THE ROLE OF IONOTROPIC GLUTAMATE RECEPTORS IN THE DORSOMEDIAL HYPOTHALAMUS IN THE INCREASE IN CORE BODY TEMPERATURE EVOKED BY INTEROCEPTIVE AND EXTEROCEPTIVE STRESSES IN RATS

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This thesis is dedicated to my parents Homero, and Maria, and to my sisters Araceli and Joyce, my husband and best friend Lee, our boys Javier, Israel and Fabian, and our growing family. Your love, confidence, and never-ending support have made this achievement possible. I will never be able to express how much I appreciate all you have done and continue to do for me.

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ABSTRACT

Maria Moreno

THE ROLE OF IONOTROPIC GLUTAMATE RECEPTORS IN THE DORSOMEDIAL HYPOTHALAMUS IN THE INCREASE IN CORE BODY TEMPERATURE EVOKED BY INTEROCEPTIVE AND EXTEROCEPTIVE STRESSES IN RATS

Brain responds to an array of diverse challenges that are defined as either exteroceptive stress, involving cognitive processing of sensory information from the external environment and or interoceptive stress, detected through sensory neural or chemical cues from the internal environment. The physiological response to most stresses consists of autonomic responses that are essential for animal survival in the face of a threatening circumstance. However, it is known that exposition to continuous situations of stress is involved in the development of a series of diseases such as hypertension, myocardial infarction and panic syndrome. Several studies have shown that cells in a specific area of the brain, the dorsomedial hypothalamus (DMH), are involved in the response produced during emotional stress. However, the role of glutamatergic transmission in the DMH in the increase in body temperature induced by experimental stress has not been examined. Research findings thus far indicate that neurons in the DMH play a role in thermoregulation and that local glutamate receptors may be involved. The hypothesis of this thesis is that ***activity at ionotropic glutamate receptors in the DMH is necessary for the thermogenic response induced by experimental stress***. In the present work, microinjections of kynurenate, an

excitatory amino acid antagonist, NBQX (2, 3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione), an AMPA/kainate receptor antagonist, DL-2-amino-5-phosphonovaleric acid (APV), an NMDA receptor antagonist, and a mixture of NBQX and APV, were delivered to the DMH before exposure to experimental stress. The stress paradigms used include models for exteroceptive stress and interoceptive stress. The results show that inhibition of both NMDA and non-NMDA receptors is necessary to abolish the thermogenic response produced by all stress paradigms tested. Furthermore, there appears to be a difference in the degree of attenuation of the thermogenic response produced by either inhibition of NMDA receptors or non-NMDA receptors. Together these results support a definite role for ionotropic glutamate receptors within DMH region in the thermogenic response to stress. These results also finally show that the DMH is involved in all the major physiological stress responses including increase in plasma ACTH, increase in heart rate, blood pressure and now temperature as well.

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ACSF	artificial cerebrospinal fluid
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
APV	2-amino-5-phosphonopentanoic acid
AS	air stress
BAT	brown adipose tissue
BMI	bicuculline methiodide
BP	blood pressure
CS	cage-switch
CNQX	6-cyano-7-nitroquinoxaline-2, 3-dione
CNS	central nervous system
DHA	dorsal hypothalamic area
DMH	dorsomedial hypothalamus
DNQX	6, 7-dinitroquinoxaline-2, 3-dione
EAA	excitatory amino acid
GABA	gamma-amino butyric acid
HPA	hypothalamic pituitary adrenal axis
IBAT	interscapular brown adipose tissue
IGLUT	ionotropic glutamate receptors
KA	kainic acid
KYN	kynurenic acid
LPS	lipopolysaccharide

MUS	muscimol
NBQX	2, 3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2, 3-dione
NCS	no cage-switch
NMDA	N-methyl-D-aspartic acid
NS	no stress
pACTH	plasma adrenocorticotropic hormone
PGE2	prostaglandin E2
PH	posterior hypothalamus
PICRO	picrotoxin
POA	preoptic area
PVN	paraventricular nucleus
RP	raphe pallidus
TNZ	thermal neutral zone
UCP1	uncoupling protein 1
VMH	ventral medial hypothalamus
XAN	xanthurenic acid

CHAPTER 1: INTRODUCTION

Temperature regulation is considered a “holistic” regulatory system because it involves the activities of all other physiological and behavioral processes (Boulant, 2000). However, to date, it is not yet clear which area(s) in the brain mediate(s) the thermogenic response to stress. Several studies suggest that cells in a specific area of the brain, the dorsomedial hypothalamus (DMH), are involved in a number of responses produced during emotional stress, including increases in heart rate and blood pressure as well as the release of adrenocorticotrophic hormone (ACTH), the neuroendocrine hallmark for stress. Furthermore, evidence suggests that glutamatergic transmission in this region may play a key role in at least some of these stress-induced changes. However, the role of glutamatergic transmission in the DMH in the increase in body temperature induced by stress has not been examined or characterized. Understanding how this increase in body temperature is mediated can provide further insight into how neuronal signaling in the brain is involved in responses to stress and/or anxiety. I suggest that neuronal activity in the DMH plays a role in the increase in body temperature seen in stress. Specifically, I ***hypothesize that neuronal activation via ionotropic glutamate receptors in the DMH is necessary for the increase in body temperature induced by experimental stress***. The purpose of this research is to test this hypothesis and characterize the role of ionotropic glutamate receptors in the DMH in the thermoregulatory response to various types of stresses. Not all stresses are alike and may be

broadly categorized as being either exteroceptive or interoceptive, both of which I examined. Exteroceptive stress involves external cues, such as noise or restraint, that evoke a stress response (Sawchenko et al., 2000). Exteroceptive stress is thought of as an ‘‘emotional’’ stress and is often species and context specific, and may be determined or modified through experience (for reviews see Refs. DiMicco et al., 2002; DiMicco et al., 2006a). Unlike exteroceptive stress, interoceptive stress, is a type of stressor detected through sensory neural or chemical cues from the internal environment; an example of such stress is a bacterial infection (Sawchenko et al., 2000).

I. Thermoregulation in the rat

Thermoregulation in mammals is a finely tuned mechanism that involves a coordinated response in various organ systems such as respiratory (increase heat evaporation through respiration, digestive (salivation as seen in rodents), cardiovascular (vasoconstriction and vasodilation), and muscular (shivering) (Gordon, 1990). A constant body temperature is maintained in humans and other mammals throughout their lifetime within a narrow range of 36.8±0.7°C under normal conditions. Any deviation from homeostasis is usually a foretelling sign of pathology in the body. This is why body temperature is one of the four major vital signs used in medical exams to assess the most basic body functions. When patients develop or have a fever, this may be a sign of serious infection or organ dysfunction in the body.

Mammals regulate body temperature using various thermoregulatory mechanisms, such as heat-producing, heat-conserving, and heat dissipating

responses that differ from species to species. With regard to thermoregulation, vertebrates can be divided into two groups: bradymetabolic vertebrates which include reptiles, fish, amphibians and others, or tachymetabolic vertebrates which include birds and mammals (IUPS Thermal Commission, 2001). Tachymetabolic animals are endothermic meaning that "their control of body temperature is dependent primarily on the generation of heat through metabolic processes", while bradymetabolic animals are ectothermic meaning that their control of body temperature is "achieved behaviorally by controlling the transfer of heat between their bodies and their environment" (IUPS Thermal Commission, 2001). Many endotherms, warm-blooded animals, are also homeothermic, maintaining a constant body temperature under most environmental circumstances. Humans are homeotherms with a body temperature maintained within a narrow range of 36.8-37.7°C (Mackowiak et al., 1992). In this respect, the laboratory rat is similar to humans because a laboratory rat is homeothermic. The Sprague Dawley rat was used in all experiments for this thesis and it has been used in various studies of thermoregulation. Surprisingly, despite the obvious differences in size, rats and humans are similar with regard to several variables including core body temperature (37°C), skin temperature (rat, ~30°C; human, 33°C), preferred ambient temperature (28°C), and upper lethal core temperature (rat, 44°C; human, 43°C) (Gordon, 1993). The rat has a large surface area-to-body mass ratio which means that it must maintain a relatively high rate of heat production to keep its core temperature at 37°C (Gordon, 1990). It is important to be aware of this physiological difference, but despite this difference, the rat has proven to be

a useful and popular model for the study of thermoregulation. A PubMed search suggests that about 65% of studies of thermoregulation use the rat, followed by studies in mice at 21% and 14% of studies that employed other rodents such as the guinea pig, hamster, or gerbil.

Temperature in homeotherms is maintained within narrow limits by a mechanism thought to be similar to a thermostat that regulates according to reference temperature known as a set-point. Set-point as described in the most recent edition of "Glossary of terms for thermal physiology" is "the value of a regulated variable which a healthy organism tends to stabilize by the processes of regulation" (IUPS Thermal Commission, 2001). This term implies that a fixed value exists, and that a set-point temperature is a threshold temperature; thus, deviation from the set-point temperature would elicit a "corrective" effector response. Romanovsky most recently suggested that the term set-point be phased out, as it is now understood and accepted that the thermoregulatory system is not a system that is unified and under control of one set-point (Romanovsky, 2007). Rather, he suggests that the thermoregulatory system is made up of thermoeffector loops that work independently of each other and even though their actions may look coordinated, these effectors can have different thresholds (Romanovsky, 2007). Thermoeffector loops as defined by Romanovsky consist of a circuit with both efferent and afferent pathways. The efferent parts of the loops differ, because each effector has its own efferent pathway. The afferent parts are also different because each effector receives a unique combination of signals from peripheral and central thermosensors

(Romanovsky, 2007). Because of its connotation, the use of the term set-point is still subject to debate, leading some researchers to adapt the term balance point. The term balance point refers to a regulated level of core body temperature. Most importantly it allows for the possibility that the thermoregulatory system operates as a collaboration of independent thermoeffector loops in order to maintain thermal balance or normal body temperature (Briese, 1998; Romanovsky, 2007). However, due to the lack of research to concretely defend the use of either set-point or balance point, set-point continues to be more customarily used.

The thermoregulatory effectors that are used to maintain a stable body temperature over a range of environmental temperatures are different in rats and humans. As mentioned earlier, a thermoregulatory system is one that integrates the functions of other systems in order to maintain a stable body temperature. In both hot and cold environments, homeotherms use both autonomic and behavioral responses to regulate body temperature. One mechanism of autonomic thermoregulation is cutaneous vasomotor tone. Dilating vessels increases heat loss via conduction. When humans are exposed to a warm environment, blood vessels of the skin dilate to allow greater convective and conductive heat loss. Sweating also increases evaporative heat loss. Behaviorally, we may seek the shade, turn on the air-conditioner or take off our clothes to allow more efficient cooling. Likewise, in the cold, blood vessels of the skin constrict to reduce heat dissipation, we generate heat through shivering, and we add layers of clothes and seek to be warm. Rodents employ analogous

autonomic and behavioral responses. However, they also have evolved several morphological and physiological adaptations that allow them to regulate body temperature more effectively than humans.

In this thesis, I examined the role of ionotropic glutamate receptors in the DMH in the increase in temperature induced by stress in the rat. The majority of studies of the thermoregulatory system have employed the rat as a model. In order to facilitate the extrapolation of results from studies in rats to humans, it is important to understand the unique characteristics of the thermoregulatory system of the rat.

A. Cutaneous vasomotor tone: Rat tail

The vasomotor tone of the rat tail is crucial to conservation or loss of body heat. During heat stress, a stress induced by exposure to ambient temperatures that exceed body temperature, the vasomotor tone of the tail permits a high rate of blood flow to allow effective heat dissipation. The tail lacks fur, and has a relatively high surface area to volume ratio, accounting for approximately 7% of the total surface area of the rat. All of the described characteristics of the tail enhance the potential for dissipation of heat (Gemmell and Hales, 1977; Lin et al., 1979; Little and Stoner, 1968). Striker and Hainsworth (1971) showed that amputation of the rat's tail reduced the rat's heat tolerance and resulted in higher body temperature after exposure to a hot environment. The vasomotor tone in the rat's tail is also extremely sensitive to ambient temperatures. The blood flow to the rat tail is near zero at standard room temperatures in the range of 20-25°C, and as the ambient temperature increases, blood flow to the rat tail increases as

well, allowing for increased dissipation of heat (for review see Gordon, 1990). In fact, the tail can dissipate up to 25% of the rat's metabolic heat at ambient temperatures ranging between 29-33°C (Young and Dawson, 1982). When the rat is exposed to cold ambient temperatures, blood flow in the tail decreases to minimize the dissipation of body heat.

In conjunction with vasoconstriction in the tail, rats recruit metabolic processes to help maintain their body temperature. When ambient temperature decreases to below 20°C, two heat-generating mechanisms are recruited, shivering and non-shivering thermogenesis.

B. Shivering and nonshivering thermogenesis in the rat

Thermogenesis falls into two types: obligative and facultative. Obligative thermogenesis involves the basal metabolic processes that are sufficient to maintain thermal homeostasis when the organism is exposed to temperatures in the thermoneutral zone (TNZ). TNZ is "the range of ambient temperature at which thermoregulation is achieved only by control of sensible heat loss, i.e., without metabolic heat production or evaporative heat loss" (IUPS Thermal Commission, 2001). Facultative thermogenesis involves metabolic responses that are required to maintain normal body temperature as ambient temperature is reduced to below TNZ and consists of shivering and nonshivering thermogenesis (Gordon, 1990). Shivering takes place in skeletal muscle, and rats like humans shiver to generate heat when exposed to cold temperatures. However, during exposure to constant cold stress, the rat can rely on the activation of brown adipose tissue, an important adaptation. Brown adipose tissue (BAT) is thought

to be the primary means by which rodents generate heat, and is responsible for nonshivering thermogenesis. Nonshivering thermogenesis is a major component of facultative thermogenesis in many mammals. In humans, BAT is seen in higher amounts during infancy and with time this tissue atrophies; active tissue is found only in restricted regions including main depots found in the supraclavicular and the neck regions with some additional paravertebral, mediastinal, para-aortic, and suprarenal localizations (Lean et al., 1986; Nedergaard et al., 2007). In rodents, this tissue becomes the primary source of heat production when the animal is exposed to a cold environment (Cannon et al., 1998; Foster and Frydman, 1978; Kuroshima, 1993). The venous and arterial circulation of BAT is positioned to facilitate heat transfer from BAT to strategic organs including the spinal cord and heart (Smith and Horwitz, 1969). BAT also serves other thermoregulatory functions in rodents including its apparent role in diet-induced thermogenesis; it provides the heat needed to elevate body temperature during a fever; and finally, it serves as a major source of heat during recovery from anesthesia-induced hypothermia, torpor or hibernation (Gordon, 1993). In the rat, BAT is found in distinct regions including the cervical, pericardial, intercostal, and perirenal sites with the highest concentration and most studied found in the interscapular region (Alexander, 1979; Gordon, 1990).

Girardier and colleagues (1983) first recorded that under stimulation by catecholamines, BAT was able to generate heat at 400W/kg which is about 80 times that of the basal metabolic rate for the rat. BAT is able to produce a vast amount of heat because of its cellular and molecular makeup. Brown fat cells

are densely packed with mitochondria, and the activity of uncoupling protein 1 (UCP1) found in the inner layer of the mitochondria is key to efficient heat production. In most tissues, mitochondria produce ATP through oxidative phosphorylation, which is driven by an electrochemical gradient across the mitochondrial inner membrane. In BAT however, UCP1 allows an alternative for proton entry so that protons bypass the ATP synthase route of entry (Argyropoulos and Harper, 2002). The transfer of protons across the mitochondrial membrane cause “uncoupling” of the electron transport chain and so a reduction in the amount of ATP produced per milliliter of oxygen consumed (Nicholls and Locke, 1984). By uncoupling oxidative phosphorylation, UCP increases the electron transport and metabolic process in an attempt to maintain an electrochemical gradient sufficient to make adequate amounts of ATP. This increases metabolic work and the energy of the electrochemical gradient is dissipated as heat (Smith and Horwitz, 1969).

C. Evaporative heat loss and other thermoregulatory effectors

Mechanisms for evaporative heat loss evolved in animals to aid in heat dissipation. As ambient temperature rises, the ability of the animal to transfer heat from the core to the environment diminishes and evaporation is a means that allows heat loss and helps maintain normal body temperature. Evaporative heat loss can occur passively through normal respiration or through convection on the skin (both contribute minimally to evaporative heat loss), or actively via sweating, panting, and application of saliva, urine and other forms of moisture to the fur and skin (Gordon, 1993).

The thermoregulatory system also employs behavior as a means for temperature regulation. For example, rodents will spread saliva over their fur and show increased grooming which allows them to dissipate their heat through evaporation (Hainsworth, 1967; Hubbard et al., 1982; Stricker and Hainsworth, 1971). Spreading saliva on their fur is thought to have an effect similar to that of sweating in humans because in both cases, exposed surfaces are covered with moisture, which during evaporation increases the level of heat loss and thereby minimizes elevations in body temperature (Gordon and Heath, 1986). A rat will increase its grooming time as the ambient temperature rises above its TNZ (Hainsworth, 1967). Rats surgically "desalivated" (salivary glands are removed) are highly susceptible to heat stress, much more so than tailless rats (rats subjected to surgical amputation of tail) (Stricker and Hainsworth, 1971). Stricker and colleagues showed an 81% reduction in heat tolerance, a measure of the time it takes for the animal to reach a core temperature of 40°C, in surgically desalivated rats versus 8% reduction in heat tolerance in rats whose tails had been amputated (Stricker and Hainsworth, 1971). A rat exposed to continuous heat stress will also show extension of the body which increases its surface area to body-mass ratio and allows for greater heat dissipation.

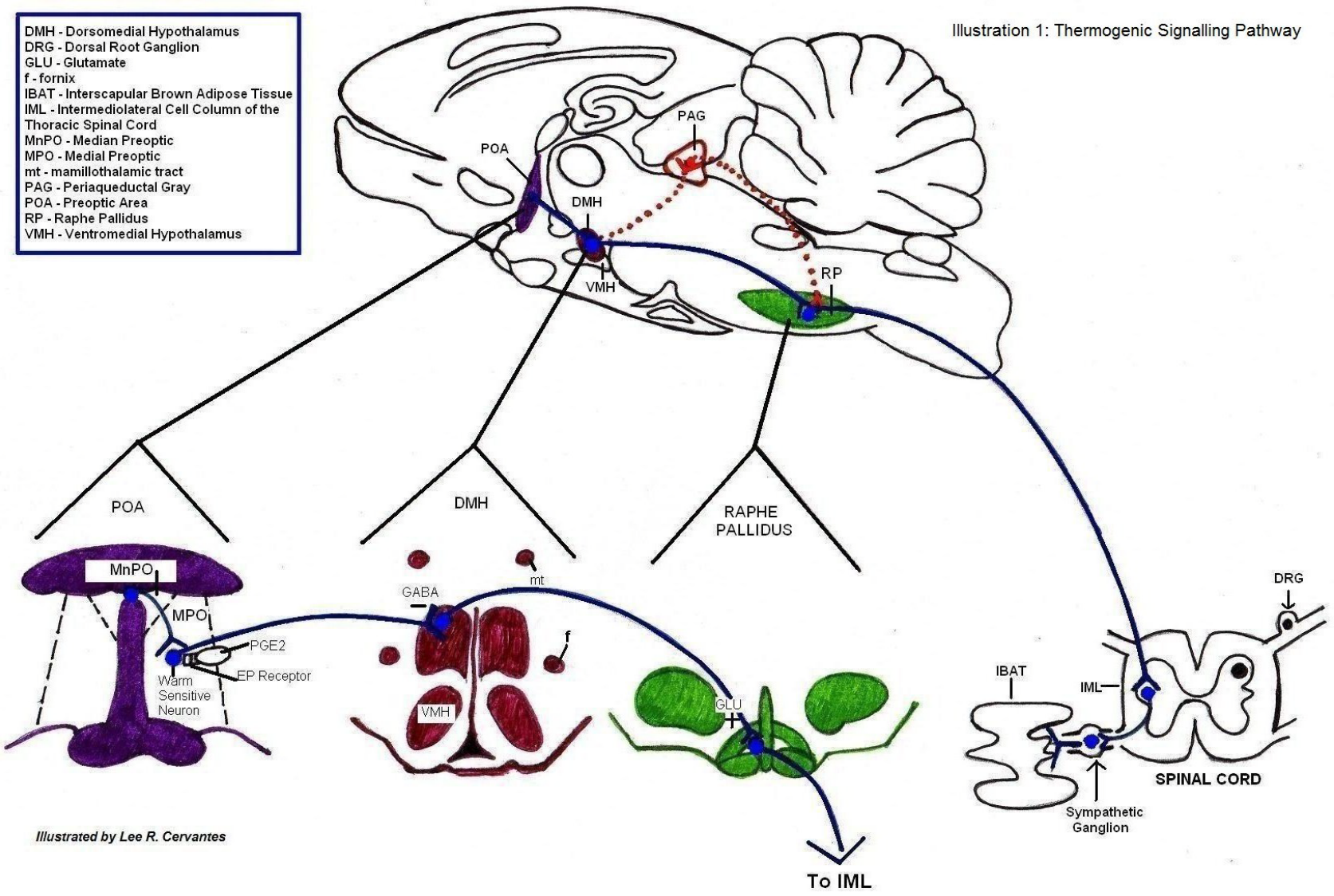
D. Neural control of thermoregulatory effectors

During the first half of the twentieth century, considerable progress was made in the search for the loci in the CNS involved in the regulation of body temperature. By the 1930s the anterior/posterior hypothalamus and preoptic area had been established as key thermoregulatory sites (for historical review

see Lomax, 1979). The thermosensitive nature of the preoptic area (POA) has also been well characterized in rats (Boulant and Dean, 1986; Boulant, 2000). Much of our understanding of the loci in the CNS that are potentially involved in thermoregulation comes from studies involving electrolytic lesions or knife cuts. Chen and colleagues (1998) showed that coronal transections caudal to the POA induced BAT thermogenesis. Electrolytic lesioning of the POA evoked an increase in metabolism, stimulated shivering thermogenesis and activated cutaneous vasoconstriction all of which led to a hyperthermia (Szymusiak and Satinoff, 1982). Therefore, evidence suggests that the POA is involved in thermoregulation and could be a key player in the increase in temperature induced by any type of stress. It is also currently understood that the preoptic area tonically inhibits neurons in caudal brain regions. In fact, inhibition of neurons in the preoptic area increases core body temperature, shivering, metabolism and heart rate (Osaka, 2004; Zaretsky et al., 2006). However, we now know that a simple, long, inhibitory pathway from the POA to the medullary sympathetic premotor neurons that activate BAT thermogenesis is not the case. In fact, transections made in the midbrain caudal to the hypothalamus did not increase the basal levels of BAT thermogenesis (Rothwell et al., 1983) and instead reversed increases in BAT sympathetic nerve activity (SNA) and thermogenesis evoked by PGE_2 (Morrison, 2004; Rathner and Morrison, 2006). Therefore, observations from experiments involving transections of the neuraxis suggest that a hypothalamic input is necessary for BAT thermogenesis in response to the effects of PGE_2 acting within the POA (Morrison et al., 2008).

