employed to determine the specificity or selectivity of the evoked response. Abrupt neuronal activity changes were reported in rostral ventromedial medullary (RVM) neurons of lightly anaesthetized rats to whole body thermal stimulation and were associated with concomitant changes in EEG activity (EEG desynchronization). Thermoreponsive RVM neurons increased their firing rates dramatically to thermal stimulation but always following a transition in EEG activity from a low-frequency, high-amplitude pattern to a high-frequency, low-amplitude EEG pattern, (i.e. EEG desynchronization) (Grahn and Heller, 1989a). Similar EEG correlative results were also observed in thermoreceptive neurons from the subcoeruleus area of the pontine reticular formation of rats (Grahn et al, 1989b). The switching responses of thalamic and PO/AH neurons induced by thermal stimulation (31-40°C) of the rat’s scrotum have also been found to coincide with changes in EEG activity (Kanosue et al, 1985). The latter study claimed that thermal responses of temperature-responsive neurons in the ventrobasal complex (VB) of the thalamus or within the PO/AH were not specific to only thermal stimulation of the scrotum, but were also modified by noxious stimulation. The above studies all suggested that peripheral thermal stimulation does not evoke selective or specific changes in neuronal activity of CNS (thalamic, hypothalamic, medullary or pontine) thermoreceptive neurons.
In the initial study of this work (Section 3-2) using hot water and ice packs, non-specific activity changes of VMH neurons to scrotal thermal stimulation could not be ruled out because painful scrotal stimuli were not tested and EEG activity changes (eg, desynchronization, indicative of increased arousal) were not measured. However, a high proportion of VMH neurons appeared to change their neuronal activity due to specific thermoreceptor activation because the percentage of VMH WRNs and CRNs was not excessively high, compared to TNRNs, as would occur with non-specific activation. In fact, the ratio of WRNs:CRNs:TNRNs in the normothermic group was similar to that of PO/AH thermosensitive and insensitive neurons (Boulaux, 1980), and was also similar to that of later experiments in this study when a better controlled more localized thermode was used. Moreover, the majority of classified VMH WRNs and CRNs of this initial study reciprocally altered their firing rates to both warm and cold scrotal thermal stimulation (ie, biphasic activity), neuronal responses that did not occur with noxious or mechanical scrotal stimulation (e.g. a VMH WRN increased its firing frequency to scrotal warming (hot pack) and, most often, also decreased its firing rate to scrotal cooling(ice pack).

The results presented in Section 3-2-3 demonstrated that 50% of recorded VMH neurons were thermoresponsive to localized, physiological incremental changes in scrotal temperature. Of this total of VMH thermoresponsive neurons, 32% of these were classified as WRNs and 18% were classified as CRNs. VMH thermoresponsive neurons altered their neuronal activity to localized scrotal temperature changes between 10 to 40°C inferring that VMH neurons responded to physiological temperature changes sensed by cutaneous warm or cold receptors on the scrotum (Hellon et al, 1975). Moreover, these changes in firing rate of VMH WRNs and CRNs were specific to localized changes in scrotal temperature as they occurred without associated changes in EEG activity, inferring these neuronal responses to scrotal thermal stimulation were not evoked due to a general arousal response.

The present work demonstrates that changes in VMH WRNs and CRNs neuronal
activity were not associated with concomitant changes in EEG activity when scrotal temperatures were maintained between 10 and 40°C (Figure 19-24). Moreover, mechanical stimuli (brushing) of the scrotum caused no change in VMH neuronal activity. VMH neuronal activity of all WRN, CRN and TNRNs was abruptly and transiently changed (increased) by noxious stimulation (tail pinch) only. Changes in EEG activity (↑ frequency, ↓ amplitude) did occur only when scrotal temperatures reached 45°C (outside normal physiological limits) or when noxious stimuli (tail pinch) were applied to the animal. These "non-specific" VMH neuronal responses to noxious (temperature or mechanical) stimuli have also been observed in thalamic (Schingnitz and Werner, 1980a; Kanosue et al 1985) and rostral ventromedial medullary neurons (Grahn and Heller, 1989a) when painful stimuli were applied to the animal. Such painful stimuli have the capacity to abruptly change the neuronal activity of most CNS neurons (Kanosue et al, 1985) due to their massive and widespread discharge of reticular neurons. Abrupt, transient changes in neuronal activity of all VMH neurons in this study to painful stimuli (tail pinch) does not detract from the specificity shown by thermo-responsive VMH neurons to localized, physiological changes in scrotal temperature with no associated changes in EEG activity. Other work (Dostovsky and Hellon, 1978) has shown that most cold or warm-responsive neurons of the trigeminal nucleus caudalis of the cat exhibit greater responsiveness to changes in facial temperature than to mechanical stimulation within the same facial area.