Most recently the dorsomedial hypothalamus and the raphe pallidus have been suggested to play a key role in thermoregulation and together with the preoptic area are involved in a signaling pathway that regulates body temperature (DiMicco et al., 2006a; DiMicco and Zaretsky, 2007). Disinhibition of the neurons in the DMH by microinjections of bicuculline methiodide (BMI), a gamma-amino butyric acid (GABA) receptor antagonist increased BAT SNA and thermogenesis (Cao et al., 2004b; Zaretskaia et al., 2002) further suggesting a tonic inhibitory input to the DMH that may originate in the POA. Evidence for a signaling pathway from the POA to the DMH includes the observation of GABAergic axon swellings that make close appositions with DMH neurons that include those projecting to the raphe pallidus (Nakamura et al., 2005b). Likewise inhibition of neurons in the DMH blocked 1) the febrile response to microinjections of PGE_2 in the POA (Cao et al., 2004b; Nakamura et al., 2005b; Zaretskaia et al., 2003), 2) excitation of BAT SNA and thermogenesis induced by cold exposure and 3) shivering thermogenesis (Tanaka et al., 2001). However, neurons in the DMH itself do not send direct projections to the sympathetic preganglionic neurons, but have been shown to project to the sympathetic premotor neurons responsible for thermogenesis including neurons in the raphe pallidus. Neurons in the raphe pallidus are the putative sympathetic premotor neurons for BAT thermogenesis (Morrison et al., 2008). In fact, evidence suggests that activation of glutamate receptors within the raphe pallidus is required for activation of the BAT SNA and BAT thermogenesis induced by disinhibition of neurons in the DMH (Cao and Morrison, 2006). Sarkar and colleagues also observed that some neurons in the

DMH labeled by microinjection of a retrograde tracer into the raphe pallidus express FOS, a marker for neuronal activation, in response to endotoxin administration or stress (Sarkar et al., 2007). Likewise neurons in the DMH that are labeled with a retrograde tracer from the raphe pallidus receive GABAergic appositions from neurons in the POA (Nakamura et al., 2005b). The proposed model for a signaling pathway for thermogenesis from the DMH to the raphe pallidus also includes another region of interest, the periaqueductal gray area (PAG). The PAG may be involved in transmitting a thermogenic signal from the DMH to the raphe pallidus. Indeed, many neurons in the caudal PAG have been shown to project directly to the medullary raphe (Hermann et al., 1997). Likewise Cano and colleagues have shown that neurons in the caudal PAG (cPAG) were labeled by microinjection of pseudorabies virus into BAT, and neurons within the cPAG expressed FOS in response to cold (Cano et al., 2003). In addition, pretreatment with microinjection of muscimol into the cPAG attenuated the increase in body temperature induced by microinjection of bicuculline into the DMH (de Menezes et al., 2006). This evidence places the cPAG as a likely player in the thermogenic signaling pathway as shown in Illustration 1. However, there is still a need for further investigation of the pathways transmitting the thermogenic drive from the hypothalamus to the sympathetic premotor neurons.


DMH - Dorsomedial Hypothalamus
DRG - Dorsal Root Ganglion
GLU - Glutamate
f - fornix
IBAT - Interscapular Brown Adipose Tissue
IML - Intermediolateral Cell Column of the Thoracic Spinal Cord
MnPO - Median Preoptic
MPO - Medial Preoptic
mt - mamillothalamic tract
PAG - Periaqueductal Gray
POA - Preoptic Area
RP - Raphe Pallidus
VMH - Ventromedial Hypothalamus
Illustration 1: Thermogenic Signalling Pathway
PAG
POA
DMH
VMH
RP
POA
DMH
RAPHE PALLIDUS
MnPO
MPO
PGE2
EP Receptor
Warm Sensitive Neuron
GABA
mt
f
VMH
GLU
To IML
DRG
IBAT
IML
SPINAL CORD
Sympathetic Ganglion
Illustrated by Lee R. Cervantes

II. Stress-induced hyperthermia: Brain mechanisms, function, and pharmacology

Stress-induced hyperthermia is an acute increase in body temperature in response to exteroceptive stress. The physiological response to emotional stress in mammals consists of changes in the activity of the autonomic nervous system that are intended to increase the probability of the animal's survival in the face of a threatening circumstance. This response is seen in a wide variety of vertebrate species, both warm and cold blooded, including humans, baboons, pigs, rabbits, rodents and even reptiles (for review see Bouwknecht et al., 2007). Thus, when a lizard is gently handled, a type of exteroceptive stress, it will move under an infrared lamp to increase its core body temperature (Cabanac and Gosselin, 1993).

As mentioned, hyperthermia is evoked by a number of anxiogenic or stress-inducing stimuli. Numerous case reports suggest that "psychological stress" or "emotional stress" in humans produces elevated core temperature (Oka and Oka, 2007). Increases in temperature of 0.6° were reported in healthy adults before an examination compared to core temperature taken during a relaxed state (Briese, 1995; Marazziti et al., 1992). The phenomenon of stress–induced hyperthermia is also observed in laboratory animals in response to various exteroceptive stresses such as handling, where the rat is petted or manipulated (Bouwknecht et al., 2007; Briese and De Quijada, 1970; Pae et al., 1985; Parrott et al., 1995) or restraint stress, in which, the rat's movements are confined, usually by placing the rat in a tube (Beotra and Sanyal, 1982; Chung et al., 2000). During foot shock, another exteroceptive stress, an electrical current

is applied to a rat's foot (Bouwknecht et al., 2000; Millan et al., 1981; Pechnick and Morgan, 1987). Rats also show an increased temperature when placed in a new cage (cage-switch) or when placed in a cage previously used by another rat (cage exchange) (Groenink et al., 2003; Oka et al., 2003; Olivier et al., 2003; Spooren et al., 2002; Watanabe et al., 1999). Other exteroceptive stresses that have been shown to induce hyperthermia include fear-conditioning paradigms (Kiyokawa et al., 2004) as well as participation in resident-intruder paradigms (Bouwknecht et al., 2001; Chung et al., 1999). Behavioral stresses such as introduction to novel environment (Akutsu et al., 2002; Amico et al., 2004; Groenink et al., 2003; Pattij et al., 2002), and open-field stress (Kluger et al., 1987; Rowsey et al., 2002; Singer et al., 1986; Soszynski et al., 1998) also increase body temperature in rats. Stress-induced hyperthermia is a parameter used to model some of the physiological symptoms associated with anxiety disorders and has proven to be reliable in predicting clinical efficacy of anxiolytic drugs (Rorick-Kehn et al., 2005). A vital question concerning this effect of emotional stress is whether we are dealing with hyperthermia or fever. There seems to be a consensus that fever is beneficial to the organism and that it might increase the likelihood of survival. Likewise, animals have evolved to increase body temperature when stressed and this response may also have evolved to increase the probability of survival.

A. Fever versus Hyperthermia

Considerable debate exists about the differences between the mechanisms responsible for the increase in body temperature seen in fever and

the increase in body temperature seen with stress. Fever is defined as being the result of raised “set-point temperature”, toward which the thermoregulatory system works to raise core body temperature (Gordon, 1990). On the other hand hyperthermia is defined as an increase in body temperature above the “set-point” that does not require resetting of the temperature set point (Gordon, 1993). Fever is a response to a bacterial or viral infection seen in all mammals, and the hallmark of infection. It is considered to be a centrally-regulated increase in core temperature that is due to a raised “set point”. Exposure to endotoxins such as lipopolysaccharide (LPS), a component of the bacterial cell wall, induces the release of cytokines from macrophages (i.e. Kupffer cells or hepatocytes). These cytokines are then thought to act centrally to produce fever (Kluger et al., 1995; Kluger et al., 1998). One model suggests that cytokines cross from the blood into the brain through the organumvasculosum lamina terminalis (OVLT) (Blatteis, 1992; Saper and Breder, 1992). The OVLT is a circumventricular organ located near the region of the POA and most importantly, it is outside the blood brain barrier, and so neurons in this region can respond to factors that are present in the systemic circulation such as cytokines (Saper and Breder, 1992). Cytokines act on the neurons of the OVLT to promote the synthesis and release of prostaglandin E2 (PGE_2) (Blatteis, 1992). The neurons in the OVLT project to the region of the POA and PGE_2 diffuses from the OVLT to the region of the POA where PGE_2 binds to prostaglandin E receptor subtype EP3 receptors in this region (Blatteis, 1992). Following activation of the EP3 receptors in the POA, the brain orchestrates changes in the autonomic, neuroendocrine, and behavioral

thermoregulatory responses to increase heat-producing mechanisms and inhibit heat-dissipating mechanisms. Microinjection of PGE_2 in the POA is considered a reproducible and reversible experimental model for fever. The POA is a region that contains thermosensitive neurons that not only receive somatosensory information from the skin and spinal thermoreceptors but also integrate central and periphery thermal information from these ascending neural pathways (Boulant and Dean, 1986; Boulant, 2000; Hori et al., 1988; Lipton and Clark, 1986).

According to the Commission for Thermal Physiology of the International Union of Physiological Sciences (IUPS Thermal Commission), hyperthermia is a core temperature above its range specified for the species at thermal neutral zone (TMZ) ambient temperature (2001). Evidence suggests that stress-induced hyperthermia (cage-change, cage-exchange and open-field stresses) can be attenuated by pretreatment with indomethacin and sodium salicylate (Kluger et al., 1987; Singer et al., 1986; Stewart and Eikelboom, 1979). This finding suggests that, like fever evoked by endotoxin, the stress-induced increases in core temperature are also dependent on the actions of PGE_2 (Kluger et al., 1987; Morimoto et al., 1991; Oka et al., 2001; Singer et al., 1986). Like endotoxin-induced fever, open-field and cage exchange stress-induced increases in core temperature in rats seem not to be affected by ambient temperature (Briese and Cabanac, 1991; Long et al., 1990a). Cage-switch stress also produces an increase in core body temperature, heart rate, blood pressure, levels of plasma adrenocorticotrophic hormone (pACTH) and locomotor activity similar to the

responses seen with endotoxin induced fever (Morimoto et al., 1991). Furthermore, the increase in core temperature of rats following cage exchange does not correlate with the associated increase in physical activity suggesting that the increase in temperature is not due to muscular activity (Long et al., 1990b).

Compelling evidence also indicates that the mechanisms involved in stress hyperthermia and fever are not identical. Oka and colleagues (2003) have shown that in EP1 and EP3 receptor knockout mice the febrile response to systemic administration of lipopolysaccharide is suppressed but that stress-induced hyperthermia caused by handling is unchanged. Likewise, rats made tolerant to endotoxin do not exhibit an altered hyperthermic response to an open-field stress (Soszynski et al., 1998). Finally, lesions to the anteroventral third ventricle (AV3V), a region in the brain specifically shown to be involved in the production of fever, suppressed the febrile response to systemic administration of interleukin-1β, another experimental model for fever, but had no effect on the hyperthermic response to open-field stress or cage-switch stress (Hunter, 1997). This evidence suggests that the mechanisms involved in the production of fever and of stress hyperthermia may be different.

B. DMH and stress response

A role for the DMH in thermoregulation was theorized by DiMicco and colleagues (2006a), following an evaluation of the role of the DMH in the sympathetically mediated tachycardia induced in mammals by acute emotional stress. The DMH appears to mediate a broad range of physiological and

behavioral changes seen in stress, which include tachycardia and activation of the hypothalamic-pituitary adrenal (HPA) axis (DiMicco et al., 2006a). Activation of the HPA axis is reflected by acute increases in pACTH levels and is a neuorendocrine marker for stress (DiMicco et al., 2002; Selye and Fortier, 1950). The increase in heart rate and pACTH are physiological changes associated with the fight-or-flight response which includes the most immediate changes in physiological and behavioral state seen in exposure to exteroceptive stress, and is centrally mediated (McDougall et al., 2005; Selye and Fortier, 1950). Microinjection of BMI, a GABA receptor antagonist into the region of the DMH in urethane-anesthetized rats, elicits a marked increase in heart rate and modest increases in blood pressure (DiMicco et al., 1986). At baseline (unstressed), neurons in the region of the DMH are under tonic inhibition. This can be demonstrated by the finding that microinjection of muscimol, a GABA agonist inhibitor in nearly all adult central mammalian neurons that produces acute reversible inactivation of neurons (Johnston et al., 1968), into the DMH has little or no significant effect on baseline heart rate or mean arterial pressure. Conversely removal of GABA tone with microinjection of BMI evokes increases in heart rate and mean arterial pressure (Samuels et al., 2002). Likewise, microinjection of BMI into the DMH of anesthetized or conscious rats elicits increases in pACTH; (Bailey and Dimicco, 2001; Keim and Shekhar, 1996; Zaretskaia et al., 2002). Microinjections of muscimol into the DMH abolishes the increase in heart rate, arterial pressure, and increases in pACTH seen in an air-jet stress paradigm (De Novellis et al., 1995b; Lisa et al., 1989a; Stotz-Potter et

al., 1996a; Stotz-Potter et al., 1996b). Neurons in the ventrolateral subregion of the DMH send direct projections to the parvocellular hypothalamic paraventricular nucleus (PVN) and are activated in experimental stress (Cullinan et al., 1996). The PVN is a region that has been shown to mediate increases in pACTH levels evoked by stress or infection (Cullinan et al., 1996; Herman and Cullinan, 1997; Rivest and Rivier, 1991). These studies suggest that the DMH through signaling to the PVN may be responsible for the increase in pACTH associated with stress.

Evidence also suggests a role for the DMH in the increase in heart rate induced by stress that may be mediated through signaling to the raphe pallidus. Neurons in the region of the raphe pallidus were found to be heavily retrogradedly labeled following microinjection of pseudorabies virus (PRV), a transsynaptic viral tracer, into the stellate ganglion, the primary location of sympathetic neurons innervating the heart (Jansen et al., 1995). In the same study, infected cells were also found in the dorsal area of the DMH known as the dorsal hypothalamic area (DHA). Various exteroceptive stresses including restraint, swim stress, and noise stress, increased FOS expression or FOS mRNA in the raphe pallidus (Campeau and Watson, 1997; Cullinan et al., 1996). These anatomical studies suggested a possible role for the raphe pallidus in the cardiovascular response seen in stress. Indeed, microinjections of BMI into the raphe pallidus produced marked sympathetically mediated increases in heart rate and arterial pressure in anesthetized and conscious rats (Cao and Morrison, 2003; Morrison et al., 1999; Samuels et al., 2002; Zaretsky et al., 2003a).

Increases in heart rate, and arterial pressure produced by microinjection of BMI into the DMH of anesthetized rats were attenuated by microinjection of muscimol into the raphe pallidus (Cao et al., 2004b; Samuels et al., 2002; Samuels et al., 2004). These results were also confirmed in conscious rats (Zaretsky et al., 2003b). In conjunction, these studies suggest that neuronal activity in the raphe pallidus mediates the cardiovascular responses induced by disinhibition or activation of neurons in the region of the DMH. Conversely, Zaretsky and colleagues showed that microinjections of muscimol into the raphe pallidus did not alter baseline heart rate but caused dose-related attenuation of the increase in heart rate associated with air-jet stress (Zaretsky et al., 2003c). These studies showed that the projection from the DMH to the raphe pallidus plays a key role in stress-induced cardiac stimulation. As mentioned earlier, these functional studies are supported by the already existing anatomical studies. A more recent tracing study aimed to enhance the resolution of the area of the DMH most likely involved in the increases in heart rate evoked by disinhibition of neurons in the DMH. Samuels and colleagues, using the retrograde tracer cholera toxin B, confirmed that neurons in the DHA of the DMH sent numerous projections to the raphe pallidus (Samuels et al., 2004). Most importantly, the greatest increases in heart rate were produced by microinjections of BMI in this exact region in the DMH, the DHA, where the neurons projecting to the raphe pallidus are most densely localized (Samuels et al., 2004). Taken together, these studies suggest that the DMH through signaling from the DHA to the raphe pallidus and through signaling through the PVN may be responsible for mediating different

components of the physiological responses to stress including the increases in heart rate and in pACTH, respectively. Evidence suggests that the projection from the DMH to the raphe pallidus may also play a key role in the thermogenic response to stress, another physiological component of the stress response as described next.

C. DMH and its role in thermoregulation

Until recently the DMH was overlooked as a region of interest in thermoregulation and was instead mostly studied for its apparent role in metabolic regulation associated with ingestive behavior (Bernardis and Bellinger, 1987), a behavior that is also related to thermogenesis (Himms-Hagen, 1995), 1995). Thermoregulatory research focused mainly on the nearby regions of the ventromedial hypothalamus (VMH) (Holt et al., 1987; Hugie et al., 1992; Kelly and Bielajew, 1991; Perkins et al., 1981) and the posterior hypothalamus (PH) (Thornhill and Halvorson, 1994). However, in microinjection studies involving the VMH and PH, the lack of anatomical specificity due to large injection volumes used, or lack of appropriate anatomical control injections calls into question the role of these regions in thermoregulation. Instead, functional studies with the appropriate anatomical controls singled out the region of the DMH as a region containing neurons likely to be involved in the signaling pathway for thermoregulation (DiMicco et al., 2006b). Disinhibition of neurons in the DMH by blockade of $GABA_A$ receptors resulted in increases in core body temperature, brown adipose tissue (BAT) temperature, and BAT sympathetic nerve activity (SNA) (Cao et al., 2004b; Zaretskaia et al., 2002). This evidence suggested a

possible role for neurons in the DMH in thermoregulation. In addition, inhibition of neurons in the DMH blocked the febrile response to microinjections of PGE_2 in the POA, cold-evoked excitation of BAT SNA and increase in BAT temperature, as well as shivering (Cao et al., 2004b; Morrison, 2004; Nakamura and Morrison, 2007; Nakamura et al., 2005b; Tanaka et al., 2001; Zaretskaia et al., 2003). Neurons in the DMH do not project directly to sympathetic preganglionic neurons; however, these neurons may contribute to thermogenic sympathetic outflow by influencing the activity of the sympathetic premotor neurons found in the raphe pallidus responsible for BAT thermogenesis. In fact, Sarkar and colleagues found that some DMH neurons that were retrogradedly labeled from the raphe pallidus also express FOS, a marker for neuronal activity, in response to either stress or systemic administration of LPS (Sarkar et al., 2007). Furthermore, Nakamura and colleagues found that some neurons in the DMH that were labeled by microinjection of a retrograde tracer into the raphe pallidus receive close GABAergic appositions (putative synapses) from neurons in the POA. This evidence suggests a possible role for a monosynaptic pathway from neurons in the DMH to the raphe pallidus in thermogenesis.

The anatomical connections between the DMH and the medullary raphe pallidus are also consistent with a role for the DMH in thermoregulation. As shown using transsynaptic neuronal tracing studies, the raphe pallidus is the location of sympathetic premotor neurons that directly control sympathetic preganglionic neurons (SPNs) in the intermediolateral cell column (IML) of the thoracic spinal cord that innervate the interscapular brown adipose tissue (IBAT)

and thermoregulatory cutaneous blood vessels (Bamshad et al., 1999; Nakamura et al., 2004; Smith et al., 1998). Anterograde tracing studies have shown that the DMH sends direct projections to the raphe pallidus (ter Horst and Luiten, 1986). Retrograde tracing studies also show that these neurons that project to the raphe pallidus are concentrated in the DHA (Hermann et al., 1997; Hosoya et al., 1987; Hosoya et al., 1989). Thus, anatomical evidence places the DMH at the crossroads of a thermoregulatory signaling pathway, downstream from the POA and upstream from the raphe pallidus.

The region of raphe pallidus is a brain region that when chemically stimulated increases core body temperature in part through stimulation of IBAT temperature and regulation of cutaneous vasoconstriction. Neurons in the raphe pallidus were infected following transsynaptic viral tracing from the IBAT, the primary thermogenic organ in rodents (Bamshad et al., 1999). Also, cold exposure produced a dramatic increase in c-fos expressing neurons in the region of the raphe pallidus (Bonaz and Tache, 1994). Together these anatomical studies identified the raphe pallidus as a region of interest in thermoregulation. In 1999, Morrison and coworkers reported that microinjections of BMI in the raphe pallidus of anesthetized rats produced dramatic increase in SNA to IBAT (Morrison et al., 1999). Similarly Blessing and colleagues reported that microinjections of BMI into the raphe pallidus produced cutaneous vasoconstriction in the tail, a mechanism used for heat conservation (Blessing and Nalivaiko, 2001). Electrical stimulation of neurons in the region of the raphe pallidus caused vasoconstriction in the ear pinna in rabbits, an important heat-

conserving mechanism in this species (Blessing et al., 1999; Nalivaiko and Blessing, 2002). In a similar study, electrical stimulation of, the DMH, evoked vasoconstriction in the ear pinna of rabbits, a response blocked by microinjection of muscimol into the region of the raphe pallidus (Nalivaiko and Blessing, 2001). This finding suggested a possible role for neurons in the region of the DMH that projected to the raphe pallidus in thermoregulatory cutaneous vasoconstriction.

Neurons in the raphe pallidus and the DMH seemed to be cooperatively involved in stress-induced tachycardia. Therefore, it seemed possible that the DMH and even the raphe pallidus could also be involved in stress-induced increases in body temperature. In support of this idea, disinhibition of neurons in the DMH produced an increase not only in core body temperature but also in IBAT temperature (Zaretskaia et al., 2002). In fact, the increase in IBAT temperature was greater than the increase in core temperature and preceded it as would be expected considering the role of IBAT as a thermogenic organ. These findings suggested that activation of neurons in the DMH produces increases in body temperature that are mediated, at least in part, by activation of IBAT. These results were later confirmed when Morrison and coworkers reported increases in sympathetic nerve activity to IBAT, IBAT temperature and core body temperature following disinhibition of neurons in the DMH (Cao et al., 2004b). Most importantly these increases in both core and IBAT temperature were abolished when neurons in the raphe pallidus were inhibited (Cao et al., 2004a). This evidence supports a monosynaptic signaling pathway from the DMH to the raphe pallidus that may be involved in thermogenesis.

Neurons in the medial POA are also known to send direct neuronal projections to the DMH. As mentioned earlier, neurons in the DHA send direct projections to the raphe pallidus, (DiMicco and Zaretsky, 2007; Jepson et al., 1988; Morrison et al., 1999). Activation of IBAT and cutaneous vasoconstriction are two mechanisms responsible for the increase in body temperature associated with bacterial infection (Fyda et al., 1991; Romanovsky and Blatteis, 1998).

One of the prevalent hypotheses on how a bacterial infection (interoceptive stress) induces fever is that lipopolysaccharide (LPS), a component of the bacterial cell wall, binds to a CD14 receptor found on macrophages and other immune response cells and elicits the release of cytokines, such as TNF-α and IL-1 (Conti et al., 2004; Dinarello, 2004; Kluger et al., 1995; Kluger et al., 1998; Kozak et al., 1995; Kozak et al., 1997; Kozak et al., 1998). These cytokines are then thought to act on the brain to induce the synthesis of PGE_2 in the POA (Boulant and Dean, 1986; Kluger et al., 1995). The release and activity of PGE_2 on warm sensitive neurons in the POA is thought to be involved in resetting the temperature set-point to a higher body temperature, a critical role of the hallmark of febrile response seen in infection (Hori et al., 1988; Kozak et al., 1968; Kozak et al., 2000; Negishi et al., 1995; Oka et al., 1997; Romanovsky and Szekely, 1998; Romanovsky et al., 2000; Scammell et al., 1998; Simons et al., 1998; Szekely et al., 2000). Microinjection of PGE_2 in the medial POA of anesthetized and conscious rats elicits increases in body temperature that are accompanied by tachycardia and activation of the

HPA axis (Fyda et al., 1991; Malkinson et al., 1988; Morrison, 2003; Nakamura et al., 2002; Zaretskaia et al., 2003; Zaretsky et al., 2006).

Interestingly, fever associated with infection is usually accompanied by tachycardia in mammals, and tachycardia is also reported following microinjection of PGE_2 into the POA (Karjalainen and Viitasalo, 1986; Osborne and Kurosawa, 1994; Romanovsky et al., 1997; Xia and Krukoff, 2001). The response to an interoceptive stress such as a bacterial infection in mammals also includes increases in blood pressure and pACTH (Harris et al., 1987; Karjalainen and Viitasalo, 1986; Xia and Krukoff, 2001; Xia and Krukoff, 2003). The central nervous mechanisms that mediate these changes have not been fully elucidated, and it is possible that more than one brain region may serve as a target for the action of cytokines. Considering that disinhibition of neurons in the DMH evokes an increase in temperature, heart rate, blood pressure and pACTH, the DMH seems a logical target for investigation.

Considering that the pathway from the DMH to the raphe pallidus appears to constitute a key relay mediating the increase in heart rate associated with "emotional stress," this same relay could be responsible for the increase in body temperature associated with emotional stress (DiMicco et al., 2006a). The evidence suggests that neurons in the region of the DMH may play a key role in thermogenesis generated by emotional stress. In my studies, the temperature response to both interoceptive and exteroceptive stressors was studied, and I hypothesized that the increase in core temperature seen with either stress is mediated by neurons in the region of the DMH. I examined specifically the role

of ionotropic glutamate receptors in the DMH in experimental fever (microinjections of PGE_2 in the POA or microinjections of BMI in the DMH) and stress-induced hyperthermia (i.e. air-jet stress and cage-switch stress). As discussed next, ionotropic glutamate receptors in the DMH seem to play a key role in thermoregulation.

D. Glutamate as a neurotransmitter in the mammalian brain

It was thought at one point that all central synaptic transmission in the brain was electrical and not chemical. In the quest to understand brain function, the relationship between chemicals already known to exist in the brain and their role in brain mechanisms was examined. Glutamate and similar compounds were known to be metabolites and precursors for other molecules such as GABA and glutamine (Johnson, 1972; Watkins and Jane, 2006). Work spearheaded by Curtis and colleagues allowed for glutamate and other structurally similar compounds referred to as excitatory amino acids to be recognized as neurotransmitters (Curtis and Eccles, 1960; Curtis and Phillis, 1960; Curtis and Watkins, 1960; Curtis and Watkins, 1961; Hayashi, 1954).

Early studies showed that L-glutamate and other naturally occurring acidic amino acids excited virtually all neurons in the mammalian central nervous system. However, it was not until a review by Frode Fonnum that a case for glutamate as a neurotransmitter was widely accepted (1984). In order to be considered as a neurotransmitter, glutamate had to satisfy four main criteria. Glutamate had to be shown (1) to be localized presynaptically in specific neurons where it is stored and released from synaptic vesicles, (2) to be released

in calcium-dependent fashion by physiological stimuli in concentrations high enough to elicit post-synaptic responses, (3) to have pharmacologic identity of action with naturally occurring transmitter, including response to antagonists, and (4) to have reuptake mechanisms that rapidly terminate its transmitter action (Bennett et al., 1973; Fonnum, 1984; Stallcup et al., 1979).

Both in vivo and in vitro preparations have been used to show that glutamate is present in and released from presynaptic terminals. Storm-Mathisen and colleagues used immunohistochemistry procedures to provide evidence that glutamate is localized to presynaptic plasma membrane (Storm-Mathisen et al., 1983; Storm-Mathisen and Ottersen, 1990). They suggested that the intraterminal concentration for glutamate is at least 10mM which is a 10,000 fold concentration difference across the presynaptic plasma membrane (Ottersen and Storm-Mathisen, 1984; Ottersen and Storm-Mathisen, 1987). Glutamate is released in a Ca^{2+} dependent manner from brain slices or synaptosomes (pinched off nerve endings) by electric field depolarization (De Belleroche and Bradford, 1972; Potashner, 1978) or high potassium concentration (Nadler et al., 1977; Nadler et al., 1978).

More than twenty years ago it was also demonstrated that some neurons contain synaptic vesicles that store glutamate (Storm-Mathisen et al., 1983). More recently, two vesicular transporters VGLUT1 and VGLUT2 were found to be responsible for the uptake of glutamate into vesicles (Bellocchio et al., 2000; Takamori et al., 2000; Varoqui et al., 2002). The uptake mechanism for glutamate is electrogenic, requiring two sodium ions for the uptake of one

glutamate molecule, and thus is driven by the ion gradients of K^+ and Na^+ (Bennett et al., 1973; Fonnum, 1984; Stallcup et al., 1979). Using autoradiographic studies of isolated glial cells (Henn and Hamberger, 1971), or primary cultures of astrocytes (Hertz, 1979) or rat brain slices (Balcar and Johnston, 1972) high affinity uptake of glutamate was verified. Likewise, glial cells in the hippocampus were also preferentially labeled following intracerebral injection of [^{3}H] glutamate (Hokfelt and Ljungdahl, 1972). It is now known that glutamate is taken up into neurons by excitatory amino acid carrier 1 EAAC1 (or excitatory amino acid transporter, EAAT3), EAAT4, and EAAT5 (Danbolt, 2001). This glutamate uptake system plays an important role in terminating the excitatory effect of exogenous glutamate, an important criterion for classification of glutamate as a neurotransmitter.

The mechanism of action for the central excitatory transmitter(s) has proven to be the more difficult criterion to prove. Cotman and coworkers showed that glutamate was release from slices of the dentate gyrus in a Ca^{+2} dependent manner. Furthermore, this release and the high affinity uptake of glutamate were diminished following lesions of the major input to the dentate gyrus (Cotman, 1995). In fact, the discovery of specific agonists and glutamate analogues such as N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), D- and L-homocysteate, and kainate, has been important in the characterization and identification of multiple glutamate receptors (Curtis et al., 1961; Curtis et al., 1972; Curtis, 1974; Evans et al., 1979; Krogsgaard-Larsen et al., 1980; McLennan et al., 1968; Watkins et al., 1981; Watkins and Evans,

1981). However, it was with the development and use of highly selective NMDA receptor antagonists that the existence of glutamatergic synaptic transmission in the central nervous system was unequivocally established (Biscoe et al., 1977a; Biscoe et al., 1977b; Davies et al., 1981a; Davies et al., 1981b; Davies et al., 1982; Evans et al., 1979; Evans and Watkins, 1981; Evans et al., 1982; Olverman et al., 1984).

E. Ionotropic glutamate receptors in the DMH

Ionotropic glutamate receptors in the DMH have been shown to play a role in stress-induced tachycardia and experimental fever. Dose-related increases in heart rate and arterial pressure were seen after microinjection of excitatory amino acids NMDA, AMPA and kainic acid (KA) into the DMH (Soltis et al., 1991a; Soltis et al., 1992; Soltis et al., 1991b). Furthermore, co-injection of kynurenate, a non selective glutamate receptor antagonist that blocks excitation, into the DMH blocked or reversed the increases in heart rate and blood pressure induced by microinjection of BMI into the DMH of anesthetized rats (Soltis et al., 1991b). This suggests that the disinhibition of neurons in the DMH by bicuculline produces sympathetic responses similar to those seen with stress that are also dependent on excitation at glutamate receptors in the DMH. Microinjection of kynurenate in the region of the DMH also reduced the increases in heart rate and blood pressure seen in experimental air-jet stress (Soltis et al., 1992). Collectively, these studies suggest that glutamate receptors in the region of the DMH play a key role in these same effects evoked by stress. Microinjection of kynurenate into the DMH of anesthetized rats also attenuates increases in body

temperature induced by microinjection of PGE_2 into the POA (Madden and Morrison, 2004). However, the role of glutamate receptors in the DMH in the increase in body temperature induced by air stress or other exteroceptive or interoceptive stresses in conscious rats has not been studied.

III. Ionotropic glutamate receptors

A. Receptor mechanism

Understanding the function and regulation of glutamate and other excitatory amino acids was further advanced by the identification, characterization and localization of their receptors. L-glutamate is a major neurotransmitter in the brain that acts through both ligand-gated ion channels (ionotropic) and G-protein coupled receptors (metabotropic). Because of its abundance and role as a major neurotransmitter, glutamate receptors play a vital role in excitatory synaptic transmission. So far two main types of glutamate receptors have been identified which include the ionotropic glutamate receptor subtypes, and the metabotropic glutamate receptor subtypes (Cooper, 2003). Although an important part of the glutamate story, for the purpose of this thesis, metabotropic receptors will not be further discussed in detail.

The ionotropic receptors on binding glutamate that has been released from a companion cell, allow charged ions such as Na^+ Ca^{2+} and K^+ to pass through a channel in the center of the receptor complex. This flow of ions produces a depolarization of the plasma membrane. The ionotropic glutamate receptors can be separated further into three groups, 1) NMDA receptors, 2) AMPA receptors, and 3) kainate receptors, based on the sequences of receptor

subunits and the agonist activity of several ligands. The agonists NMDA, ibotenate, kainate and AMPA differentially activate at each receptor. Until recently these receptors were only subdivided into either NMDA or non-NMDA receptors, but the development of selective agonists and antagonists has helped characterize these receptors further (Cooper, 2003). Even though these receptors share a similar mechanism of action, they are diverse in structure and agonist activity.

Ionotropic glutamate receptors are made up of multimeric assemblies of four or five subunits, and like other ligand-gated ion channels share a common structure including four transmembrane domains (TMI-IV) (Cotman and Monaghan, 1986; Cull-Candy, 2002). Unlike other receptor subunits, the TMII forms a re-entrant loop giving these receptors an extracellular N-terminus and an intracellular C-terminus (Cotman and Monaghan, 1986). The diversity in the ionotropic glutamate receptors actually stems from the extensive splice variation at the C-terminus. It is this extensive splice variation at the C-terminus that gives each of the ionotropic glutamate receptors its unique pharmacological qualities that have helped differentiate each receptor. Their unique qualities and agonist activity are described below. By far, the NMDA receptor is the best characterized of the ionotropic glutamate receptors and accumulating evidence suggests that it may play a role in a wide range of both physiological and pathological functions from memory acquisition, to epilepsy, neurotoxic effects of brain ischemia, Alzheimer's disease and schizophrenia (Bergink et al., 2004; Follett et al., 2000; Hertz, 2006; Javitt, 2004; Kehne et al., 1991).

B. NMDA receptor complex: Distribution, mechanism and pharmacology

NMDA receptor is widely distributed in the mammalian brain and spinal cord. According to van den Pol (1990) and colleagues their evidence suggested that glutamate accounted for the majority of excitatory synapses in the hypothalamus. Using in situ hybridization and Northern blots, the ionotropic subtypes of the glutamate receptor in the rat hypothalamus were studied and widespread expression of AMPA, kainate, and NMDA receptor RNA was found in the hypothalamus with the transcripts the same size and number as found in other regions of the brain (van den Pol et al., 1994). Anatomical distribution of L-[^{3}H] glutamate-binding sites displaced by NMDA was found throughout the CNS and within the hypothalamus they (Monaghan and Cotman, 1985). Various techniques have been used to verify the existence of ionotropic glutamate receptors within the hypothalamus. Receptor autoradiography confirmed the widespread presence of all major ionotropic glutamate receptor subtypes within the hypothalamus with the greater regional density found in the ventral and dorsomedial hypothalamus (Monaghan et al., 1983). Likewise, subtype expression varied regionally, with rostral hypothalamic and preoptic regions having proportionally higher levels of non-NMDA vs. NMDA binding (Monaghan et al., 1983; Monaghan et al., 1984; Monaghan et al., 1985). More recently in situ hybridization was used with ^{35}S-labeled cRNA probes for the different ionotropic glutamate receptor subunits, including those for AMPA, kainate, and NMDA receptor subtypes, and results showed that all three glutamate receptor subtypes and their subunits were distributed widely but were most abundant in the hypothalamus (Eyigor et al., 2001).

Two distinctive features of the NMDA receptor are its voltage-gated block by Mg^{2+} ion, which is present under physiologic conditions at resting membrane potential, and its need for glycine as a co-agonist (Cooper, 2003). The voltage-dependent block suggests that fast transmission is mediated mainly by non-NMDA receptors and this is reflected in the two component time course of many synaptic currents (Cooper, 2003). The NMDA receptor's voltage block keeps it from opening during the initial depolarization. Only after the post-synaptic cell is depolarized is the voltage-dependent block relieved allowing this receptor to function as a "coincidence receptor" (Silver et al., 1992). Early on in the study of excitatory amino acid receptors it was observed that magnesium cations produced a selective depression of excitatory amino acid depolarizations (Ault et al., 1980). This observation was further verified using spinal cord neurons where it was shown that as theorized magnesium ions do selectively block NMDA receptor activity at depolarized potentials (Mayer and Westbrook, 1984). Since then other divalent cations have also been found to modulate NMDA receptor activity. While some cations act like Mg^{+2} ions, others do not. In fact, Ni^{+2}, Co^{+2} and to a lesser extent Mn^{+2} mimic the effects of magnesium ions (Ascher and Nowak, 1988) while Ba^{+2}, Cd^{+2}, Sr^{+2} and Ca^{+2} do not (Ascher and Nowak, 1988). The difference in how these cations work is thought to be due to their hydrophilicity (Ascher and Nowak, 1988; Mayer and Westbrook, 1987). The Mg^{+2}-like ions are more hydrophilic and thus water exchange occurs less rapidly delaying their permeation into the cell and allowing these cations to act as inhibitors (Ascher and Nowak, 1988; Mayer and Westbrook, 1987; Nowak et al.,

1984). Breakthroughs in understanding the structure of the receptor lead to better understanding of its function.

The NMDA receptor is composed of two subunits, NR1 and NR2, which can be encoded by four different gene variants, and expression of both protein subunits is necessary for functional channels (Cull-Candy, 2002). To further add to the complexity, the NMDA receptor has at least six pharmacologically distinct sites through which compounds can alter the activity of this receptor (1) a binding site for L-glutamate, NMDA and related agonists, (2) a strychnine-insensitive glycine-modulatory site, 3) a binding site for phencyclidine (PCP site) and other related non-competitive antagonists such as ketamine, (4) a voltage-dependent Mg^{2+}-binding site, (5) a divalent cation site near the mouth of the channel that binds Zn^{2+} to produce a voltage-independent block, and (6) a polyamine regulatory site that is activated by spermine and spermidine and facilitates NMDA receptor-mediated transmission (Cooper, 2003).

i. NMDA receptor agonist binding sites

Binding of L-[^{3}H] glutamate to NMDA receptors was described using quantitative autoradiographic techniques. Because of the development of more specific agonists and antagonists, researchers were able to distinguish other functional binding sites on the receptor, each with its own ligand-binding domain. These other ligand-binding domains thus can alter the activity of this receptor. Furthermore, the transmitter binding site itself is known to have two distinct sites with one that is agonist-preferring and one that is antagonist preferring (Monaghan and Cotman, 1985).

a. Glycine binding site

Glycine binding is required for binding of L-[^{3}H] glutamate or NMDA and other agonists to the NMDA receptor. Johnson and Asher (1987) first postulated that NMDA receptor activation required occupation of a positive allosteric site by low micromolecular concentrations of glycine. The glycine binding site is strychnine-insensitive and studies of the NMDA receptor expressed in Xenopus oocytes indicate that the presence of glycine is an essential pre-requisite for NMDA receptor function (Kleckner and Dingledine, 1988). Experiments carried out by Johnson and Archer (1987) showed that glycine is required for binding of the agonist but has no action itself. Furthermore, these studies showed that glycine increases the frequency of the channel opening and not the amplitude, or period of time that the channel is open (Johnson and Ascher, 1987). The observations regarding channel opening were made with outside-out patch clamp preparations suggesting that glycine did not require an intracellular second messenger but rather facilitated excitatory transmission in the brain through an allosteric activation of the NMDA receptor.

Given that the glycine binding site is intimately related to the action of the transmitter site, it is not surprising that its distribution was found to correlate highly with that observed for the NMDA receptor. Some antagonists actually inhibit the action of NMDA receptors by competitively inhibiting the glycine binding site, and kynurenate is one such antagonist (Ganong and Cotman, 1986). Kynurenate is a drug used in this thesis because of its non-specific antagonism at all three ionotropic glutamate receptor subtypes and will be discussed below (see Section III. D. i.). Another antagonist used in thesis is 2-amino-5-

phonsphonovalerate (APV), which unlike kynurenate acts as a potent and selective antagonist at the glutamate binding site of the NMDA receptor.

b. Phencyclidine binding site (PCP)

Both ketamine and PCP are known to act as selective antagonists that can completely block NMDA receptor function without affecting responses at the AMPA or kainate receptors (Anis et al., 1981; Duchen et al., 1985). Other similar compounds such as MK-801 (dibenzocycloheptenеimine) and TCP are more potent and selective than PCP with MK-801 being the most potent and selective (Vignon et al., 1983; Wong et al., 1986). These compounds act as noncompetitive antagonists at the NMDA receptor by blocking the cation channel gated by the NMDA recognition site and not at the NMDA-binding site itself (Martin and Lodge, 1985; Wong et al., 1986; Woodruff et al., 1987). Using whole-cell techniques, Huettner and colleagues discovered that recovery from MK-801 blockade was voltage-dependent being faster at more positive potentials (Huettner and Bean, 1988). We now know that the PCP binding site is found proximal to the ion channel and that the presence of magnesium ions inhibits blockade of MK-801 at negative potentials (Huettner and Bean, 1988; Loo et al., 1986). Several studies also suggest that the PCP-binding is directly related to the simultaneous binding of L-glutamate and glycine. Binding of glutamate and glycine has been shown to enhance stimulation of [^{3}H] TCP or [^{3}H] MK-801 binding (Benavides et al., 1988; Thomas et al., 1988). A proposed explanation for this phenomenon is that binding of agonists at the glutamate and glycine sites allow a conformational change within the receptor that exposes the ion channel

and provides access of ligands to the PCP binding site (Kloog et al., 1988; Monaghan et al., 1989).

c. Polyamine regulatory site

Polyamines including spermidine and spermine are found in high concentrations in mammalian tissue. As mentioned previously, [^{3}H] MK-801 binds specifically to the activated state of the NMDA receptor at a site perhaps within the ion channel of the receptor. Likewise, divalent cations have been shown to block [^{3}H] MK-801 binding. Besides these interactions, it has also been shown that the polyamines spermidine and spermine increase the affinity of the NMDA receptor for [^{3}H] MK-801 binding (Ransom and Stec, 1988). Later, it was shown that both spermidine and spermine potentiate the binding of [^{3}H] MK-801 at maximally effective concentrations of L-glutamate and glycine (Williams et al., 1989). Other polyamines such as putrescine and cadaverine were shown to inhibit binding of [^{3}H] MK-801 in the presence of spermine, L-glutamate and glycine but not in the presence of L-glutamate and glycine alone, suggesting that both putrescine and cadaverine are antagonists at the polyamine site (Williams et al., 1989). Unlike glycine, spermidine and spermine are not required for NMDA receptor activation. However, under pathological conditions, concentration of polyamines increases, which suggests a role for these compounds in mediating or potentiating the excitotoxic mechanisms responsible for the neuronal damage produced (Cooper, 2003). A study in vivo using neonatal rats found that polyamines decreased NMDA-induced neurotoxicity which suggests that

polyamines or related compounds may have important therapeutic potential as neuroprotective agents (Munir et al., 1993).

C. Non-NMDA receptor complex (AMPA and Kainate): Distribution, mechanism, and pharmacology

It is known that excitatory amino acid depolarizations appear to be mediated by NMDA, AMPA and kainate receptors, and these receptors are found in similar distributions throughout the brain. Using autoradiography, Monaghan and colleagues quantified AMPA and KA binding sites by analyzing displacement of [^{3}H] AMPA binding and [^{3}H] KA binding respectively (Monaghan and Cotman, 1982; Monaghan et al., 1984). Both AMPA and KA receptors are widely distributed ubiquitously in the brain and follow a distribution pattern similar to that of NMDA receptors.

Despite the general trend for similar distributions of all three ionotropic glutamate receptors, some differences in distribution are known. For example, unlike NMDA and KA receptor sites, AMPA receptor sites are more enriched in the molecular layer of the cerebellum with lower levels in the granule cell layer. Unlike AMPA binding sites, KA sites are found in high concentrations in the deep layers of the neocortex, caudate-putamen, and stratum lucidum of hippocampus (Chittajallu et al., 1999; Monaghan and Cotman, 1982; Monaghan et al., 1984). Besides these differences in distribution, for the most part both AMPA receptors and KA receptors are found in very similar distribution patterns to each other (Monaghan and Cotman, 1982; Monaghan et al., 1984). However, finding regions in the brain that contain higher concentrations of either AMPA receptors or kainate receptors has become easier thanks to more advanced visualization

techniques (Barrera et al., 2008; Chittajallu et al., 1999; Fisahn, 2005; Jane et al., 2009).

Because AMPA receptors and KA receptors are voltage-independent, unlike NMDA receptors, these receptors mediate the fast excitatory synaptic transmission (Cooper, 2003). Kainate was first discovered and purified from the algae *Digenea simplex* (kainate) and quisqualate from seeds of a plant *Quisqualis fructus* (AMPA). These compounds are potent glutamate agonists and early on it was theorized that these two agonists acted on a specific subset of receptors (Watkins, 1981; Watkins et al., 1981; Watkins and Evans, 1981). Both AMPA and kainate receptors are also found throughout the region of the DMH and inhibition of these receptors in the DMH has been shown to attenuate the increase in temperature induced by microinjections of PGE_2 in the POA of anesthetized rats (Madden and Morrison, 2004). Furthermore, inhibition of these receptors by microinjections of 6-cyano-7-nitroquino-xaline-2,3-dione (CNQX) in the DMH also attenuates the increase in heart rate induced by stress (Soltis and DiMicco, 1992b).

AMPA receptors are composed of subunits GluR1-4, products from separate genes. GluR subunits have an extracellular N-terminus and an intracellular C-terminus, and the ligand binding domain is made up from N-terminal regions, and like the NMDA receptor, it is possible that the binding site is spread across more than one subunit. Native AMPA receptor channels are impermeable to calcium, a function controlled by the GluR2 subunit. This, along

with the interactions with other intracellular proteins, makes GluR2 perhaps the most important AMPA receptor subunit (Isaac et al., 2007).