Neuronal activity changes of thermo-responsive RVM neurons and associated changes in EEG activity were evoked from whole animal thermal stimuli (Grahn and Heller, 1989a). Non-specific thermo-responsive activity changes seen in thalamic and PO/AH neuron studies were induced from relatively large scrotal thermodes (900 mm²) (Kanosue et al, 1985). In this study, a much smaller thermode (3 mm²) was used to stimulate a more localized area of the scrotum with regulated, incremental changes in temperature of 5 - 10°C to evoke absolute scrotal temperatures of between 5 to 45°C.

It should be noted that different types of physiological stimuli can evoke neuronal
activity changes in the same VMH neuron (plurality response neurons). For example, VMH glucose-responsive neurons have also been found to respond to local temperature changes (Ono et al, 1982; Nakayama & Imai-Matsumura, 1984). In addition, Ono et al (1987a) classified VMH neurons recorded from conscious rats into two major groups: (1) EEG-related VMH neurons and (2) EEG-independent VMH neurons. The VMH EEG-independent neurons were proposed to contribute to long-term processing of information concerned with the regulation of the internal homeostasis including feeding, osmoregulation, and thermoregulation (Ono et al, 1987b). In this study, although VMH neurons were not tested for their responsiveness to glucose or NaCl, neuronal activity changes of VMH thermoreceptive neurons may be similar to those classified earlier as VMH EEG-independent neurons (Ono et al, 1987b).

4-3 Characteristics of VMH Neurons of Cold Acclimatized Rats to Scrotal Thermal Stimulation

Little is known about the neuronal basis of thermal adaption in mammals during long term cold exposure. It has been postulated that the shivering threshold is shifted downwards in cold adapted guinea-pigs (Bruck, 1981). The downward displacement of the shivering threshold may be associated with changes in the neuronal activity of peripheral thermoreceptors. However, during long-term thermal adaptation Hensel and Banet (1978) reported that there is no change in the characteristics (dynamic or static response) of facial cold receptors of cats acclimatized to cold at 5°C for 8 weeks, as compared to room temperature acclimatized cats. It is suggested that adaptation to long term cold exposure occurs in the central nervous system.

The present study demonstrated that cold acclimatization changed the thermoresponsiveness of VMH neurons. The results of Section 3-3 indicated that VMH CRNs of cold acclimatized rats became more responsive to scrotal cooling as these VMH CRNs showed a great change in firing rate to scrotal cooling (see Figure-35 and Figure-38), compared to respective CRNs recorded from room temperature (21°C) acclimatized rats.
Using metabolic mapping techniques, Morimoto and Murakami (1985) reported that rat VMH neurons showed a significant increase in [¹⁴C]-2-deoxyglucose incorporation during local preoptic and peripheral cooling, suggesting the activation of VMH neurons in response to central or peripheral cold stimulation. The present results also showed that VMH neurons of cold acclimatized rats, regardless of their thermoresponsiveness, increased their basal firing rates during the initial (20°C) control period before scrotal heating and cooling began.

The present results appear to be an agreement with the thermoregulatory neuronal model proposed by Hammel (1965) and Boulant (1980, 1996). In their model, it is suggested that PO/AH warm sensitive neurons are excited by peripheral warm receptors and in turn, these warm sensitive neurons synaptically inhibit the activity of PO/AH cold sensitive neurons. Alternatively, warm sensitive neurons are inhibited by activation of peripheral cold receptors and in turn, would have less synaptic inhibition on cold sensitive neurons in the PO/AH. Thus, cold sensitive neurons, on the one hand, can be directly activated by peripheral cold stimulation and, on the other hand, receive less inhibitory synaptic input from warm sensitive neurons during cold acclimatization. This model could account for the increased number of VMH CRNs vs WRNs of CA-group, as compared to the ratio in the 21°C acclimatized rats.

However, the present results disagree with findings observed in cold adapted guinea-pigs. Hinckel and Schröder-Rosenstock (1982) reported that from a total of 17 neurons recorded from the subcoeruleus area of cold adapted guinea-pigs (4°C for 5 weeks), 5 were excited by cooling of the abdomen while the remaining neurons were thermally non-responsive. In addition, CRNs from the subcoeruleus nucleus showed reduced peak activity during abdominal cooling, compared to subcoeruleus CRNs of normothermic guinea-pigs. Hinckel and Perschel (1987) also reported that only one CRN was isolated in the nuclei raphe magnus of 14 cold adapted guinea-pigs. However, 6 warm thermoresponsive raphe magnus neurons showed a significant increase in peak activity when abdominal temperatures were elevated to between 35°C and 45°C. In contrast, the
present results indicated that VMH CRNs outnumbered VMH WRNs in the cold acclimatized group, individual VMH CRNs showed a greater response to localized scrotal cooling (their thermal coefficients were more negative) whereas the thermoresponsiveness of VMH WRNs of cold acclimatized rats did not significantly differ from that of VMH WRNs of room temperature acclimatized (21°C) rats following localized scrotal heating. Interestingly, using scrotal thermal stimulation, Werner et al (1981) demonstrated that more WRNs were isolated in the thalamus and midbrain of cold acclimatized male rats, compared to that of WRNs in the thalamus and midbrain of room temperature acclimatized rats. It should be noted that the subcoerulous and raphe nucleus are thought to be two important loci involved in the regulation of shivering thermogenesis in animals (Hinckel et al, 1983), whereas the VMH is involved in nonshivering thermogenesis.

The present study has shown for the first time that VMH CRNs of cold acclimatized rats increased their thermoresponsiveness to scrotal cooling, compared to that of VMH CRNs of room temperature acclimatized rats. These results indicate that adaptive changes in VMH neuronal activity during long term cold exposure is associated with increased thermoresponsiveness of VMH CRNs. It is probably premature to link the observed increased thermoresponsiveness of VMH CRNs of cold acclimatized rats to the associated increased IBAT temperatures following scrotal cooling in that group. However, it has been suggested that activation of PO/AH cold sensitive neurons by cold exposure is associated with enhanced heat production (Hammel 1965; Boulant, 1980, 1996).

Preoptic cooling or exposure of the entire skin surface to 10°C temperature increased metabolic activity and induced the expression of c-fos in VMH neurons as well (Morimoto and Murakami, 1985, Kiyohara et al 1995). These results suggested activation of VMH neurons during cold exposure. In the present study, the increase in basal firing rates of all VMH neurons, regardless of their thermoresponsiveness, indicated that modulation of neuronal activity of VMH neurons had occurred in cold acclimatized rats. Why all recorded VMH neurons showed increased basal firing rates after long term cold exposure is unknown. However, this finding is consistent with that observed in cold
adapted (4°C) guinea pigs in which thermoresponsive neurons in the nuclei raphe magnus showed higher basal firing rates compared to those in nonthermally acclimatized or warm-adapted guinea pigs (Hinckel and Perschel, 1987). It is also noted that increased basal firing rates occurred with acute cold exposure (Figure-14) as colonic temperature decreased from 37°C to 35°C and finally 33°C during the 3 trials of scrotal cooling and heating. It is suggested that changes in basal firing rates of VMH neurons may be an important (first) step in the development of neuronal adaptation (ie, changes in thermoresponsiveness) seen in VMH CRNs of CA-rats with long-term cold exposure.

4-4 Transmission of Scrotal Thermal Signals Through the MPO to the VMH - Electrophysiological Evidence

In rats and primates scrotal thermal information originating from warm and cold receptors projects to the PO/AH thermosensitive neurons (Nakayama et al, 1979) via the anterolateral tract. Moreover, in vivo and in vitro hypothalamic studies have shown that VMH neurons respond to local PO/AH thermal stimulation (Imai-Matsumura et al, 1988; Imai-Matsumura and Nakayama 1984; Nakayama et al, 1981). Horseradish peroxidase (HRP) and autoradiographic findings (Conrad and Pfaff, 1976a; 1976b) have shown direct projections from the PO/AH, in particular the medial preoptic nucleus, to VMH neurons.