Kainate receptors share many of the same structural characteristics with NMDA receptors and AMPA receptors; nonetheless, kainate receptors constitute a group of proteins distinct from AMPA and NMDA receptors. Watkins and coworkers first described the kainate receptor as a unique and distinct receptor from the binding sites activated by NMDA and AMPA. Mainly, they reported that kainate produced selective depolarization of isolated dorsal root fibers (Davies and Watkins, 1981; Watkins, 1981). Early studies based solely on pharmacological and radioligand binding assays (Monaghan et al., 1989; Verdoorn et al., 1989), did support the existence of a separate class of molecules selective for binding kainate. However, the fact that a given neuron could exhibit a rapidly desensitizing response upon the application of AMPA and a non-desensitizing response to kainate was interpreted as an indication that each ligand was acting on a separate molecular entity. It was cloning of glutamate receptor subunits, specifically of the kainate receptor subunits, which led to the unequivocal discovery that kainate receptors indeed showed a strong preference for kainate over AMPA (Dingledine et al., 1999; Hollmann and Heinemann, 1994).

Like the other ionotropic glutamate receptors, kainate receptors have an extracellular N-terminus that helps form the ligand binding domain and a re-entrant loop that forms the lining of the pore region of the ion channel. Because of the lack of kainate receptor-selective agonists, research into kainate receptors has lagged behind that for AMPA and NMDA receptors. To date, five kainate

receptor subunits have been identified which include GluR5-7 and KA1 and KA 2 (Jane et al., 2009). These kainate receptor subunits are subject to both alternative splicing and RNA editing which increase the number of subunit isoforms (Chittajallu et al., 1999; Huettner, 2003). However, it was the discovery and use of specific antagonists at the AMPA and kainate receptors that helped clarify this receptor's function.

In 1982, McLennan, studied the action of six different antagonists of the ionotropic glutamate receptors and found that glutamic diethyl ester (GDEE) blocked depolarizations produced by AMPA but did not affect KA-induced responses (McLennan, 1982a; McLennan, 1982b). Likewise, several compounds affect KA activity but have less effect on AMPA activity (Monaghan et al., 1989). Until recently, there were not specific antagonists that could clearly separate KA and AMPA responses and thus these two receptors were often referred to collectively as non-NMDA receptors. However, the availability of specific agonists and antagonists for the KA receptor now permits investigation both in vivo and in vitro of possible physiological roles for this receptor (Pinheiro and Mulle, 2006). For this thesis work, a non-NMDA receptor antagonists was used, 6-nitro-7-sulfamoylbenzoquinoxaline-2, 3-dione (NBQX), a potent and competitive antagonist at both AMPA and kainate receptors (Honore et al., 1988; Honore, 1989; Verdoorn et al., 1989). NBQX was either microinjected alone or mixed with APV, the NMDA competitive antagonist, into the DMH of conscious rats to investigate the role of these receptors in the increase in temperature produced by stress. All of these ionotropic glutamate receptor subtypes have

been observed in the region of the DMH and are targets for the study of the role of the DMH in the thermogenesis produced by stress.

D. Ionotropic glutamate receptor antagonists used: Kynurenate, APV and NBQX

i. Kynurenate

Kynurenate is a general ionotropic glutamate receptor antagonist, and one of the drugs used in this thesis to inhibit ionotropic glutamate receptors in the region of the DMH. Kynurenic acid or kynurenate is an endogenous metabolite of tryptophan degradation. The products of tryptophan metabolism via the kynurenine pathway include both quinolinic acid and kynurenic acid (Heyes, 1993). Evidence for nonspecific blockade by kynurenate of excitatory amino acid sensitivity on some neurons was confirmed by work in the spinal cord, hippocampus, neocortex, and caudate nucleus (Curry et al., 1986; Peet et al., 1986a; Peet et al., 1986b) in which kynurenate was shown to be capable of antagonizing responses to quinolinic acid, NMDA, kainate, and AMPA. Since the recognition of kynurenate as a non-specific excitatory amino acid antagonist, it has become a popular agent because of its ease of use, efficacy and inexpensiveness. However, the use of kynurenate to antagonize ionotropic glutamate receptors nonspecifically is an accepted and widely used technique.

Kynurenate has complicated actions on the NMDA receptor complex. Low concentrations act selectively at the glycine site, whereas high concentrations act directly at the NMDA recognition site (Ganong and Cotman, 1986). It has been observed that kynurenate's mode of action is not simple competitive antagonism at the NMDA receptor. Birch and colleagues (1988a; 1988b) first observed that

kynurenate acts as a competitive antagonist of kainate and AMPA receptors without any selectivity but acted as an insurmountable antagonist of NMDA in the rat hemisected spinal cord. They also reported that the insurmountable antagonism could be reversed by superfusion with L-serine or glycine, and in the presence of these agents, kynurenate then acted only as a weak or competitive antagonist (Birch et al., 1988a, b). In fact, in some situations in which glycine does not enhance basal NMDA sensitivity, glycine can reverse the kynurenate-inhibition of the NMDA receptor suggesting that it acts as a competitive antagonist at the glycine site (Pralong et al., 1992; Stone, 1991).

On postsynaptic receptors, kynurenate exhibits a dual mode of action, partly blocking NMDA by an action at its receptor recognition site on the receptor and partly by displacing glycine from its allosteric modulatory site associated with the receptor (Ascher et al., 1988; Evans et al., 1987; Kemp et al., 1988; Mayer et al., 1988). Using patch-clamp technique in cortical cultures, NMDA responses were antagonized noncompetitively by kynurenate with an IC_{50} of 70µM, whereas kainate response were antagonized competitively at higher concentrations (ID_{50} 500µM) (Bertolino et al., 1989). Binding studies suggested that kynurenate was able to displace glutamate binding at NMDA receptors (IC_{50} 184 µM) and AMPA binding (IC_{50} 101µM), but it was less effective at displacing kainate binding (IC_{50} 2082 µM). However, in the same study, Kemp and colleagues (1988) confirmed that kynurenate antagonized electrophysiological responses to kainate, suggesting that perhaps kynurenate antagonizes kainate by acting at a site other than the ligand-binding site. Overall, kynurenate has been proven to be an

effective excitatory amino acid receptor antagonist, and many studies have proven its efficacy, including work in our lab that has helped establish the importance of the use of this drug in microinjection studies.

Microinjections of kynurenate 10nmol/100nL (0.1 M) into the DMH have shown that blockade of glutamate receptors in the region of the DMH attenuates the cardiac response to either stress or to disinhibition of neurons in the DMH (Soltis and DiMicco, 1991a, 1992). Similarly, microinjections of kynurenate into the DMH abolished the increase in temperature produced by microinjection of PGE_2 in the POA of anesthetized rats (Madden and Morrison, 2004). For this thesis, kynurenate was employed as one of the antagonists to study the role of ionotropic glutamate receptors because of its previous utility. Likewise, using this antagonist in conscious and freely moving rats specifically would permit direct comparisons with results published using this same antagonist in the anesthetized rat (Madden and Morrison, 2004).

ii. 2-amino-5-phosphonopentanoic acid (APV)

In 1979, Davies was the first to describe APV as a potent and selective NMDA receptor antagonist. APV blocked L-aspartate and dorsal root-evoked excitation of spinal neurons, but it had no effect on the cholinergic excitation of Renshaw cells evoked by exogenous acetylcholine or ventral root stimulation (Davies and Watkins, 1979; Davies et al., 1981b). Microinjections of APV have been used previously in our lab at a dose of 100pmol/100nL (1mM) and shown to specifically inhibit increases in heart rate induced by microinjection of NMDA into the DMH (Soltis and DiMicco, 1991a; Soltis and DiMicco, 1992b). This same

dose of APV was shown to effectively attenuate the increase in heart rate induced by air-jet stress (Soltis and DiMicco, 1992b). APV has also been microinjected into the raphe pallidus at a dose of 300pmol/60nL (5mM) and shown to effectively inhibit the increase in temperature induced by disinhibition of neurons in the DMH (Cao and Morrison, 2006).

iii. 6-nitro-7-sulfamoylbenzoquinoxaline-2, 3-dione (NBQX)

Some of the most selective and potent non-NMDA antagonists available are a series of dihydroxyquinoxaline derivatives which include 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione 9 (NBQX), 6-cyano-7-nitroquino-xaline-2,3-dione (CNQX), and 6,7-dinitroquinoxaline-2,3-dione (DNQX). These drugs are potent antagonists that competitively block both AMPA and KA receptors as was shown in electrophysiological and binding studies in rat cortical membrane (Honore et al., 1988). NBQX was shown to block responses of spinal neurons in vivo to kainate, quisqualate, and AMPA in parallel but had little effect on responses to NMDA (Lodge et al., 1991). This drug has been microinjected into various regions of the hypothalamus at doses as high as 20mM (Busnardo, 2009; Deolindo et al., 2008; Jardim and Guimaraes, 2004). Recently, de Menezes and colleagues showed that microinjection of a combination of the glutamate receptor antagonists APV and NBQX at a dose of 1mM into the caudal lateral/dorsal lateral PAG decreased stress-induced increases in heart rate (de Menezes et al., 2006). For this thesis, the dose used was 100pmol/100nL (1mM) microinjected into the DMH.

IV. Thesis Goals

The studies in this thesis investigated the role of ionotropic glutamate receptors in the of the region of the DMH in the thermoregulatory response to stress. Stress can be categorized as either interoceptive or exteroceptive and there is no consensus about how these two apparently different types of stresses are mediated by the central nervous system. Compelling evidence supports the idea that the DMH plays a key role in the physiological responses to both exteroceptive and interoceptive stress, including the increase in temperature. However, whether the DMH mediates thermoregulatory responses to different types of stress has not been studied. The work presented here can lead to better understanding of the hypothalamic mechanisms involved in the central control of not just the increase in body temperature but of the other physiological responses to stress. A better understanding of the central control of these responses can lead to new drug discovery and perhaps better treatments or prevention of stress-related illnesses. Therefore the specific aims of this thesis are

1) To determine the role of ionotropic glutamate receptor subtypes in the DMH play in the thermoregulatory response to exteroceptive and interoceptive stresses in the conscious rat
2) To differentiate the role of NMDA and non-NMDA receptor subtypes in the thermoregulatory response to exteroceptive and interoceptive stresses in the conscious rat.

CHAPTER 2: METHODS

For this thesis, I studied the role of ionotropic glutamate receptors in the DMH in the temperature response to both interoceptive and exteroceptive stressors in conscious untethered rats. Most of the techniques used in this study were previously used in our lab and/or adapted for use in conscious untethered rats. All of the surgical and experimental procedures were approved by the Indiana University's Institutional Animal Care and Use Committee (IUACUC).

I. Animals

Male Sprague-Dawley rats (270-330g, Harlan Industries, Indianapolis, IN) were individually housed under controlled temperature, humidity and light periodicity (12 hour light-dark cycle with the lights turning on at 0700 hr) with free access to rat chow and water in the university's Laboratory Animal Resource Center (LARC). All experiments were carried out between 9am and 2pm to reduce the effects of circadian rhythm variation. On days of surgery or experimentation, the rats were transferred within-building to the laboratory and returned to LARC thereafter.

II. Experimental Design

In order to study the role of ionotropic glutamate receptors in the DMH in the temperature response to stress, I designed experiments that studied the thermogenic response to both exteroceptive and interoceptive stresses. The three major subtypes of ionotropic glutamate receptors have been defined pharmacologically and are named for the relatively selective agonists NMDA,

AMPA, and kainate. Of all three, NMDA receptors are the best characterized pharmacologically. AMPA and KA receptors are not as well characterized because of the lack of selective agonists that can differentiate sufficiently between the AMPA and KA receptor-mediated responses. For this reason, NBQX, an AMPA/KA receptor antagonist, and APV, an NMDA receptor antagonist, were used. Kynurenate a non-specific ionotropic glutamate receptor antagonist was also used.

Previous studies in conscious animals indicated that under the experimental conditions used, 2-amino-5-phosphonopentanoic acid (APV) is a selective antagonist for the NMDA receptor. Similarly, the dose of 6-nitro-7-sulfamoylbenzoquinoxaline-2,3-dione (NBQX), a selective antagonist of AMPA and KA receptors, was chosen based on the results of preliminary experiments. For each experiment described, animals served as their own control, so that each animal received all treatments (control and experimental) in random order on alternate days.

The first two sets of studies were designed to assess the role of ionotropic glutamate receptors in the DMH in the thermogenic response to an exteroceptive stress. The first experiment examined the role of ionotropic glutamate receptors in the DMH in the effect of air-jet stress on body temperature. Air-jet stress is a stress paradigm that has been used in stress studies in a number of variations (Blanc et al., 1991; Lam et al., 1995; Martin et al., 1996). The particular paradigm for air-jet stress that I employed has been previously used in stress studies in this laboratory (Lisa et al., 1989b; Morin et al., 2001; Sarkar et al.,

2007; Soltis and DiMicco, 1992a). Because of the unexpected finding that this paradigm might be complicated by cold stress, I employed cage-switch stress as my experimental paradigm for the remainder of my studies of exteroceptive stress. Cage-switch stress paradigm does not have the cold stress confound discovered in the air-jet stress paradigm. In addition to employing kynurenate as a non-selective glutamate antagonist in these experiments, I also used a combination of ionotropic glutamate receptor antagonists NBQX and APV. I hoped that this would allow me to differentiate the role of specific glutamate receptor subtypes, AMPA/kainate versus NMDA receptors, in the changes noted. Also, these agents are effective at much lower concentrations than kynurenate which, because of the high concentration needed for effective blockade of glutamate receptors, is much more likely to produce non-specific effects. Thus, the second set of experiments was designed to determine the effect of microinjection of a combination of NBQX and APV into the DMH on the thermogenic response to cage-switch stress.

The third and fourth set of experiments were designed to study the role of ionotropic glutamate receptors in the DMH in the thermogenic effect of microinjection of PGE_2 in the POA, a well known model for experimental fever, or effect of microinjection of BMI in the DMH. Both experimental paradigms have been used extensively with reproducible results in our lab and by others (Cao et al., 2004a; Madden and Morrison, 2004; Morrison, 2004; Nakamura et al., 2005b; Zaretskaia et al., 2002; Zaretskaia et al., 2003; Zaretsky et al., 2006). For each

paradigm, I examined the effects of microinjections of NBQX and APV, both individually and in combination.

A. Chronic studies: Effects of microinjection of ionotropic glutamate receptor antagonists into the DMH on increases in temperature produced by exteroceptive stress

The experiments described here were all conducted in conscious and freely moving rats using the microinjection technique developed in our lab. For these experiments, both air-jet stress and cage-switch stress were used as models for exteroceptive stress. All animals for the exteroceptive stress experiments were implanted with telemetric probes for the real-time monitoring of core body temperature. After at least three days of recovery, bilateral guide cannulas targeting the DMH were implanted. Finally, all rats were allowed at least three days of recovery before experimentation.

On the day of the experiment, the rats were brought to the testing facility and placed in their home cages inside the experimentation rooms. Their cages were placed on telemetric receiver plates, and dummy wires were removed from the guide cannulas. The rats were then left undisturbed for at least two hours to establish resting baseline parameters.

i. Effects of microinjection of kynurenate into the DMH on stress hyperthermia produced by air-jet stress

Microinjection of kynurenate has been used in our lab effectively to inhibit ionotropic glutamate receptors in the DMH. Bilateral microinjection of kynurenate into the DMH of conscious rats was shown to attenuate the tachycardia evoked by air-jet stress (Soltis and DiMicco, 1992a). Considering that both tachycardia

and increase in temperature are physiological responses evoked by stress, the goal of this experiment was to investigate the hypothesis that the increase in body temperature seen in this paradigm was also mediated by ionotropic glutamate receptors in the DMH. Seven rats were used, each serving as its own control. In unstressed trials, the rat remained in its home cage and the only manipulation or interaction that occurred was during the microinjections. During microinjections, the dummy wires were unscrewed and the microinjectors were then placed into the cannula. The rat was not restrained or held at any point during the microinjections.

ii. Effects of microinjections of ionotropic glutamate receptor antagonists into the DMH on stress hyperthermia produced by cage-switch stress

The goal of these experiments was to assess the role of ionotropic glutamate receptors in the DMH in the thermogenic response known to be induced by cage-switch stress. Cage-switch stress involves removing a rat from its "home" cage and placing it in a new clean cage with new bedding. The novel environment appears to constitute a mild stress that has been shown to produce an increase in core temperature (Kluger et al., 1987; Rowsey et al., 2002; Singer et al., 1986; Soszynski et al., 1996). Cage-switch stress, unlike air-jet stress, does not involve a cold-stress (see Results), and therefore is the stress-paradigm used for all other studies of effects of exteroceptive stress.

Rats were placed in the experimental rooms and left undisturbed for at least two hours to establish a resting baseline for core temperature. The cage-switch stress paradigm consisted of quickly removing the rat from its home cage and placing it in an identical cage with fresh bedding. Once the rat was switched

to a new cage it remained in it for the remainder of the experiment. In unstressed trials, the rat remained in its home cage and the only manipulation or interaction that occurred was during the microinjections. Animals were pretreated five minutes before the cage-switch stress with bilateral microinjections into the DMH of either 1) kynurenate (10pmol/100nL), 2) combination of NBQX (100pmol/100nL) and APV(200pmol/100nL), 3) NBQX (100pmol/100nL) alone, or 4)APV (200pmol/100nL) alone.

The pretreatment of animals with microinjections of NBQX alone or APV alone in the DMH followed by exposure to cage-switch were included to study the role of NMDA and non-NMDA receptor subtypes in the thermogenic response to cage-switch stress. The assumption is that all ionotropic glutamate receptors are required for the thermogenic response to stress. If so, then blockade of either NMDA or non-NMDA receptors alone would produce less attenuation of the thermogenic response to cage-switch compared to the attenuation seen when all ionotropic glutamate receptors are inhibited.

iii. Effects of microinjection of ionotropic glutamate receptor antagonists into the DMH on increases in body temperature produced by interoceptive stressors

Microinjections of PGE_2 in the POA and bilateral microinjections of BMI into the DMH have been shown to produce an increase in body temperature. I hypothesized that microinjection of glutamate receptor antagonists would attenuate increase produced by either of these interventions. As mentioned in the introduction of this thesis, microinjections of PGE_2 in the POA produce an increase in temperature that is abolished by microinjections of muscimol in the DMH, suggesting a vital role for the DMH in the neural signaling pathway for the

thermogenic response to this interoceptive stressor. The main goal of the studies described below was to determine whether ionotropic glutamate receptors in the DMH are involved in the increase in temperature evoked by either microinjection of PGE_2 in the POA or unilateral microinjection of BMI in the DMH.

All animals for these experiments were implanted with telemetric probes for the real-time monitoring of core body temperature. After at least three days of recovery, bilateral guide cannulas targeting the DMH and a single cannula targeting the POA were implanted. Finally, all rats were allowed at least three days of recovery before experimentation.

On the day of the experiment, the rats were brought to the testing facility and placed in their home cages inside the experimentation rooms. Their cages were placed on telemetric receiver plates, and dummy wires were removed from the guide cannulas. The rats were then left undisturbed for at least an hour to establish resting baseline parameters.

In order to understand the role of ionotropic glutamate receptors in the DMH in the thermogenic response to an interoceptive stress, either a combination of NBQX and APV or saline vehicle was microinjected into the DMH five minutes before microinjection of PGE_2 in the POA in conscious and freely moving rats. Rats served as their own controls and each rat received all four treatments in random order: 1) bilateral microinjection of vehicle (aCSF) in the DMH followed by a microinjection of vehicle (aCSF) in the POA; 2) bilateral microinjection of vehicle in the DMH followed by microinjection of PGE_2 in the

POA; 3) bilateral microinjection of the combination of NBQX and APV in the DMH followed by microinjection of vehicle in the POA; 4) bilateral microinjection of NBQX and APV in the DMH followed by microinjection of PGE_2 in the POA. In order to differentiate effects mediated by NMDA and non-NMDA receptor subtypes, two other sets of animals were microinjected with either NBQX alone or APV alone followed by microinjection of PGE_2 in the POA in a similar experimental design.

Finally, to investigate whether ionotropic glutamate receptors in the DMH play a role in the increases in body temperature produced by the disinhibition of neurons in the DMH, a set of rats received the following treatments in random order and on alternate days: 1) unilateral microinjection of aCSF in the DMH followed by unilateral microinjection of BMI into the same side of the DMH five minutes later or 2) unilateral microinjection of NBQX or APV followed by unilateral microinjection of BMI in the DMH five minutes later. The goal of this experiment was to determine the role that NMDA and non-NMDA glutamate receptors play in the thermogenic effect seen with microinjection of BMI in the DMH.

III. Chronic Preparations: Studies in conscious rats

A. Anesthesia

Animals were anesthetized using a combination of ketamine/xylazine (80 mg/kg ketamine, 11.5 mg/kg xylazine; i.p.; supplement as needed) in preparation for all of the surgical procedures performed. After the surgical procedure, the rats were placed in their home cage atop a heating plate in order

to support their core temperature until they recovered from the anesthesia. Animals undergoing multiple surgical procedures were allowed at least three days of recovery between procedures.

B. Stereotaxic Surgery: Implantation of chronic guide cannula targeting specific brain regions

The purpose of implanting chronic guide cannula is to allow acute delivery of drug solutions to specific brain regions. This technique of implanting chronic guide cannulas has been used successfully and extensively in our laboratory (Bailey and Dimicco, 2001; De Novellis et al., 1995a; Shekhar et al., 1990; Stotz-Potter et al., 1996a; Stotz-Potter et al., 1996b; Zaretsky et al., 2006).

In preparation for the surgery, the top of the head and nape of the neck were shaved, and Betadine was then applied to the shaved areas. The anesthetized rat was then placed in a sterile stereotaxic apparatus (Kopf). To fix the rat on the apparatus, the metal bars were inserted firmly into the bony processes of the ear canal creating an imaginary line between the two ear bars, known as the inter-aural line. The inter-aural line was used as a line of reference to determine appropriately the position of the head in space. Next, the rat's skull was further fixed and aligned by positioning the upper incisor bar behind the rat's incisors and the rat's nose is held firmly down against the bar. The position of the incisor bar determined the vertical-horizontal angle of the skull and thus the angle at which the guide cannula entered the brain at time of implantation. The choice of angle was dependent on the experimental design; therefore, the incisor bar was positioned at 3.3 mm below the inter-aural line for implantation of guides

targeting the DMH or 5.0 mm above the inter-aural line for implantation of the guides targeting the POA.

The skin overlying the dorsal surface of the skull was cut with a scalpel and retracted, and soft tissue was removed from the exposed surface. Cotton swabs wet with hydrogen peroxide were used to clean out any remaining connective tissue or fascia covering the skull and the dorsal surface of the skull was rinsed off with sterile saline. The use of hydrogen peroxide not only clears away remaining tissue but it makes the suture lines more visible. Using the tip of a 23 gauge needle, bregma, the point of intersection between the frontal-sagittal and coronal suture lines, was marked. Determination of the coordinates for both the DMH and the POA was determined using established atlases of the rat brain (Paxinos, 2007). Using bregma as a reference point for all coordinate measurements, a sterilized 26 gauge guide cannula (Plastics One Inc., Roanoke, VA, USA) was mounted in the arm of the stereotaxic device. For placement of guide cannula targeting the DMH, the arm was angled at 10° from the sagittal plane. The arm was lowered to the skull until the tip of the guide was centered on bregma. The coordinates for this position were taken and target coordinates were calculated. The arm was moved to the calculated coordinate position and lowered on to the skull. A pencil was used to mark the position of the guide at the specified coordinate. After all markings were made, the arm with the guide cannula was moved up and away to allow room to drill the hole in the skull. A Dremel drill with a carbide bur (Miltex) was used to bore a 3-5 mm diameter hole in the skull at the approximate position where the guide would enter the brain.

To prevent the drill bit from becoming too hot, saline was continuously applied to the skull. Using the same drill bit, additional small holes were placed in the frontal and parietal bones for the insertion of anchoring stainless steel screws (Plastics One, Inc.).

The guide cannula was then placed above the predetermined coordinate positions and lowered into the brain. For targeting the DMH, two guide cannulas targeting the right and left side of the DMH were positioned for microinjection targeting sites 3.1 mm posterior, 2.0 mm lateral, and 7.2 mm ventral with respect to bregma. The tip of the guide cannula was positioned 1 mm above the desired targeted region. The microinjector itself extended exactly 1 mm beyond the guide cannula to target the exact region of interest. For some experimental protocols implantation of three guide cannulas was required including bilateral guide cannulas targeting the DMH and a single guide cannula targeting the POA. For these animals, two guide cannulas were placed in the DMH first at the coordinates described above. Three sterilized stainless steel screws were placed on the right and left side of the exposed posterior skull behind the two guide cannula and one on the right side of the drilled hole made for the implantation the guide to the POA. Vetbond tissue adhesive (3M, Inc.) and dental acrylic (Lang Dental Manufacturing Co., Inc) were then added to the posterior region of the skull surrounding the guide cannula and the two screws to fix and anchor the two guide cannulas. Once the two guide cannulas were firmly anchored, the incisor bar initially set at 3.3 mm below the inter-aural line, was moved to 5.0 mm above the inter-aural line. Using bregma as a reference point,

a single guide cannula was inserted at a 10° angle from the sagittal plane and targeted to 1.9 mm anterior, 1.9 mm lateral, and 6.9 mm ventral coordinates. The guide was then secured with Vetbond and dental acrylic. Dummy cannulas were then inserted to seal the guide cannulas and prevent clogging. The animals were removed from the stereotaxic frame and allowed to recover from anesthesia in their individual cages set on a warm plate.

C. Implantation of telemetric probes

For this thesis, a Dataquest telemetry system (Data Sciences, MN, USA) was used for measurement of core body temperature and locomotor activity. (Locomotor activity is calculated from changes in strength of the transmitter signal over time, where an activity unit is approximately equivalent to movement of 1 cm s^{-1}). Telemetric probes allow monitoring of physiological parameters via radio transmission in the undisturbed, conscious animal. Rats were anaesthetized (see Section III.A) and their abdomen was shaved and then painted with Betadine solution. A one-inch incision was made in the abdomen along the linea alba with scissors exposing the peritoneal cavity. The body of a telemetric probe (PhysioTel® TA-F40, Data Sciences Int.) previously sterilized with Cidex solution (Advanced Sterilization Products) and flushed clean with sterile saline was placed into the peritoneal cavity. The abdominal muscle wall was then closed with 3-0 suture followed by suturing of the abdominal skin. Animals were allowed to recover from anesthesia in their individual cages set on a warm plate.

D. Post-operative care

Per the training guidelines and survival surgery protocols established by the Institutional Animal Care and Use Committee (IACUC), all animals were closely monitored for their well-being before and after any surgical procedure. Following any surgical procedure, an analgesic was administered (buprenorphine, 0.02 mg/kg, s.c.) while the rats recovered on a warming plate to minimize hypothermia caused by the anesthesia. The animals were monitored throughout the recovery period and only after they regained consciousness were the animals returned to LARC. The animals were weighed daily until the day of experimentation and then every other day during experimentation. Their well being was assessed every day until the first day of experimentation. Rats that were deemed unhealthy were excluded from experimentation. Persistent weight loss (i.e. 20 grams of weight loss daily over a period of three days), inflammation or infection of surgical incisions, or changes in behavior warranted exclusion from experimentation and euthanasia. Before experimentation, dummy wires were moved in and out of the guide cannulas to prevent clogging of the guide as well as to habituate the animal to this manipulation before microinjection experiments. Animals with clogged guide cannulas were excluded and euthanized.

Post-operative care was imperative to ensure that each rat used in the experimentation protocols was healthy. After the last surgical procedure, the animals were allowed to recover for at least three days before undergoing any experimental protocols.

IV. Experimental Techniques

A. Testing facilities

All experimentation took place inside two isolated rooms within the laboratory that were temperature controlled (21-25°C). The rooms were equipped with video cameras that allowed continuous monitoring of the rats in their cages during the experimentation process. The telemetric receivers were kept inside these rooms and configured in such a way that the real-time reading from the telemetric probes was received in the central processing units (CPUs) kept outside the rooms. This configuration was optimal in that the rats could be left completely undisturbed by persons walking into the lab, noise, or smells. Each room could accommodate up to six rat cages comfortably, which allowed me to run experiments for all groups in each experimental protocol at the same time.

B. Telemetric monitoring

All animals were instrumented with telemetric probes that were vital for the results presented in this thesis. I used a Dataquest telemetry system (Data Sciences Int.) to monitor both temperature and motor activity of the rat. Data was transmitted via radio signal from the implanted probe (PhysioTel® TA-F40, Data Sciences Int.) to a receiver plate (RPC-1, Data Sciences Int.) placed under the home cage of the animal. The receiver plate transferred the data via a data cable to the hard drive of a central processing unit (CPU) located outside of the testing room. The accompanying Dataquest software converted the signal from

the receiver into a real time readout that recorded the parameters, in this case the minute-by-minute change in temperature or motor activity of the animal.

C. Microinjection technique

On the day of the experiment, rats were placed in their home cages on the telemetry receiver plates. When a stable baseline of core body temperature had been attained (1-2 hours), dummy cannula(s) were then removed, the microinjector(s) (33 gauge, Plastics One Inc.) connected to a 10 µl Hamilton syringe with Teflon FEP tubing (i.d. = 0.12 mm; o.d. = 0.65 mm; BAS, USA) was inserted into the guide cannula(s). The Hamilton syringes were loaded with appropriate solutions for microinjection. All solutions for microinjections contained 4% fluorescent microspheres used to mark sites of injection. The Hamilton syringe was mounted in an infusion pump (Sage, Boston, MA, USA) that was used to deliver a volume of 100 nL/side of injectate over 30s. At the end of the infusion, the microinjector was left in place for 1 min. The microinjection was considered successful if, immediately after removal of the microinjector, flow appeared within 5 s after the pump was reactivated, indicating that the injector was not clogged and that injectate had been successfully delivered.

V. Stress Paradigms

A. Air-jet stress

The air-jet stress paradigm is an experimental procedure that has been described previously and used in our laboratory (Stotz-Potter et al., 1996a,b; Zaretsky et al., 2003). I learned from the results of this experiment that the

continuous stream of air significantly dissipates body heat, meaning that the air-jet stress paradigm may have a potentially confounding effect of a “cold stress”. Therefore, the air-jet stress paradigm includes a combination of both restraint and cold-stress.

The air-jet stress paradigm consists of placing the rats to be stressed in a Plexiglas tube (21 cm in length, 7 cm in diameter), sufficiently restrictive as to prevent them from reversing direction, with an aperture (1 cm) at one end of the cylinder connected by latex tubing to an air-jet so that a stream of air at a constant and specific flow rate (40 L/min) was directed at the rat’s face. The aperture was placed approximately 5 cm in front of the rat’s head. The stream of air was delivered for a period of 10 min, starting 5 min after the microinjection. At the end of the 10-min period, the air was turned off and the rat released from the tube into its home cage and continuously monitored for 120 min. In unstressed trials, the rat remained in its home cage and the only manipulation or interaction that occurred was during the microinjections.

B. Cage-switch stress

The cage-switch stress paradigm consisted of quickly removing the rat from its home cage and placing it in an identical cage with fresh bedding. Once the rat was switched to a new cage it remained in the new cage for the remainder of the experiment. In unstressed trials, the rat remained in its home cage and the only manipulation or interaction that occurred was during the microinjections (Groenink et al., 2003b; Oka et al., 2003; Olivier et al., 2003; Spooren et al., 2002; Watanabe et al., 1999).

VI. Drugs

Stock solutions of all drugs used in the experimental protocols were prepared ahead of time. Aliquots of the stock solutions were stored at −20°C, until the day of the experiment. Enough stock solution of each drug needed was made so that the same drug solution(s) were used for each trial of any of the experimental protocols described above minimizing any effect due to differences in drug makeup. In addition, to help with verification of site injection, all solutions used in the microinjections contained different colored fluorescent-embedded, polystyrene microspheres (4% v/v, Molecular Probes). For each treatment I used a different color of fluorescent microspheres. I then used fluorescent microscopy to look at brain slices and verify drug delivery to the targeted regions. Because of the different colors used, I could also verify the delivery of each treatment.

A. Artificial cerebrospinal fluid (aCSF)

For some controls artificial cerebrospinal fluid was used as a vehicle for the microinjection of APV, BMI, NBQX and PGE_2. The aCSF solution (122 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 20 mM NaH_2PO_4, 11 mM $C_6H_{12}O_6$ in deionized water; Osmolality 300 mOsm/kg) was sterilized with a 22 µm sterile filter (Millipore) and stored at −20°C in sterilized Eppendorf tubes for up to one year.

B. Kynurenate and vehicle for kynurenate

Kynurenic acid (1 g; Sigma Aldrich, Cat no K3375) was first dissolved in 10 mL of 1 N NaOH and pH was adjusted to 7.4 by adding acid 4N HCl. Finally, 0.9% sterile saline was added gradually to a final concentration of 0.1 M. The pH was checked continuously and adjusted to a final pH 7.0-7.4. The solution was then sterilized with a 22 µM sterile filter (Millipore). The solution was stored in 1mL aliquots at −20°C to ensure stability of the solution. On the day of the experiment frozen kynurenate aliquot was thawed and then microinjected at 10nmol/100nL. The vehicle control for kynurenate was prepared as just described without the drug.

C. APV, NBQX, and APV+NBQX combination solution

A 25 mM stock solution of D-2-Amino-5-phosphonovaleric acid (APV; Sigma Aldrich, Cat no. A-8054) was prepared with aCSF as the solvent. The stock solution was stored in 20µL aliquots at −20°C. On the day of the experiment, the stock solution was reconstituted and diluted with aCSF to a final concentration of 2 mM or 200 pmol/100nL. Similarly, a 50 mM stock solution of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX; Tocris Biosciences, Cat no. 1044), was dissolved in aCSF, aliquoted, and stored at -20°C. It was later diluted to a final concentration of 1 mM or 100 pmol/100nL. A combination of both APV and NBQX was prepared in aCSF so that the final solution contained 200 pmol APV/100pmol NBQX in 100nL. Artificial CSF was used as the vehicle control for APV, NBQX and the combination of the two.

D. Bicuculline methiodide (BMI)

A 4 mM stock solution of (-)-bicuculline methiodide (BMI; Sigma Aldrich, Cat no: B6889) was prepared ahead of time. BMI has a molecular weight of 509.3g/mol with 20mg/1mL solubility in water. The stock solution was prepared using aCSF, previously prepared (see Section IV. A.), as the diluent. The stock solution was aliquoted into sterile microcentrifuge tubes and stored at −20°C until the day of the experiment. On the day of the experiment the stock solution was reconstituted and diluted further with aCSF to a final molar concentration of 200 µM or 20 pmol/100nL.

E. Prostaglandin E_2 (PGE_2) and vehicle for PGE_2

Prostaglandin E_2 (PGE_2)-dinoprostone (Cayman Chemicals, Cat no: 14010) was first dissolved in 1-methyl-2-pyrrolidinone (0.15% v/v, Sigma-Aldrich). It was then stored in 2µL aliquots in 6x50 mm glass tubes under argon gas. On the day of the experiment, the stock solution was further diluted with aCSF to a final molar concentration of 1.5 mM or 150 pmol/100nL.

VII. Histological Procedures

A. Perfusion and in situ fixation of brain tissue

Rats were deeply anesthetized with pentobarbital (100 mg/kg, i.p.). Rats were then subjected to transcardial perfusion. A 60 mL syringe containing 50mL of 0.9% normal saline and 15,000 U/I heparin sulphate was placed above the animal to allow gravity to set the speed of perfusion. The syringe was connected to a 2 foot plastic tube connected to a blunt 14 ga needle. After cutting open the pleural cavity, a small cut was made at the apex of the heart and the needle was

quickly threaded into the left ventricle and clamped in place with a pair of hemostats. Following perfusion with 50 mL of 0.9% normal saline and heparin, the animals was perfused with 120 ml of ice cold 4% buffered paraformaldehyde in 0.1M phosphate-buffered saline (PBS). The brain was then removed, and post-fixed in 4% buffered paraformaldehyde in 0.1M phosphate buffer overnight at room temperature, then transferred to 30% sucrose in 0.01M PBS solution and stored overnight at 4°C until saturation. Afterwards, brains were quick-frozen with dry ice and store at −80°C.

B. Preparation of brain sections for histology

Frozen brains were removed from −80°C storage and allowed to thaw to −20°C. Coronal sections (45μmicrons) in the region of hypothalamus and POA were cut on a cryostat. The sections were collected in 96 well plates filled with 0.01 M phosphate buffer (pH7.4). Afterwards the sections were mounted on glass slides and air dried overnight.

C. Verification of sites of microinjection

All solutions microinjected into the brain contained fluorescent-embedded, polystyrene microspheres (5% v/v, Molecular Probes) which facilitated locating the sites of injection with precision. Using a Leica microscope equipped with different absorbance filters for detection of fluorescence, the sites of injections were identified as the locations with the most intense fluorescence. The location of these intensely fluorescent sites was approximated using the atlas of Paxinos and Watson (2007) as reference.

VIII. Statistical Analysis

Results are expressed as means ± standard error of the mean (SEM). Data were analyzed by repeated measure One-way ANOVA (Fisher's LSD test used for post hoc analysis) or student t-test. Level of significance was set at $P < 0.05$. The baseline values for temperature and motor activity were calculated by taking the average of 20 minutes prior to the first microinjection.

CHAPTER 3: RESULTS

I. Role of ionotropic glutamate receptors in the DMH in increases in body temperature produced by exteroceptive stressors: Air-jet stress and cage-switch stress

The stress paradigms used for the following experiments are examples of exteroceptive stress paradigms. Both air-jet stress and cage-switch stress have been shown to produce significant increases in core body temperature. The following are the results of studies that address the potential role of ionotropic glutamate receptors in the DMH in the hyperthermia produced by exteroceptive stress in rats.

A. Pretreatment with kynurenate attenuates the increase in body temperature caused by exposure to air-jet stress

Microinjections of ionotropic glutamate receptor antagonists into the DMH attenuate the increase in heart rate and blood pressure produced by experimental stress (Soltis and DiMicco, 1992a). Experimental stress also produces an increase in core body temperature. In the following series of experiments, I examined the hypothesis that ionotropic glutamate receptors are involved in the increase in body temperature evoked by stress. Sprague Dawley rats were instrumented with bilateral guide cannulas targeting the DMH and a telemetric probe as described in the methods. In these experiments, either 100nL of vehicle or 10nmol/100nL of kynurenate was microinjected bilaterally into the DMH five minutes before exposure to a period of ten minutes of air-jet stress (experimental) or to no stress with rats left undisturbed in their home cage

(control; N=7 rats). Real time temperature changes were observed for a period of two hours. Statistical analysis was done for the average change in temperature during the 20 minute period immediately following stress or the same time period during which control rats were left undisturbed in their home cage and this period is depicted in all graphs with a gray box.

The average body temperature at baseline for all rats for which data are reported was 36.96°C±0.13. In vehicle treated animals, average body temperature at baseline was 36.88°C±0.07, and compared to control, exposure to air-jet stress produced a significant increase in body temperature (average = 0.66±0.11°C) over the 20 minute period after stress, with an average peak of 1.04°C ±0.11 (Figure 1). Temperature did not return to baseline levels for the duration of the observation period. This increase in temperature began almost immediately after exposure to the air-jet stress.

Microinjections of kynurenate into the DMH of control rats produced a decrease in temperature that was not significantly different from baseline but significantly different from the response to vehicle in control rats. However, the most dramatic and unexpected effect observed was the hypothermia seen during and after exposure to air-jet stress in rats microinjected with kynurenate in the DMH.

After microinjection of kynurenate into the DMH air-jet stress produced a dramatic and significant average decrease in temperature (−0.92±0.10°C) with a peak decrease of −1.12±0.09°C over the twenty minute period after stress. Temperature gradually returned to baseline levels during the observation period.

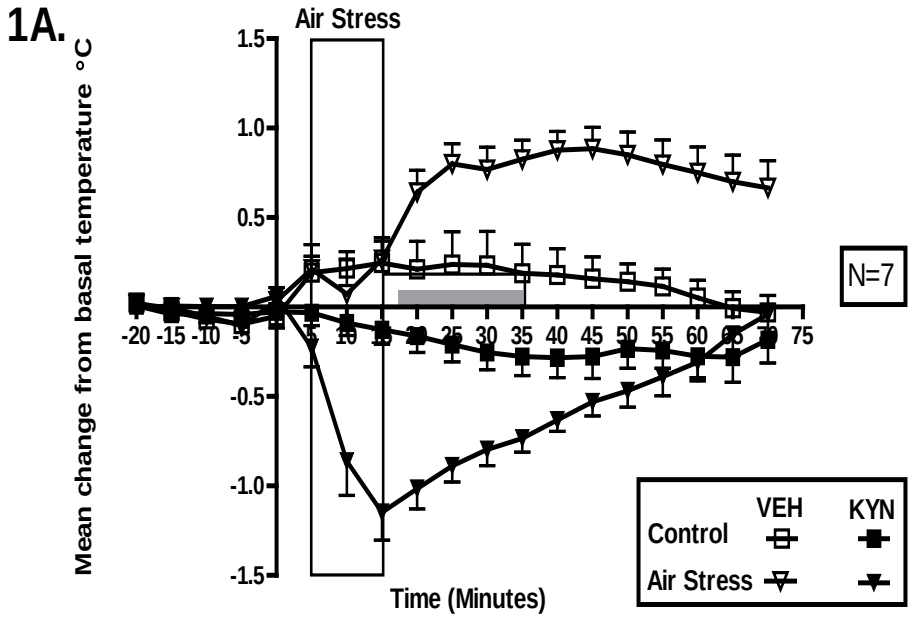


FIGURE 1A. Real time temperature response to pretreatment with kynurenate (10nmol/side) into the DMH followed by exposure to air-jet stress. Time zero indicates point of microinjection of kynurenate. White box depicts exposure to air-jet stress.

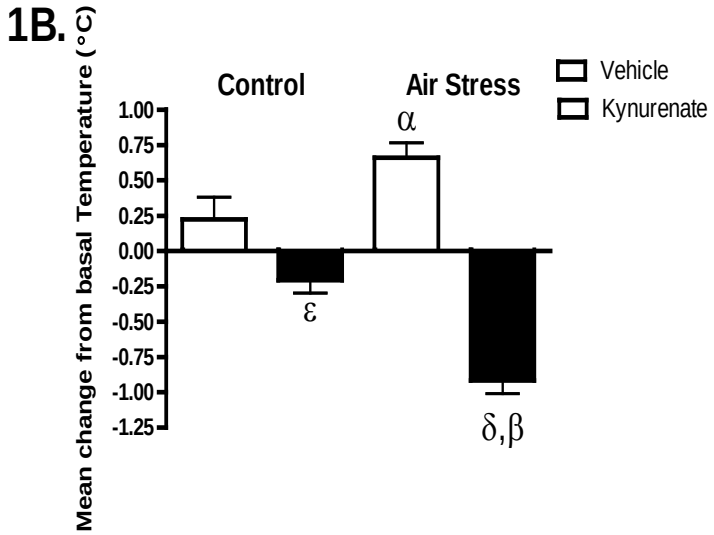


FIGURE 1B. Effects of bilateral microinjection of kynurenate (10 nmol/side) into DMH on hyperthermia induced by air-jet stress. Results are expressed as means ± S.E.M of the 20 minute period following stress period (gray box). (α) Significant difference between VEH/No Stress and VEH/Air Stress. () Signi-ficant difference between VEH/No Stress and KYN/ No Stress. (δ) Significant difference between VEH/Air Stress and KYN/Air Stress. (β) Significant difference between KYN/No Stress and KYN/Air Stress. The same set of animals was used for all trials serving as their own control and was randomly treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

Temperature tended to increase in unstressed rats treated with vehicle in this study, suggesting that experimental manipulation that includes placement of the guide cannula for the microinjection and the microinjection of vehicle itself may elevate body temperature. Therefore, I tested this hypothesis by examining the effect of (1) experimental manipulation, which involves placement of the guide cannula alone, and (2) experimental manipulation followed by injection of vehicle (Figure 2A, B). Experimental manipulation produced a significant change in temperature from baseline, an effect sustained during the first twenty minutes following the manipulation (5-20min, +0.20±0.08°C). After manipulation plus bilateral microinjection of vehicle into the DMH, a greater increase in body temperature (5-20min, +0.48±0.12°C) was generated and temperature remained significantly elevated during the subsequent 20 minute observation period (20-40min, +0.40±0.10°C; see Figure 2A, B).

B. Effect of microinjection of kynurenate in the DMH on hyperthermia produced by cage-switch stress

In the animals pretreated with kynurenate and exposed to air-jet stress, a dramatic decrease in temperature was seen. Pretreatment with kynurenate may block the animal's ability to generate a thermogenic response to the cooling effect of the stream of air resulting in a significant decrease in temperature. Therefore, it was important to find another stress paradigm that did not produce this confounding element of cold-stress. Therefore, a cage-switch stress paradigm was adapted instead. Cage-switch stress has been reported to produce an increase in body temperature of 0.75°C to 1°C. Cage-switch stress has also been shown to evoke an increase in heart rate, blood pressure and

pACTH levels, all physiological components of the response to stress (Morimoto et al., 1991). As in the protocol used in the air-jet stress studies, all rats received all four treatments in random order including pretreatment with either bilateral microinjections of vehicle 100nL or microinjections of 10nmol/100nL of kynurenate in the DMH. Five minutes after the second microinjection, rats were exposed to either cage-switch stress or to no stress (i.e. left undisturbed in their home cage).