With in vivo neuronal recording (Imai-Matsumura et al. 1988; Nakayama et al 1981), VMH neurons responded to local PO/AH thermal stimulation. In addition, Nakayama and Imai-Matsumura (1984) demonstrated that VMH neurons facilitated by electrosomotic application of glucose also responded (with both facilitation and inhibition) to scrotal thermal stimulation and preoptic warming, whereas only 50% of glucose non-responsive VMH neurons responded to local or peripheral thermal stimulation. That study correlated the glucose and thermal responsiveness of VMH neurons to morphological differences between VMH neurons (Ono et al., 1982) whereby glucose-responsive neurons predominantly had multipolar dendrites whereas half of the glucose non-responsive neurons had bipolar dendrites and half had multipolar dendrites.
The results presented in the section 3-2-4 indicate that a functional MPO is essential for scrotal thermal stimulation to evoke neuronal activity changes of VMH cold and warm thermoresponsive neurons. These findings infer that the MPO is part of the thermoafferent pathway for temperature signals originating from the scrotum to reach thermoresponsive VMH neurons. Rendering the MPO dysfunctional with lidocaine attenuated or abolished thermoresponsiveness of VMH neurons to scrotal thermal stimulation. All neuronal activity of VMH WRNs and CRNs to scrotal thermal stimulation was reversibly blocked by pretreatment with lidocaine into the MPO. However, thermal coefficients of VMH temperature non-responsive neurons to scrotal thermal stimulation were not significantly changed by MPO lidocaine administration.

It is felt that the neuronal blockade by lidocaine into the MPO of VMH warm and cold thermoresponsive neurons was due to its action within the MPO nucleus and not due to diffusion onto the VMH neurons. Studies indicate that a 1.0 μl volume of lidocaine into the brain has a diffusion distance of approximately 1.0 mm from the injection tip (Albert and Madryga 1980; Connors et al., 1982; McKenna and Melzack, 1992; Mizumori et al., 1989). Since our injection volume was a third of this volume and the diffusion distance between injection and recording sites was almost doubled, it is thought these findings with lidocaine are due to inhibition of neuronal activity of MPO neurons. As well, neuronal activity of all recorded VMH neurons was not consistently suppressed by MPO lidocaine administration, as would have occurred if the injection site was too close to the VMH recording neuron. The duration of blockade of neuronal activity of thermoresponsive VMH neurons by MPO lidocaine administration of approximately 60 minutes in this study is also consistent with previous reports. Prior studies have indicated that a microinjection of lidocaine into CNS sites attains a maximum effect within 10-15 minutes and wanes in approximately 40-50 min (Albert and Madryga, 1980; Albert and Richmond, 1976; Sandkuhler and Gebhart, 1984; Sandkuhler et al, 1987; Thornhill et al, 1994).

It is suggested from this study that there are at least two important relays that thermal
afferent signals originating from physiological changes in scrotal temperature must pass to evoke specific neuronal activity changes of thermoresponsive VMH neurons. Thermal afferent signals from the scrotum ascend the spinal cord (Hensel 1981) to the brainstem, notably the nucleus raphé magnus, NRM (Brodal et al, 1960; Jahns, 1976; Taylor, 1982; Zemlan et al, 1978). There, parallel signals are sent to (a) the thalamus, and onto the somatosensory cortex and (b) to the PO/AH (Jenssen, 1990). This study infers that scrotal thermal signals projecting to the hypothalamus will first project to the MPO and then are relayed onto thermoresponsive VMH neurons. This study also indicated that the lateral preoptic area (LPO) probably is not essential for scrotal thermal information to reach the VMH. As shown in Figure-31, VMH warm responsive neurons did not lose their thermoresponsiveness to scrotal thermal stimulation after lidocaine pretreatment into the LPO.