Core body temperature was significantly increased compared to control over the twenty minute period following cage-switch ($\bar{X}$ = +0.82±0.17°C; see Figure 3). Microinjection of kynurenate into the DMH of unstressed rats left undisturbed in their cage abolished the increase in temperature seen in unstressed rats pretreated with vehicle.

Microinjection of kynurenate into the DMH completely abolished the increase in body temperature produced by cage-switch stress, generating an average temperature change ($\bar{X}$ = −0.43±0.16°C) that was not significantly different from baseline. In the following experiments, I examine the specific subtypes of ionotropic glutamate receptors that were responsible for this effect.

C. Effect of bilateral microinjection of NBQX and APV in combination in the DMH on the hyperthermia produced by cage-switch stress

The lack of hyperthermic response to the cage-switch stress after microinjection of kynurenate into the DMH suggests that ionotropic glutamate receptors in the region play a role in the increase in body temperature evoked by experimental stress. In order to examine which specific subtypes of ionotropic

2A.

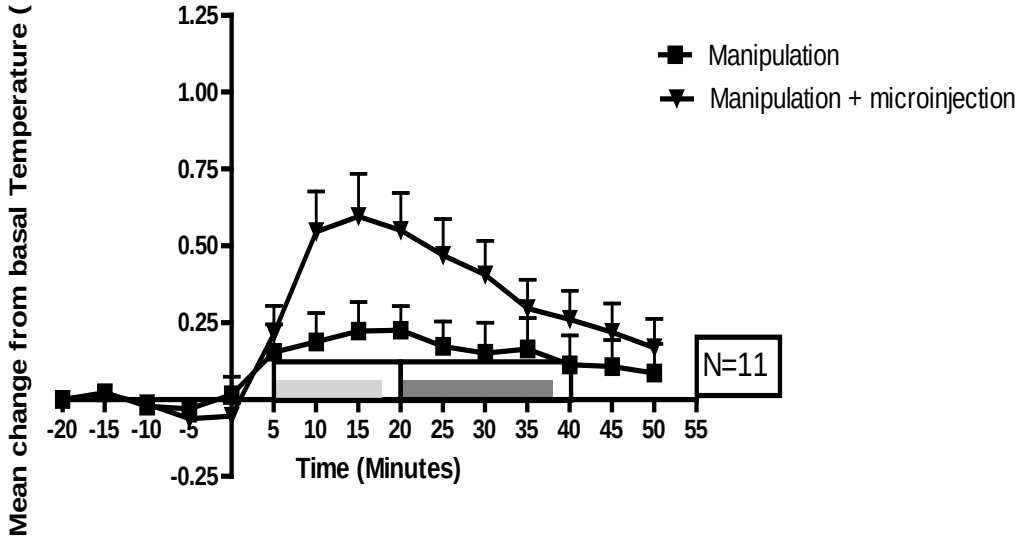


FIGURE 2A. Real time temperature response to rats exposed to manipulation alone or manipulation followed by microinjection of vehicle into DMH. Time zero indicates point of exposure to either paradigm. Light gray box is the 15 minute time period following either paradigm and dark gray box depicts a 20minute time period, 20 minutes after exposure.

2B.

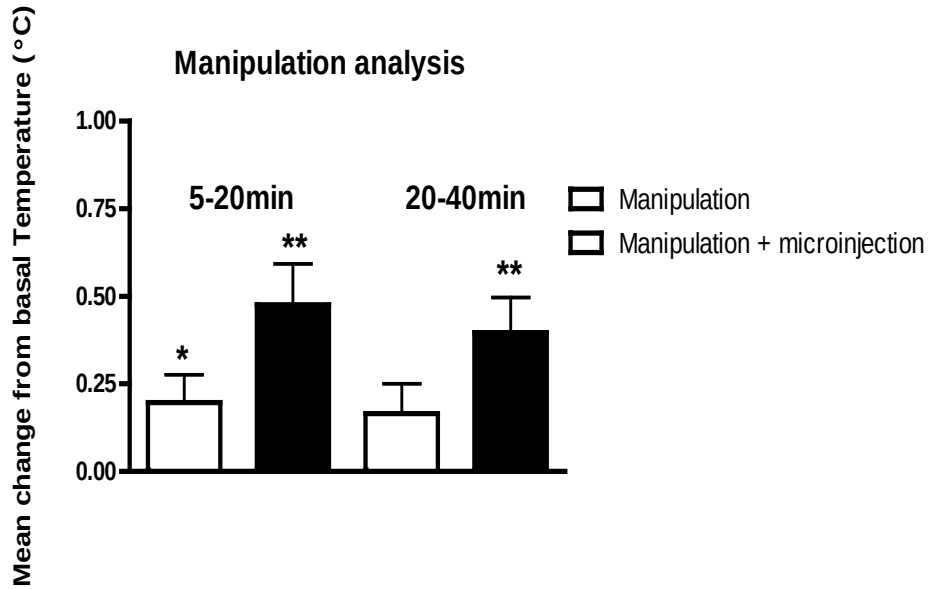


FIGURE 2B. Effect of experimental manipulation and bilateral microinjection of vehicle into DMH on body temperature. Results are expressed as means ± S.E.M of the 15 min interval 5 minutes after the exposure to the stress and means ± S.E.M of the 20min period after 20 minutes of the stress exposure (gray box and dark gray box, respectively). *Significant difference from baseline. **Significant difference between manipulation and aCSF in DMH. The same set of animals was used for all trials and were randomly treated serving as their own control and treated every other day. Significant differences on mean temperature change over the two periods were determined using student t-test. Limits of probability considered significant were 5%.

glutamate receptors mediate this response, I decided to use two drugs, NBQX, an AMPA and kainate receptor antagonist, and APV, an NDMA receptor antagonist, first in combination and then individually. As with previous experiments, exposure to cage-switch stress evoked an increase in temperature of +0.72±0.06°C in animals microinjected into the region of the DMH with aCSF, an effect significantly greater than that seen in animals under control conditions (Figure 4). The mean change in body temperature in control rats pretreated with aCSF in the DMH ($\bar{X}$ = +0.40±0.12°C) versus the mean change in body temperature in rats pretreated with NBQX and APV under control conditions ($\bar{X}$ = +0.08±0.06°C) was significantly different as determined using One-Way Anova and LSD post-hoc test. As with kynurenate, microinjection of NBQX and APV into the DMH of rats when exposed to cage-switch stress, completely abolished the increase in temperature seen in vehicle treated animals exposed to cage-switch stress. The change in body temperature in animals pretreated with NBQX and APV after cage-switch (−0.07±0.14°C) was not significantly different from baseline. These results suggest that activity at ionotropic glutamate receptors in the DMH is required for the increase in temperature produced by cage-switch stress, an exteroceptive stressor.

D. Effect of bilateral microinjection of NBQX or of APV individually in the DMH on the hyperthermia produced by cage-switch stress

To characterize the role of specific ionotropic glutamate receptor subtypes in the DMH in the hyperthermic response produced by cage-switch stress, I studied the effects of bilateral microinjections of either APV or NBQX alone. The

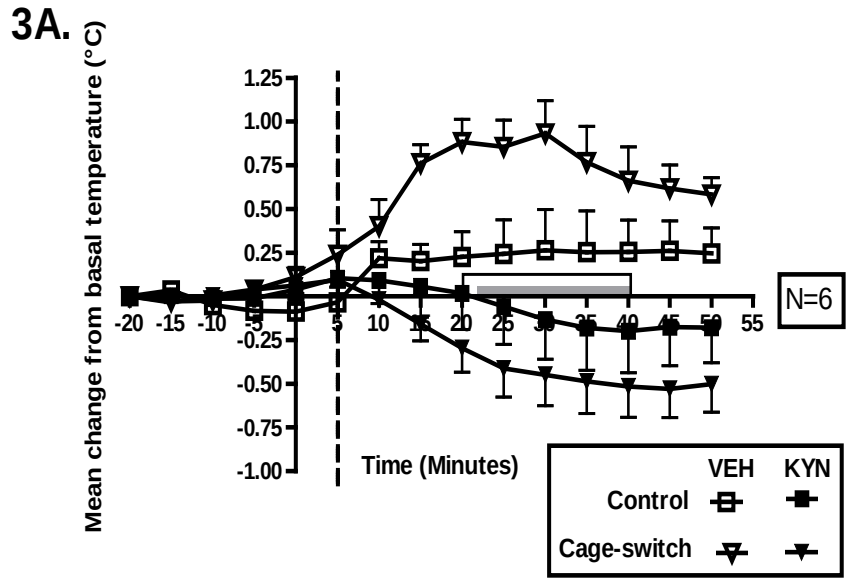


FIGURE 3A. Real time temperature response of rats either unstressed (control) or exposed to cage-switch stress following pretreatment with either vehicle or kynurenate into DMH. Time zero indicates point of microinjections. Light gray box is the 20 minute time period that was analyzed statistically.

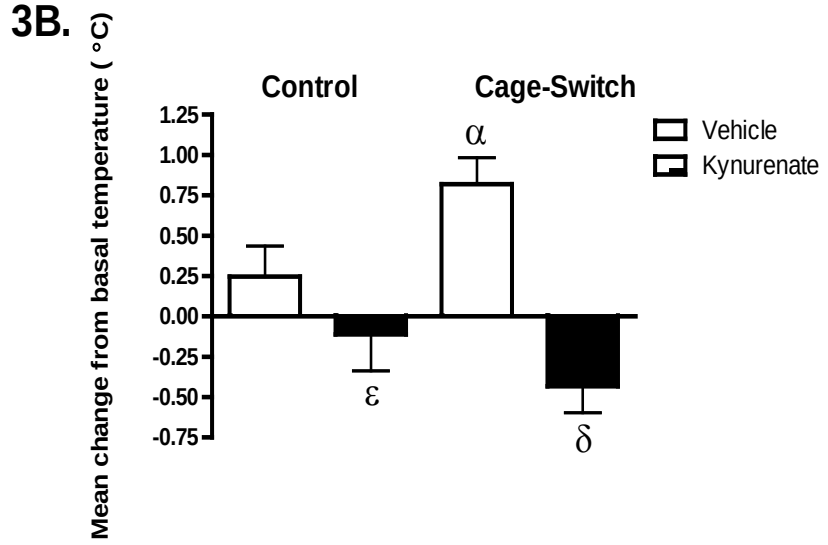


FIGURE 3B. Effects of bilateral microinjection of kynurenate (10 nmol/side) into DMH on hyperthermia induced by cage-switch. Results are expressed as means ± S.E.M of a 20 minute interval 15 minutes after cage-switch stress (gray box). (α) Significant difference between VEH/NCS and VEH/CS. (ε) Significant difference between VEH/NCS and KYN/NCS. (δ) Significant difference between CSF/CS and KYN/CS. The same set of animals was used for all trials and were randomly treated serving as their own control and treated every other day. The same set of animals was used for all trials serving as their own control and was randomly treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

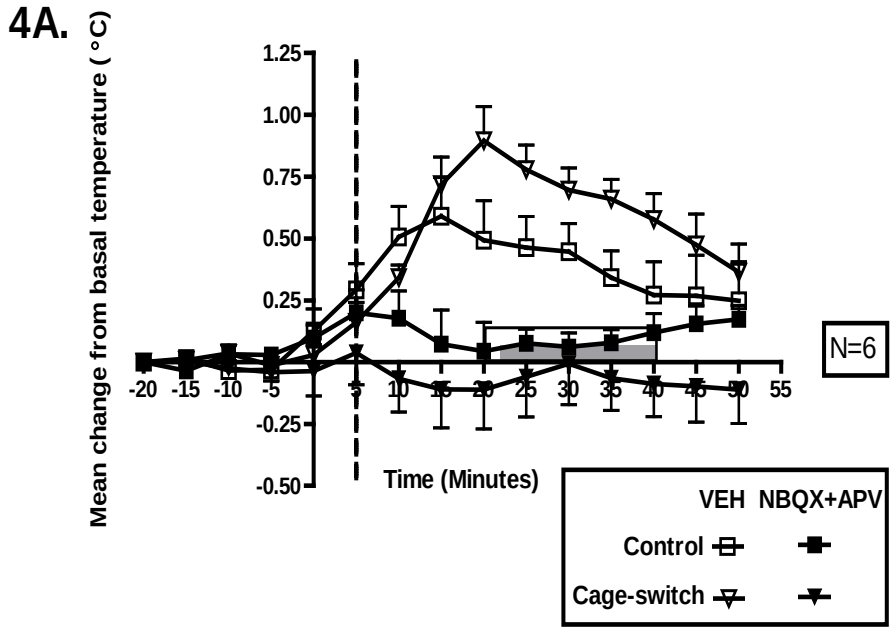


FIGURE 4A. Real time temperature response of rats either unstressed (control) or exposed to cage-switch stress following pretreatment with either vehicle or NBQX+APV into DMH. Time zero indicates point of microinjections. Light gray box depicts the 20 minute time period that was analyzed statistically.

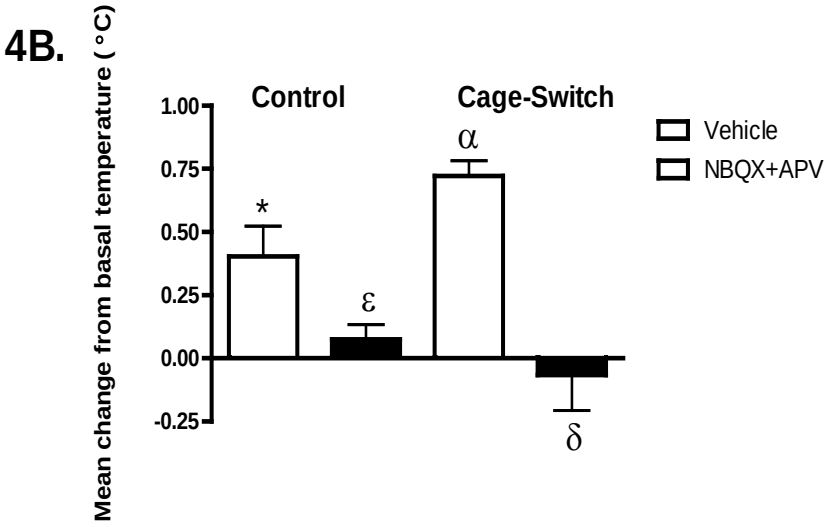


FIGURE 4B. Effects of bilateral microinjection of NBQX (100pmol/side) + APV (200pmol/side) into DMH on hyperthermia induced by cage-switch stress. Results are expressed as means ± S.E.M of a 20 minute interval 15 minutes after cage-switch stress (CS; gray box) or no cage-switch stress (NCS) (left undisturbed in their home cage). (*) Significant difference from baseline. (ε) Significant difference between Vehicle/NCS and Vehicle/CS. (α) Significant difference between Vehicle/NCS and Cocktail/NCS. (δ) Significant difference between Vehicle/CS and NBQX/CS. The same set of animals was used for all trials and were randomly treated serving as their own control and treated every other day. The same set of animals received all treatments in staggered manner each serving as its own control and were treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

microinjection procedure and experimental protocol used were identical to those used for the microinjection of the combination of these agents. The increase in body temperature seen after treatment with vehicle followed by cage-switch was not significantly different from that seen after treatment with APV (200pmol/100nL) followed by cage-switch ($\bar{X}$ = +0.79±0.11°C vs. $\bar{X}$ = +0.55±0.08°C; Figure 5), although increases tended to be smaller after APV.

I also studied the effects of bilateral microinjections of NBQX (100pmol/100nL) into the DMH on the increase in body temperature induced by cage-switch. As seen in Figure 6, after microinjection of vehicle, body temperature after cage-switch stress (+0.94±0.14°C) was significantly greater than that seen under control (unstressed) conditions. After microinjection of NBQX the increase in body temperature produced by cage-switch (+0.40±0.12°C) was significantly less than that seen after microinjection of vehicle. In fact, the increase seen after microinjection of NBQX and cage-switch was similar to that seen after microinjection of vehicle in the control (unstressed) group (+0.36±0.10°C).

Microinjection of the combination of both antagonists completely abolished the increases in body temperature produced by cage-switch, and also attenuated the increase in body temperature seen after vehicle injection under control conditions. As indicated in Figure 7, these results suggest that both NMDA and non-NMDA receptors in the DMH play a role in the increase in body temperature produced by cage-switch stress, an exteroceptive stress paradigm.

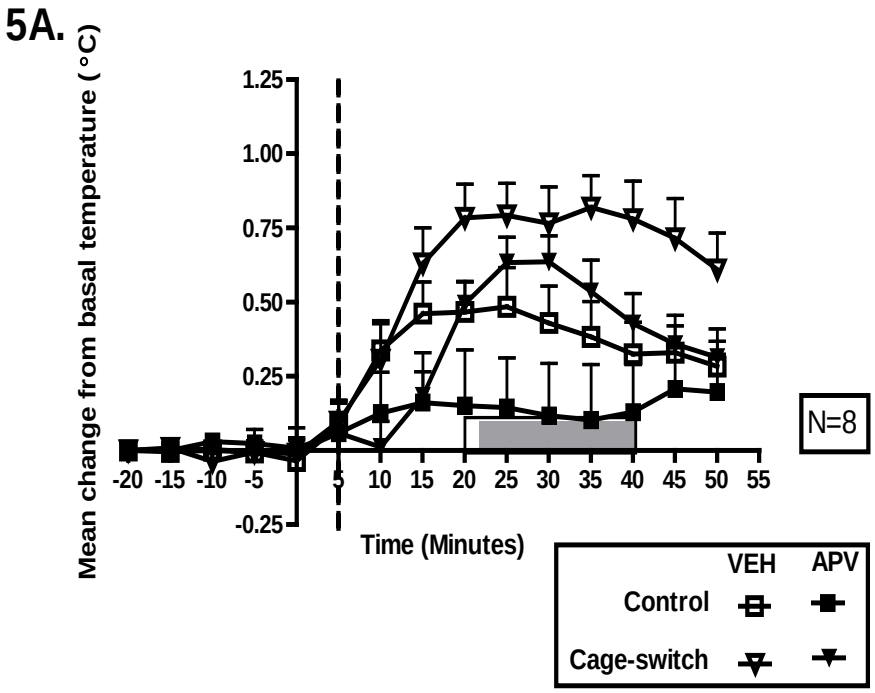


FIGURE 5A. Real time temperature response of rats either unstressed (control) or exposed to cage-switch stress following pretreatment with either vehicle or APV into DMH. Time zero indicates point of microinjections. Light gray box depicts the 20 minute time period that was analyzed statistically.

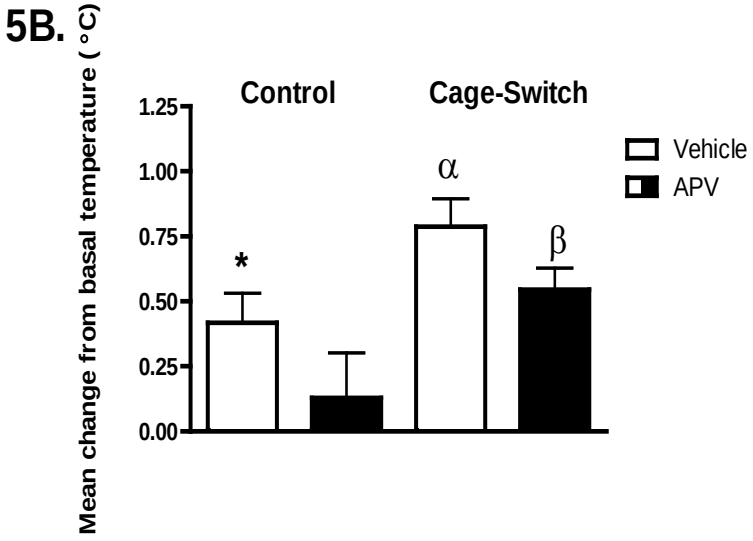


FIGURE 5B. Effects of bilateral microinjection of APV (200pmol/side) into the DMH on increase in body temperature induced by cage-switch. Results are expressed as means ± S.E.M of a 20 minute interval 15 minutes after cage-switch stress (CS; gray box) or no cage-switch stress (NCS) (left undisturbed in their home cage). (*) Significant difference from baseline. (α) Significant difference between Vehicle/control and vehicle/CS. (β) Significant difference between APV/control and APV/CS. The same set of animals received all treatments in staggered manner each serving as its own control and were treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

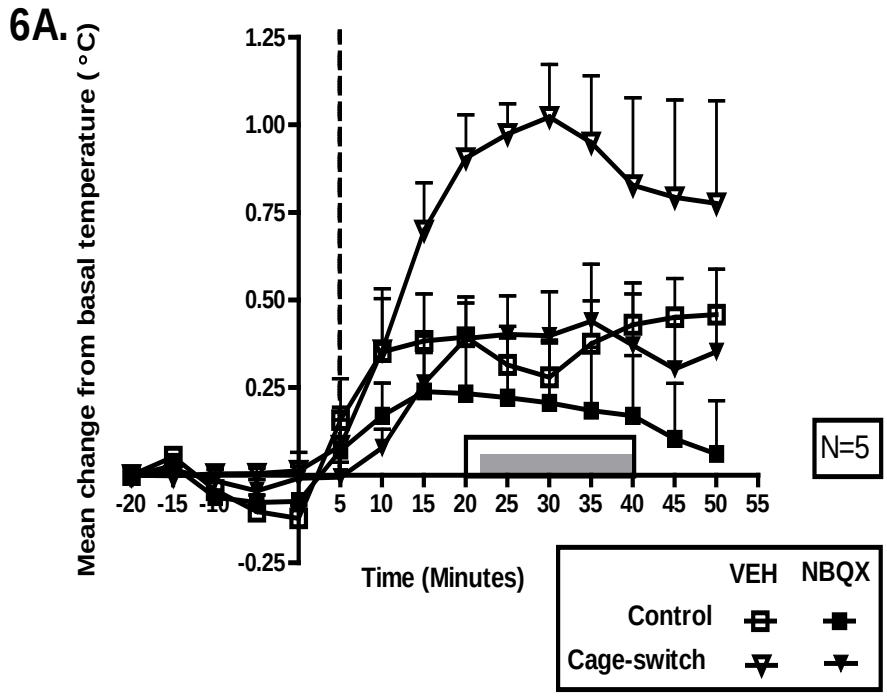


FIGURE 6A. Real time temperature response of rats either unstressed (control) or exposed to cage-switch stress following pretreatment with either vehicle or NBQX into DMH. Time zero indicates point of microinjections. Light gray box depicts the 20 minute time period that was analyzed statistically.

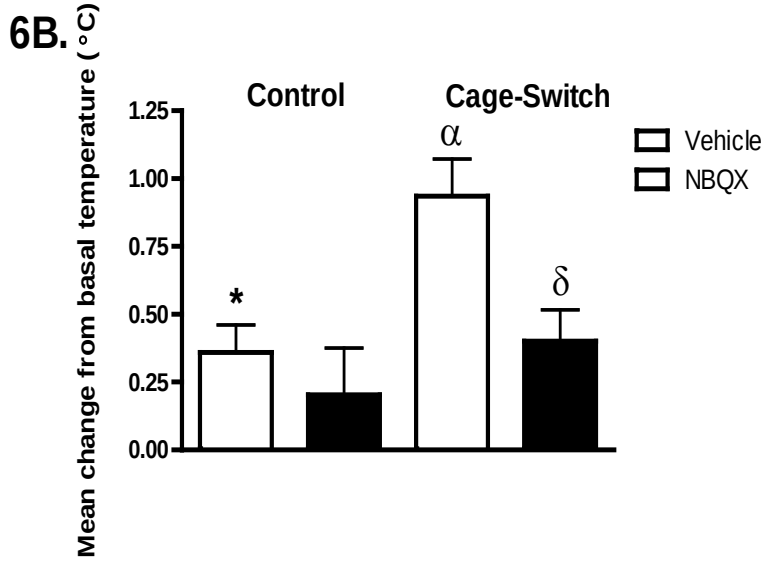


FIGURE 6B. Effects of bilateral microinjection of NBQX (100pmol/side) into the DMH on increases in body temperature induced by cage-switch. Results are expressed as means ± S.E.M of a 20 minute interval 15 minutes after cage-switch stress (CS; gray box) or no cage-switch stress (NCS) (left undisturbed in their home cage). (*) Significantly different from baseline. (α) Significant difference between Vehicle/NCS and Vehicle/CS. (δ) Significant difference between CSF/CS and NBQX/CS. The same set of animals received all treatments in staggered manner each serving as its own control and were treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

Figure 7 summarizes the results of experiments above involving effects of microinjection of NBQX and APV alone and in combination into the DMH on body temperature under baseline conditions and after cage-switch. Microinjection of APV alone did not produce a significant effect on the increase in body temperature produced by cage-switch, whereas microinjections of NBQX in the DMH attenuated these increases.

II. Role of ionotropic glutamate receptors in the DMH in increases in body temperature produced by microinjection of PGE_2 in the POA and BMI in the DMH

Interoceptive stress is detected through sensory neural or chemical cues from the internal environment such as the stress caused by a bacterial infection. Microinjection of PGE_2 into the POA is considered a model for experimental fever. It has been shown that during the course of a bacterial infection, PGE_2 is released in the POA which is thought to initiate the febrile response in mammals (for review see Blatteis and Sehic, 1998). Therefore, microinjection of PGE_2 into the POA is an experimental model for interoceptive stress. Microinjection of BMI, a $GABA_A$ receptor antagonist, into the DMH produces an increase in temperature of at least 1°C or higher, a response similar to that produced by microinjection of PGE_2 in the POA, and the increase in temperature induced by PGE_2 in the POA is mediated through the DMH. Activation of neurons or disinhibition of neurons in the DMH seems to be a key component of the thermogenic response seen not only to microinjection of PGE_2 in the POA, but to stress in general.

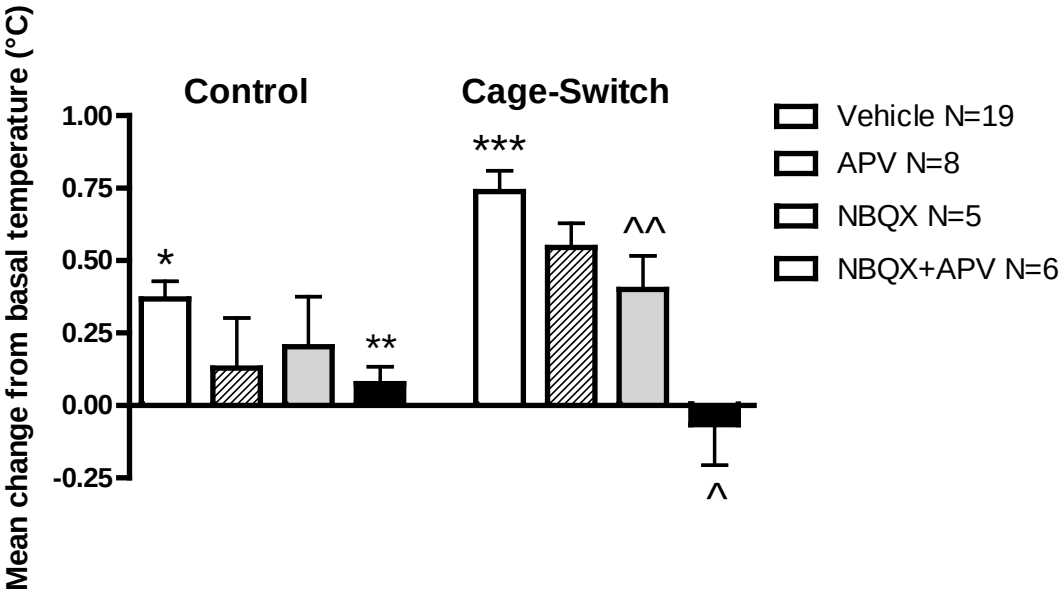


FIGURE 7. Summary of effect of microinjection of subtype-specific ionotropic glutamate receptor antagonists into the DMH on increases in body temperature evoked by cage-switch stress. Results are expressed as means ± S.E.M. **(*)** Significant difference from baseline**. (**)** Significant difference between Vehicle/NCS and NBQX+APV/NCS. **(***)** Significant difference between Vehicle/NCS and Vehicle/CS. **(^)** Significant difference between Vehicle/CS and NBQX+APV/CS. **(^^)** Significant difference between Vehicle /CS and NBQX/CS. The same set of animals received all treatments in staggered manner each serving as its own control and were treated every other day. (Data representing changes seen after treatment with vehicle have been pooled for graphic representation in this figure. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

The following results address the question of whether ionotropic glutamate receptors in the DMH play a role in the increase in temperature produced by microinjection of PGE_2 in the POA, an interoceptive stress, or microinjection of BMI into the DMH, directly disinhibiting the signaling pathway thought to be involved in the thermogenic response to both interoceptive and exteroceptive stress.

A. Effect of microinjection of combination of NBQX+APV in the DMH on the increase in body temperature produced by microinjection of BMI in the DMH

For this study either vehicle or combination of NBQX+APV (NBQX 100pmol/side and APV 200pmol/side) was microinjected unilaterally into the DMH five minutes prior to microinjection of BMI (10 pmol/100 nL) at the same site. The statistical analysis for the experiments described was done for the average change in temperature in the 20 minute period starting five minutes after microinjection of either vehicle or BMI in the DMH. Unilateral microinjection of BMI significantly increased body temperature over baseline in animals pretreated with vehicle, (+0.88±0.09°C; see Figure 8). After pretreatment with NBQX+APV the increase in temperature produced by BMI was significantly attenuated (+0.28±0.08°C).

B. Effect of prior microinjection of APV or NBQX alone on the increase in body temperature produced by microinjection of BMI in the DMH

In the next set of experiments the goal was to study the effect of pretreatment with NBQX or APV alone using the same experimental protocol. As mentioned above, the statistical analysis for these experiments was done for the average change in temperature in the 20 minute period starting five minutes after

8A

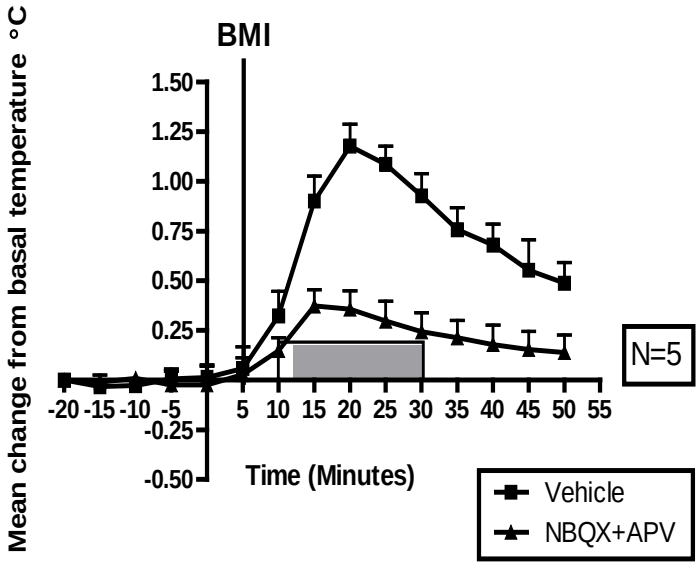


FIGURE 8A. Real time temperature response of rats pretreated with either vehicle or NBQX+APV unilaterally in the DMH followed by unilateral (same side) microinjection of vehicle or BMI in the DMH. Time zero indicates point of microinjections. Solid line depicts microinjection of BMI in the DMH. Light gray box is the 20 minute time period that was analyzed statistically.

8B.

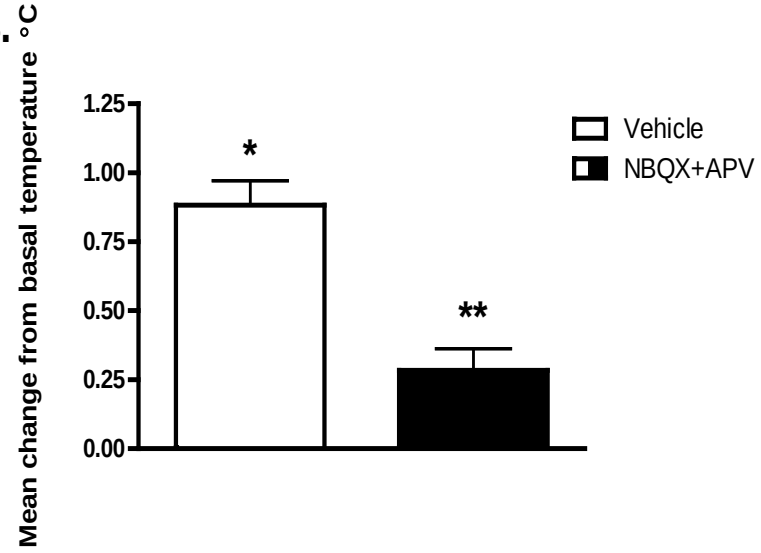


FIGURE 8B. Effects of microinjection of NBQX (100pmol/side) and APV (200pmol/side) on increase in body temperature evoked by unilateral microinjection of BMI (10pmol/side) into the DMH. Results are expressed as means ± S.E.M of a 20 minute interval 5 minutes after treatment(gray box). (*) Significant difference from baseline. (**)Significant difference between CSF/CSF and aCSF/BMI. The same set of animals was used for all trials serving as their own control and was randomly treated every other day. Significant differences on mean temperature change over the two periods were determined using student t-test. Limits of probability considered significant were 5%.

microinjection of either vehicle or BMI in the DMH. Either APV (200pmol/side) or NBQX (200pmol/side) was microinjected into either the right or left side of the DMH five minutes prior to microinjection of BMI (10pmol/100nL) at the same site. The increase in temperature seen in the vehicle-vehicle treated animals (+0.41±0.09°C) was not significantly different from that seen in the APV-vehicle treated animals (+0.22±0.07°C). The increase in temperature produced by microinjection of BMI after pretreatment with APV (+0.98±0.06°C, N=6) was not significantly different from the increase in temperature produced by microinjection of BMI after pretreatment with vehicle (0.75±0.17°C, N=6; see Figure 9).

The increase in core body temperature evoked by microinjection of BMI after pretreatment with NBQX (0.79±0.19°C, N=5) was not significantly different from the increase in temperature evoked by microinjection of BMI following pretreatment with vehicle (0.76±0.18°C, N=5; Figure 10). The increase in body temperature observed after vehicle-vehicle treatment (0.23±0.20°C, N=5) was not significantly different from the increase in temperature observed after NBQX followed by vehicle (0.03±0.09°C, N=5).

Collectively, these results suggest that blockade of both NMDA and non-NMDA subtypes of ionotropic glutamate receptors in the region is required to attenuate the increase in temperature evoked by microinjection of BMI into the DMH (see Figure 8 and Figure 9).

C. Effect of microinjection of NBQX and APV in the DMH on experimental fever produced by microinjection of PGE_2 in the POA

In a final set of experiments, I examined the role of ionotropic glutamate receptors in the DMH in the experimental fever produced by microinjections of

PGE_2 into the POA, another model for interoceptive stress (Figure 11). At t=0 min, rats were pretreated with a bilateral microinjection of either vehicle (aCSF) or drug (NBQX and APV in combination, NBQX alone or APV alone) into the DMH, and this was followed by a unilateral microinjection of either vehicle or PGE_2 in the POA at t=5min. Rats were then left undisturbed in their respective home cages and observed for a period of two hours. Increases from baseline body temperature were averaged for the thirty minute interval immediately following microinjection of PGE_2 (i.e., t=5-35 min) for all analyses.

D. Effect of bilateral microinjection of NBQX and APV in the DMH on the experimental fever produced by microinjection of PGE_2 in the POA

In the previous experiments, prior unilateral microinjection of NBQX and APV into the DMH greatly attenuated the increase in body temperature evoked by subsequent microinjection of BMI at the same site. I expected that similar pretreatment would also attenuate the febrile response evoked by microinjection of PGE_2 in the POA. After pretreatment with vehicle, microinjection of PGE_2 into the POA produced a significant increase in body temperature (+1.30±0.16°C; Figure 11). Pretreatment with NBQX+APV significantly decreased the febrile response produced by microinjection of PGE_2 in the POA (+0.24±0.20°C; Figure 11). Pretreatment with NBQX+APV also significantly attenuated the modest increase in temperature that followed microinjection of vehicle into the POA (+0.06±0.10°C after NBQX and APV versus +0.43±0.17°C after vehicle).

9A.

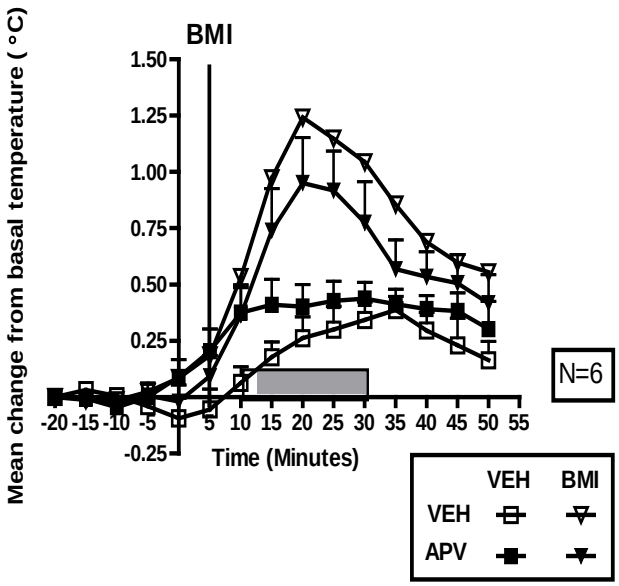


FIGURE 9A. Real time temperature response of rats pretreated with either vehicle or APV unilaterally in the DMH followed by unilateral (same side) microinjection of vehicle or BMI in the DMH. Time zero indicates point of microinjections. Solid line depicts microinjection of BMI in the DMH. Light gray box is the 20 minute time period that was analyzed statistically.

9B.

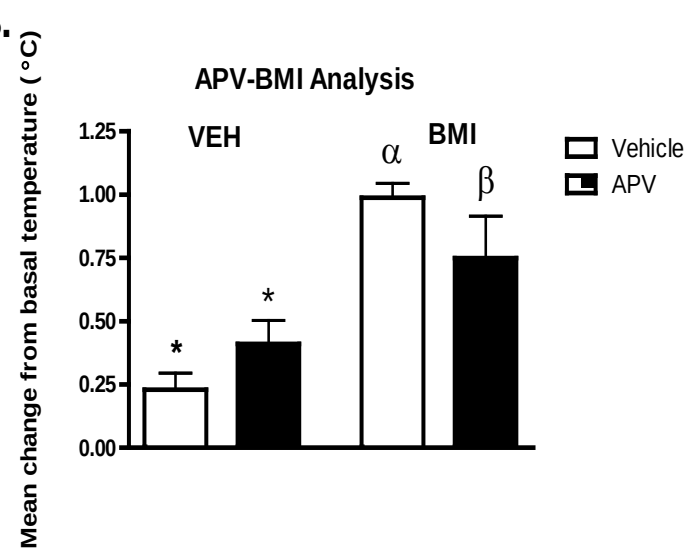


FIGURE 9B. Effect of prior microinjection of APV (200pmol/side) into the DMH on the increase in body temperature produced by microinjection of BMI (10pmol) at the same site. Results are expressed as means ± S.E.M of a 20 minute interval 5 minutes after treatment (gray box). (*) Significant difference from baseline. (α) Significant difference between Vehicle/Vehicle and Vehicle/BMI. (β) Significant difference between APV/Vehicle and APV/BMI. The same set of animals was used for all trials serving as their own control and was randomly treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

10A.

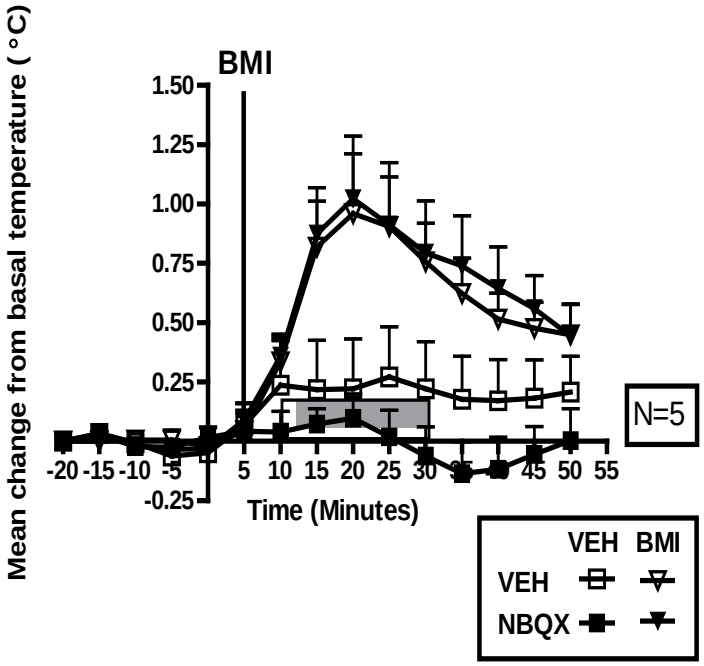


FIGURE 10A. Real time temperature response of rats pretreated with either vehicle or NBQX unilaterally in the DMH followed by unilateral (same side) microinjection of vehicle or BMI in the DMH. Time zero indicates point of microinjections. Solid line depicts microinjection of BMI in the DMH. Light gray box is the 20 minute time period that was analyzed statistically.

10B.

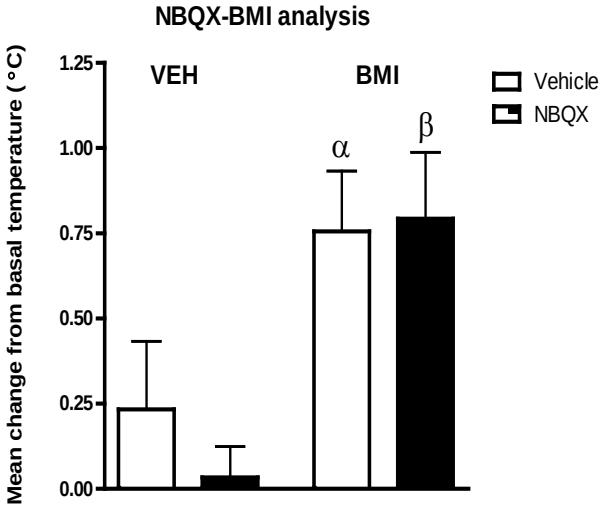


FIGURE 10B. Effect of prior microinjection of NBQX (100pmol/side) into the DMH on the increase in body temperature evoked by microinjection of BMI (10pmol) at the same site. Results are expressed as means ± S.E.M of a 20 minute interval 5 minutes after treatment (gray box). (α) Significant difference between Vehicle/Vehicle and Vehicle/BMI. (β) Significant difference between NBQX/Vehicle and NBQX/BMI. The same set of animals was used for all trials serving as their own control and was randomly treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

These results suggest that ionotropic glutamate receptors in the DMH play a role in the febrile response produced by microinjection of PGE_2 in the POA. The next series of experiments investigated the effect of NBQX and APV alone on the febrile response produced by microinjection of PGE_2 in the POA.

E. Pretreatment with NBQX or with APV evokes different degrees of attenuation on the experimental fever produced by the microinjection of PGE_2 in the POA

The role of ionotropic glutamate receptors in the DMH has been studied in various models of both interoceptive and exteroceptive stresses, and the results thus far indicate that these receptors play an important role in the stress-induced increase in temperature as seen in all models examined. Likewise, results just described show that antagonism at NMDA and AMPA/kainate receptors in the DMH attenuates the increase in core body temperature produced by microinjection of PGE_2 in the POA. I next investigated the effects of pretreatment with either APV or NBQX, individually. As shown in Figure 12A and B, a significant increase in body temperature from baseline levels (+0.44±0.08°C) was evident after treatment with vehicle in both the DMH and the POA. However, the increase in temperature seen after microinjection of APV into the DMH and vehicle in the POA was significantly reduced (−0.16±0.10°C). Microinjection of PGE_2 in the POA evoke a febrile response after pretreatment with vehicle in the DMH (+1.7±0.35°C), that was no different from that seen after pretreatment with APV (+1.6±0.44°C).

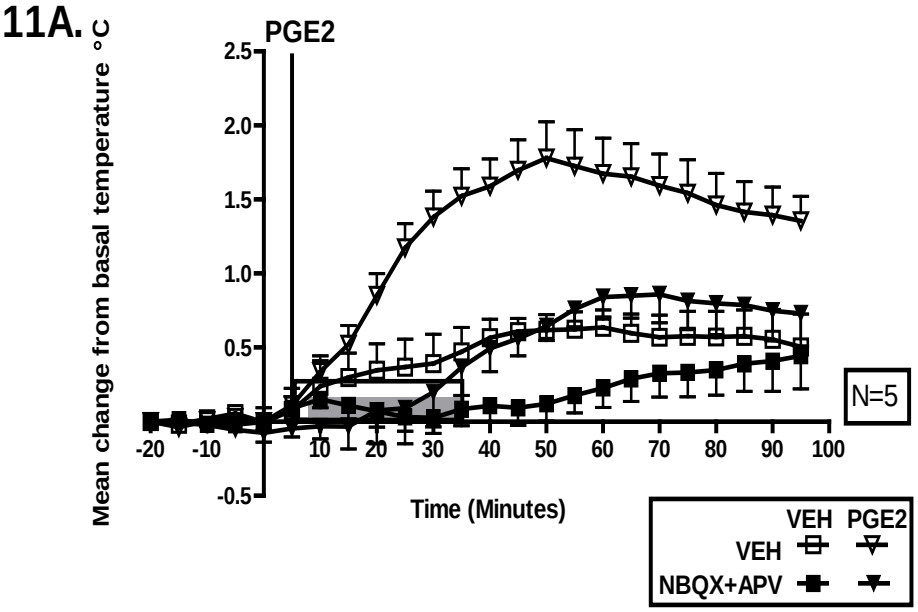


FIGURE 11A. Real time temperature response of rats pretreated with either vehicle or NBQX+APV bilaterally in the DMH followed by a microinjection of vehicle or PGE_2 in the POA. Time zero indicates point of microinjections into the DMH. Solid line depicts microinjection of vehicle or PGE_2 in the POA. Light gray box is the 30 minute time period that was analyzed statistically.