4-5 Thermoeffector Responses to Scrotal Thermal Stimulation

4-5-1 Response of IBAT Temperatures to Scrotal Thermal Stimulation

4-5-1-1 Room Temperature Acclimatized Rats

The applied cold (ice pack) to the scrotum of room temperature acclimatized rats (Figure-7) over 30 min decreased scrotal temperatures to <20°C, but this did not evoke IBAT thermogenesis, as IBAT temperatures did not increase, but rather fell. This might be expected in the normothermic group when colonic temperatures are maintained at 37°C, yet IBAT temperatures were not increased even in the hypothermic (33-35°C) group. However, the relative drop of IBAT temperatures with scrotal cooling in the hypothermic group tended not to be as great as the decrease seen in colonic temperatures in the normothermic group, possibly suggesting some IBAT activation had occurred in that group. Similarly, more localized scrotal cooling (5 - 10°C increments) did not evoke changes in IBAT temperature when colonic temperatures were maintained at 37°C (Figure-26), even though thermoresponsive VMH neurons were activated by changes in scrotal temperature to alter their neuronal activity. These results infer that a greater cold
stimulus is required for normothermic animals to have IBAT thermogenesis activated.

Kurosawa (1991) found that cooling the ears of normothermic Wistar rats acclimatized to 21°C with 4°C cold water for 20 seconds increased IBAT temperatures. The response was considered to be due to a neurally mediated increase in sympathetic output to IBAT because it was not seen in IBAT sympathetically-denervated rats. In the present studies, however, scrotal cooling (ice pack or with localized thermode) failed to evoke IBAT thermogenesis in room temperature acclimatized (21°C) rats even though a longer period of scrotal cooling was applied. The failure to evoke IBAT thermogenesis during scrotal cooling of 21°C acclimatized Sprague-Dawley rats may be due to their reduced BAT thermogenic activity. Thornhill and Halvorson (1992) reported that room temperature acclimatized Sprague-Dawley rats, compared to hooded Long Evans rats, have a reduced thermogenic capacity to increase IBAT thermogenesis, as evoked by VMH electrical or chemical stimulation.

4-5-1-2 Cold Acclimatized Rats

In contrast to room temperature acclimatized rats, Figure-33 showed a significant increase in IBAT temperatures of cold acclimatized rats maintained at 37°C colically following prolonged localized scrotal cooling (ie, at 10°C for 2 hours). The increases in IBAT temperatures were also demonstrated (Figure-34) even when shorter periods of cooling were applied to the scrotum. These findings suggest that the thermogenic capacity of IBAT of cold acclimatized rats was enhanced with sustained cold exposure (acclimatization) (Himms-Hagen, 1990) because increased IBAT temperatures due to scrotal cooling were not seen in room temperature acclimatized (21°C) rats kept colically at 37°C or when acutely lowered to 33°C (Figure-7 and 26).

4-5-2 Changes in Surface (Tail) Temperatures to Scrotal Thermal Stimulation

Scrotal thermal stimulation has been previously shown to evoke metabolic, respiratory and vasoactive responses in animals (Hales and Hutchinson, 1971; Ingram and Legge,
1972). Figure-7 and Figure-26 indicate that, using hot water and ice packs or thermal stimulation via localized thermode, tail temperatures increased or decreased in response to heating/cooling of the scrotum, respectively.

The passive diffusion of heat or cold from the scrotal thermode to the tail region probably accounted for tail temperature responses, as the delayed changes in tail temperature temporally followed the pattern of scrotal heating and cooling. Alternatively, the localized changes in scrotal temperature may have evoked centrally mediated vasoactive effector responses. Lidocaine administration into the PO/AH selectively blocked the vasodilatory response of rats undergoing heat stress (Ishikawa et al, 1980; 1984) and suggested that the PO/AH is involved in mediating reflex changes in tail temperatures. The present study shows, however, that changes in tail temperatures to scrotal thermal stimulation were not mediated by the VMH nucleus (see Figure-27), as pretreatment of the VMH with lidocaine failed to block the vasoactive responses to localized scrotal heating and cooling.

4-6 Significance of the studies

As a central effector nucleus, the VMH is well known for its activation of BAT thermogenesis. Stimulation of the VMH nucleus causes BAT thermogenesis. The present work demonstrated that VMH neurons not only activate IBAT thermogenesis but also receive peripheral (scrotal) thermal signals and specifically respond to changes in scrotal temperature. VMH neurons have the capability to sustain their thermoresponsiveness to peripheral thermal stimulation. Thermoafferent signals from the scrotum project to the VMH via the medial preoptic area (MPO), indicating that the MPO is part of the scrotal thermoafferent pathway.

The present experiments also demonstrated that VMH neurons may be involved in the neuronal adaptation of the CNS in modulating heat production of small animals to long-term cold exposure. The VMH neurons increased their basal firing rates following cold exposure but more importantly, VMH CRNs changed their thermoresponsiveness
with continued cold exposure. As well, the number of VMH CRNs increased, relative to VMH WRNs with cold. These changes in VMH neurons with cold exposure may be part of a neuronal adaptive response necessary to allow this nucleus to increase BAT thermogenesis in rodents as a result of cold acclimatization.

Due to its sensitivity to changes in environmental temperature, using the scrotum as a site for peripheral thermoreceptor activation is a useful model to investigate the thermoresponsiveness of CNS neurons and/or the activation of various thermoeffector responses.
5 SUMMARY AND CONCLUSIONS

1 Thermal stimulation of the scrotum can evoke profound thermoeffector responses in animals and alter the neuronal activity of many thermoresponsive neurons in the CNS. The present studies were designed to explore the thermoresponsiveness of VMH neurons to peripheral (scrotal) thermal stimulation of male urethane anaesthetized Sprague-Dawley rats, acclimatized for 4 weeks to either 21°C or 4°C prior to experiments, along with associated thermoeffector responses.

2 VMH extracellular recordings were made along with interscapular brown adipose tissue (IBAT) and surface (tail) temperatures following scrotal thermal stimulation induced by a) hot water and ice packs in the initial experiments and b) controlled incremental heating and cooling (5-45°C) via localized thermode in the latter experiments. In some studies, EEG and femoral arterial pCO₂, pO₂ and pH were also monitored to determine changes in cortical activity and metabolism.

3 In room temperature acclimatized rats, ~50 % of the recorded VMH neurons responded to scrotal heating and cooling with increased or decreased firing rates. Based on their thermal coefficients, VMH neurons were classified as warm-responsive (WRNs), cold-responsive (CRNs) or temperature non-responsive neurons(TNRNs). Percentages of VMH WRNs, CRNs and TNRNs were similar to those of thermoresponsive neurons observed in other brain regions (eg, PO/AH, thalamus).

4 VMH thermoresponsive neurons specifically responded to localized physiological
changes in scrotal thermal temperature between 10-40°C, as EEG activity (i.e., EEG desynchronization) did not occur with scrotal thermal stimulation. EEG changes only occurred with tail pinching as it was applied to each scrotal temperature tested.

5 Thermoresponsiveness of VMH neurons (determined by their thermal coefficients) was sustained following repeated trials of scrotal thermal stimulation with colonic temperature kept at 37°C. Furthermore, thermoresponsiveness of these VMH neurons was still maintained to repeated trials of scrotal heating and cooling when colonic temperatures were acutely lowered from 37°C to 35°C and 33°C during those trials.

6 Scrotal cooling (ice pack) and heating (hot water pack) or by incremental changes via localized thermode did not evoke changes in IBAT temperatures of room temperature acclimatized rats, inferring that BAT activation did not occur.

7 The medial preoptic area (MPO) is an important relay centre involved in transmission of scrotal thermal signals to the VMH, as the pretreatment of lidocaine, but not saline, into the MPO blocked neuronal activity of VMH thermoresponsive neurons to localized scrotal heating and cooling, but had no effect on VMH temperature non-responsive neurons.

8 In the cold acclimatized rats, however, localized scrotal cooling (prolonged or transient) increased IBAT temperatures, indicating that activation of BAT thermogenesis had occurred in this group even with colonic temperatures maintained at 37°C during testing.

9 Mean basal firing rates of VMH neurons of room temperature acclimatized rats slightly increased as colonic temperatures were acutely lowered from 37°C to 33°C. However, mean basal firing rates of all VMH neurons of cold acclimatized rats were significantly higher than those of VMH neurons of room temperature acclimatized rats.
10 VMH CRNs showed a greater thermoresponsiveness to scrotal cooling, compared to VMH CRNs of room temperature acclimatized rats. VMH CRNs outnumbered WRNs in cold acclimatized rats, suggesting that adaptive changes in VMH neuronal activity had occurred with long term cold exposure.

11 Surface (tail) temperatures showed delayed increases or decreases following scrotal heating and cooling. The VMH nucleus was not involved in the evoked tail (vasomotor) responses to scrotal thermal stimulation since bilateral administration of lidocaine into the VMH nucleus prior to testing did not block the delayed tail temperature responses to scrotal heating and cooling.
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