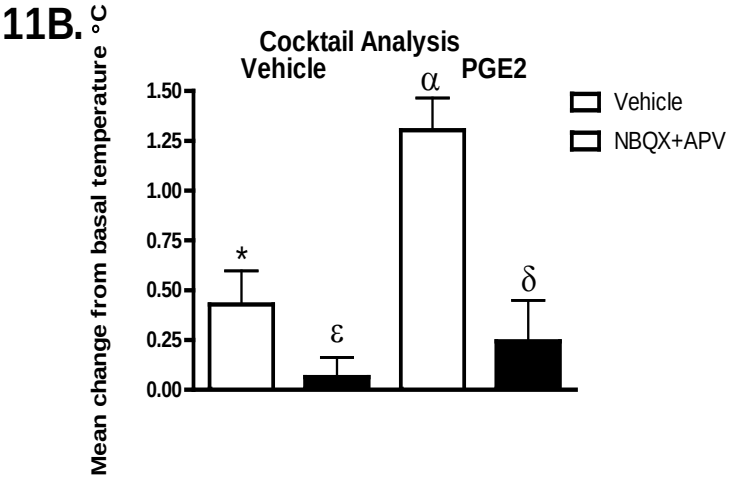


FIGURE 11B. Effects of NBQX(100pmol/side) and APV(200pmol/side) in DMH on fever evoked by microinjection of PGE_2 (150pmol) in the POA. Results are expressed as means ± S.E.M of the 30 minute period following treatment (gray box). (*) Significant difference from baseline. (ε) Significant difference between Vehicle (DMH)/Vehicle (POA) and NBQX+APV (DMH)/Vehicle (POA). (α) Significant difference between Vehicle (DMH)/Vehicle (POA) and Vehicle (DMH)/ PGE_2 (POA). (δ) Significant difference between Vehicle (DMH)/ PGE_2 (POA) and NBQX+APV (DMH)/ PGE_2 (POA). The same set of animals was used for all trials serving as their own control and was randomly treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

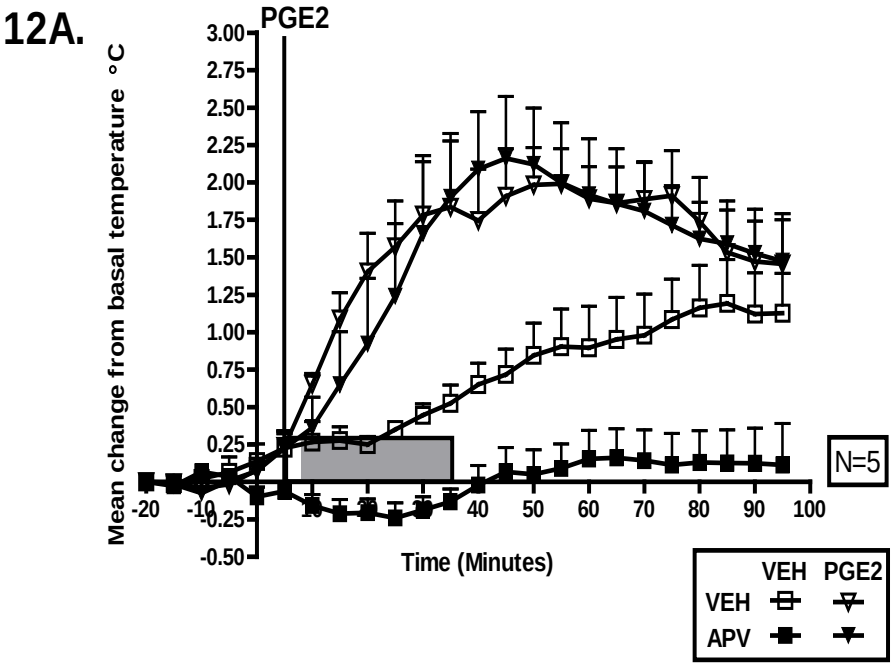


FIGURE 12A. Real time temperature response of rats pretreated with either vehicle or APV bilaterally in the DMH followed by a microinjection of vehicle or PGE_2 in the POA. Time zero indicates point of microinjections into the DMH. Solid line depicts microinjection of vehicle or PGE_2 in the POA. Light gray box is the 30 minute time period that was analyzed statistically.

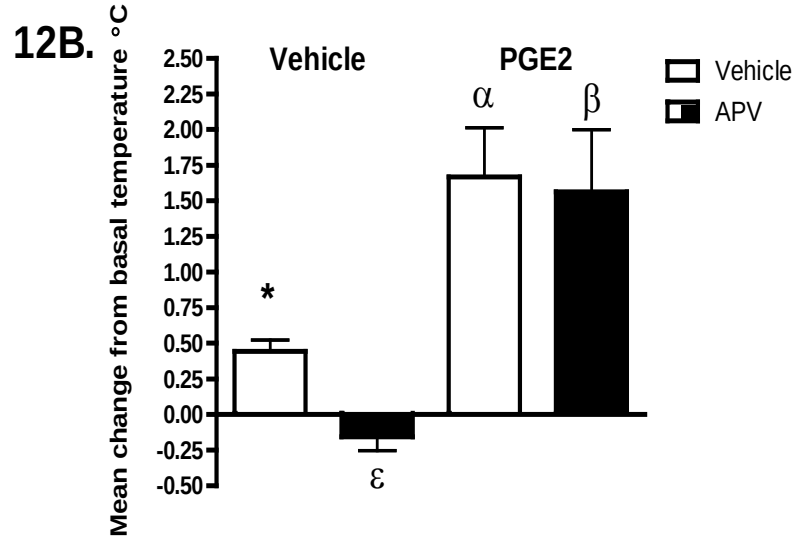


FIGURE 12B. Effects of APV (200pmol/side) pretreatment in the DMH on fever evoked by microinjection of PGE_2 (150pmol) in the POA. Results are expressed as means ± S.E.M of the 30 minute period following treatment (gray box). (*) Significantly different from baseline. (ε) Significant difference between Vehicle (DMH)/Vehicle (POA) and APV (DMH)/Vehicle (POA). (α) Significant difference between Vehicle (DMH)/Vehicle (POA) and Vehicle (DMH)/ PGE_2 (POA). (β) Significant difference between APV-DMH/VEH-POA and APV-DMH/ PGE_2-POA. The same set of animals was used for all trials serving as their own control and was randomly treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

In the last study, I investigated the effects of NBQX pretreatment alone on fever evoked by microinjection of PGE_2 in the POA (Figure 13 A and B). In this experiment, an increase in temperature was again seen after injection of vehicle into the DMH and the POA (+0.69±0.15°C; Figure13B); however, unlike pretreatment with APV, NBQX-pretreatment failed to significantly affect this response (+0.60±0.23°C; Figure 13B). Likewise, microinjection of PGE_2 in the POA after microinjection of vehicle into the DMH produced an increase in body temperature (1.7±0.17°C; Figure 13B) that was virtually identical to that seen after pretreatment with NBQX (+1.7±0.30°C, Figure 13B).

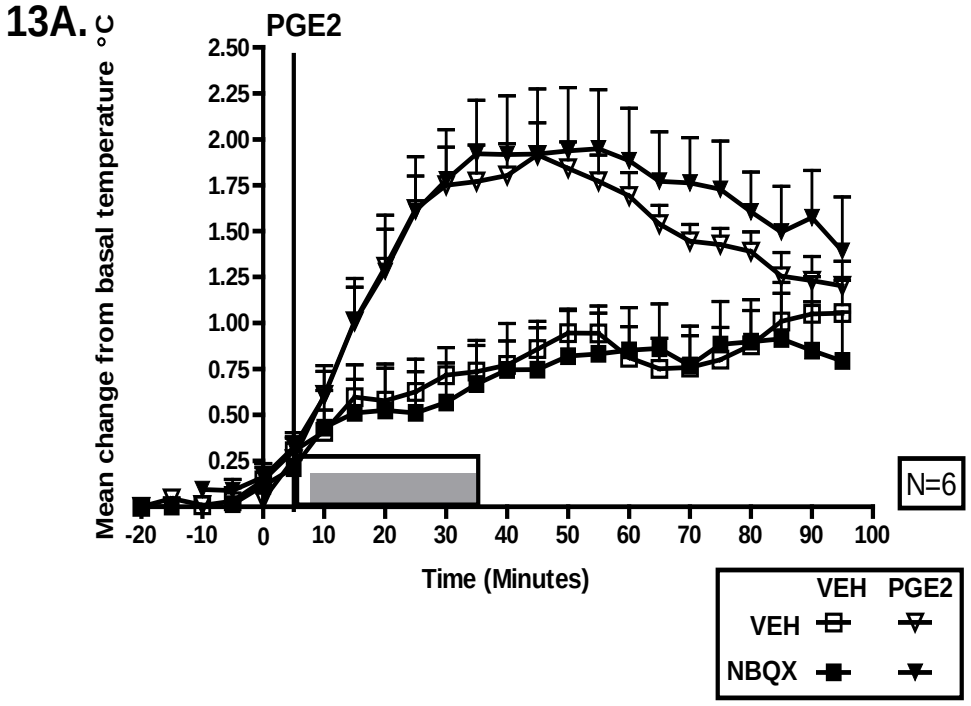


FIGURE 13A. Real time temperature response of rats pretreated with either vehicle or NBQX bilaterally in the DMH followed by a microinjection of vehicle or PGE_2 in the POA. Time zero indicates point of microinjections into the DMH. Solid line depicts microinjection of vehicle or PGE_2 in the POA. Light gray box is the 30 minute time period that was analyzed statistically.

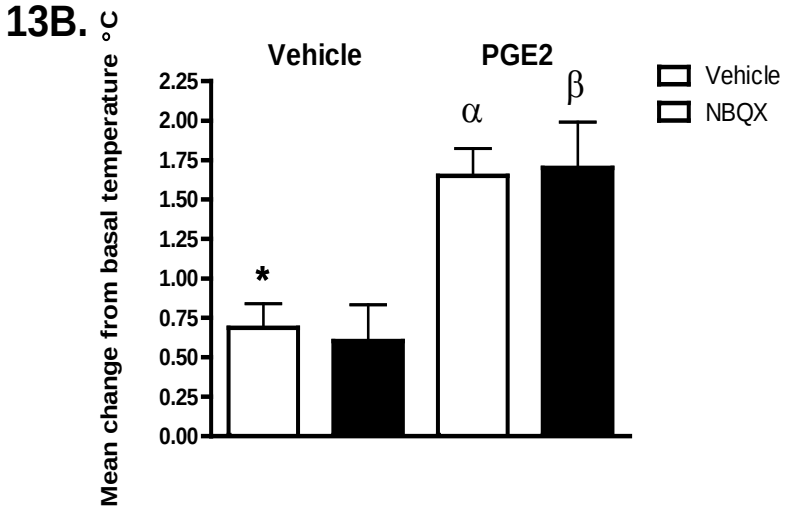


FIGURE 13B. Effects of NBQX (100pmol/side) pretreatment in the DMH on fever evoked by microinjection of PGE_2 (150pmol) in POA. Results are expressed as means ± S.E.M of the 30 minute period following treatment (gray box). (*) Significantly different from baseline. (α) Significant difference between Vehicle (DMH)/Vehicle (POA) and Vehicle (DMH)/ PGE_2 (POA). (β) Significant difference between NBQX (DMH)/Vehicle (POA) and NBQX (DMH)/ PGE_2 (POA). The same set of animals was used for all trials serving as their own control and was randomly treated every other day. Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

CHAPTER 4: DISCUSSION AND FUTURE STUDIES

The stress response is also known as the fight or flight response, and the purpose of all the physiological changes that comprise this response is thought to be to prime the body to either fight or flee a perceived threat or danger. A typical example of the fight or flight response at work is that of a zebra grazing peacefully and taking off in flight at the mere scent of a lion, which requires expenditure of a large amount of physical energy. Likewise in prehistoric times, man's fight or flight behavior was shaped by the perceived threats or dangers more likely encountered while hunting such as being confronted by a predator. The fight or flight response was vital for survival. In modern society, we have very different perceived threats which don't require expenditure of large amounts of energy yet the fight and flight response persists. Constant activation of the stress response in humans can lead to negative psychological and physical effects. Prolonged stress responses may result in an array of complications that can lead to heart disease, anxiety and panic disorders, and chronic suppression of the immune system, which leaves the body open for infection and other complications. However, to date, there is no one effective manner to deal with stress and much more research needs to be done so that we can better understand how the brain mediates the stress response.

I studied the role of ionotropic glutamate receptors in the DMH in the increase in body temperature induced by stress. The overall results show that ionotropic glutamate receptors in the DMH play a role in the increase in temperature induced by stress. Also, activation of both NMDA and non-NMDA

receptors plays a role in the increase in temperature evoked by stress, yet another important finding from these studies. I studied both exteroceptive and interoceptive stress paradigms to study the role of ionotropic glutamate receptors in the increase in body temperature associated with stress.

I. Role of ionotropic glutamate receptors in the hypothalamus in the increase in core temperature seen in response to stress

A. Blockade of both NMDA and non-NMDA receptor subtypes prevents the increase in temperature evoked by air-jet stress

Air-jet stress is a paradigm used in our laboratory and by others as a model for exteroceptive stress (de Menezes et al., 2006; Mayorov and Head, 2002; Sarkar et al., 2007; Soltis and DiMicco, 1992b). In conscious rats, exposure to this paradigm produces an increase in temperature, presumably induced as a classic component of the response to stress. Interestingly, it appears that this increase in temperature in response to air-stress may also include a component representing a compensatory response to the cooling effect produced by the stream of air. Exposure to acute cold-stress (4-5°C ambient temperature) or skin cooling (placement of a rat in a water jacket perfused with ice-cold water), induces an increase in core body temperature of 0.25 to 0.30°C (Bratincsak and Palkovits, 2005; Ishiwata et al., 2005; Morrison, 2004; Morrison et al., 2008; Saito et al., 2008) that persists for up to 30 minutes after exposure. More prolonged exposure to cold eventually leads to a decrease in body temperature. The mean maximum increase in temperature seen in rats exposed to air-jet stress was +0.66 ± 0.11°C and body temperature had still not returned to baseline an hour after exposure to the stress.

The most compelling evidence for the existence of the “cold stress” phenomenon as an unanticipated component of the paradigm is the response to air-jet stress after microinjection of kynurenate into the DMH. Pretreatment with this non-selective inhibitor of ionotropic glutamate receptors not only abolished the increase in temperature produced by air-jet stress, but actually unmasked a dramatic reduction in body temperature from baseline. The most likely explanation for this finding is that air-jet stress, which involves exposure of the rat to a stream of air, produces a cooling effect that is normally countered by adaptive thermoregulatory changes that depend upon activity at ionotropic glutamate receptors in the DMH. This cooling effect thus constitutes a cold stress associated with the air-jet stress paradigm that before this experiment had been unappreciated. Microinjection of kynurenate into the DMH results in the apparent loss of the ability to counter the cold-stress, perhaps because compensatory thermoregulatory effectors in the body are not activated when the ionotropic glutamate receptors in the DMH are inhibited. It has been shown that in rats and other small mammals exposed to cold ambient temperatures, non-shivering thermogenesis or sympathetic stimulation of brown fat acts as an essential thermoregulatory effector in cold defense by generating heat that is not associated with muscle activity of shivering (Carlson and Cottle, 1956; Foster and Frydman, 1979; Fuller et al., 1975; Golozoubova et al., 2006; Hart and Jansky, 1963; Kalter et al., 1979; Morrison, 2004; Morrison et al., 2008). Evidence supports a role for neurons in the region of the DMH in thermogenesis induced by cold exposure. In fact, cold exposure increases the expression of

Fos, a marker for neuronal activity, in neurons of the DMH (Cano et al., 2003). Furthermore, disinhibition of neurons by blockade of $GABA_A$ receptors in the DMH increases sympathetic SNA to BAT and thermogenesis (Cao et al., 2004b; Zaretskaia et al., 2002). Tonic GABAergic input to the DMH may originate in the POA (Chiba and Murata, 1985; Kita and Oomura, 1982; Simerly and Swanson, 1988; Thompson and Swanson, 1998). Likewise, inhibition of neurons in the DMH blocks the febrile and cold-evoked excitation of SNA to BAT and thermogenesis (Madden and Morrison, 2004; Morrison, 2004; Nakamura and Morrison, 2007; Nakamura et al., 2005a; Zaretskaia et al., 2003). Therefore, inhibition of neurons in the region of the DMH with microinjections of muscimol blocked shivering in rats exposed to cold (Tanaka et al., 2001). The dramatic decrease in core body temperature in the kynurenate-treated animals suggests that ionotropic glutamate receptors in the DMH are involved in the activation of thermogenic effectors that respond to “cold stress” and that blockade of these receptors may therefore prevent the animal from maintaining its body temperature when exposed to the cooling effect produced by the air-jet stream. I suggest that the increase in temperature induced by air-jet stress is probably due to the animal generating heat in response both to the cold and to being stressed from the procedure. However, I cannot differentiate those effects induced by cooling from those effects induced by stress. Therefore, in order to study the increase in body temperature produced specifically by stress, I was compelled to find another paradigm.

The results from this study also indicate that blockade of ionotropic glutamate receptors in the DMH attenuates the increase in temperature seen in unstressed animals that received injections of aCSF in the DMH. This increase in temperature may be due to manipulating the rat or its environment during the experiment (i.e., my presence in the room, placing a hand inside the rat's cage and/or handling of the rat). As can be seen from the results (Figure 2), body temperature increases during the microinjection procedure, during which the rats were briefly exposed to the experimenter and microinjectors were inserted into the guide cannulas. Several studies have reported an increase in body temperature of rats during similar experimental procedures, such as intracerebral microinjections or rectal temperature measurements (Eikelboom, 1986; Oka et al., 2001; Pae et al., 1985; Poole and Stephenson, 1977). The handling of the rat or "manipulation" during the experimentation procedure can be considered a mild stressor and, indeed, an increase in temperature was observed as a response to experimental manipulation in the present study (see Figure 2). Placement of microinjectors in the guide cannula of the conscious and untethered rat was followed by a significant increase in temperature from baseline, an effect sustained for the following twenty minutes. Likewise, bilateral microinjection of vehicle into the DMH was followed by a significant increase in body temperature during the 5-20min time interval (mean change compared to baseline, +0.48±0.12°C) that persisted for 40 minutes after the microinjections (mean for 20-40min, +0.40±0.10°C; see Figure 2A and B). The fact that this increase persisted for 40 minutes after the microinjections suggests that at least part of

the increase in temperature seen in both control and experimental rats is a consequence of these manipulations. Placement of the microinjectors into the DMH produces damage in that region of the brain which itself may be another reason for this increase in temperature (Quan and Blatteis, 1989). Handling of the rat and insertion of the guide cannula along with microinjection of vehicle produced a more pronounced and significant increase in temperature than that observed with handling of the rat and insertion of the guide cannula alone (Figure 2A). The increase in temperature induced by manipulation lasted less than twenty minutes whereas the increase in temperature induced by manipulation and microinjection of vehicle lasted for more than 30 minutes. In addition, the increase in temperature induced by experimental manipulation was completely abolished by microinjection of kynurenate in the DMH. If as suggested the increase in temperature induced during handling of the rat is a response to the mild stress of this manipulation, the inhibition of this increase by bilateral microinjections of kynurenate in the DMH suggests a role for ionotropic glutamate receptors in the DMH in stress-induced increases in body temperature. However, since the increase in body temperature was completely abolished, blockade of ionotropic glutamate receptors in the DMH also prevented any increase in temperature that may have been caused by local tissue damage.

Because of the confounding contribution of cold stress to the air stress paradigm, cage-switch stress was the paradigm chosen to clarify the role of ionotropic glutamate receptors in the DMH in the increase in temperature induced by an emotional stressor in the remainder of my studies. Cage-switch is

a mild exteroceptive stressor known to induce increases in body temperature that is uncomplicated by the confounder of the apparent “cold stress” component associated with air-jet stress, In the cage-switch stress paradigm, the animal is moved from its home cage to an identical clean cage. Several variations of this paradigm have been used and all induce a significant and rapid increase in core body temperature that has been reported to range from 0.93°C to 1.33°C (Kluger et al., 1987; Long et al., 1990a; Long et al., 1990b; Oka et al., 2001; Singer et al., 1986; Whyte and Johnson, 2005). As can be seen in Figure 3, a significant increase in body temperature was observed following cage-switch stress in my studies (mean maximum increase from baseline +0.82±0.17°C). To determine whether ionotropic glutamate receptors play a role in the increase in temperature induced by cage-switch stress, animals were pretreated with microinjection of kynurenate into the DMH. This pretreatment prevented any increase in body temperature, including the increase in temperature associated with the manipulation and microinjection as was seen in control animals. These results support the idea that ionotropic glutamate receptors in the DMH play a role in the increase in temperature seen in response to exteroceptive stress. Our laboratory has previously shown that blockade of ionotropic glutamate receptors in the DMH prevents the increase in heart rate and blood pressure associated with air-jet stress (Soltis et al 1992). Thus, increased body temperature is another physiological component of the stress response that appears to be mediated by ionotropic glutamate receptors in the DMH.

To better define the specific subtype(s) of ionotropic glutamate receptors in the DMH that was (were) relevant to the effect of kynurenate, a nonspecific antagonist of ionotropic glutamate receptors, I employed more selective antagonists in the same paradigm. In the experiments described below, I examined the effect of microinjection of a combination of NBQX (an AMPA/kainate receptor antagonist) and APV (an NMDA receptor antagonist) into the DMH on the increases in body temperature seen in cage-switch stress.

B. Microinjection of a combination of NBQX and APV into DMH attenuates increases in temperature induced by cage-switch stress

As shown in Figure 4, cage-switch stress produced a significant increase in core body temperature, and this increase in temperature was completely prevented by prior microinjection of a combination of NBQX and APV into the DMH. It is important to acknowledge various components together may account for the increase in temperature response observed in animals exposed to cage-switch stress. As discussed earlier, the microinjection alone could be responsible for an increase in temperature that may be a caused by tissue damage resulting from the insertion of the microinjectors. During the cage-switch stress procedure, rats are picked up by the base of their tails. It is possible that this type of handling alone could be responsible for the increase in temperature. More likely, grabbing the rat by the tail and then placing the animal in a new clean cage both contribute to the increase in body temperature observed. One way to differentiate between the different components of the cage-switch stress paradigm would be to add another treatment in which rats are picked up by their tails and placed back in their home cage. Likewise, it might be possible to test

for the effect of the novel environment alone by placing the rat in a cage with a divider that could be lifted to allow the rat to explore the “new” side of its cage. Nonetheless, microinjection of either a combination of NBQX and APV or kynurenate abolished the increase in temperature induced by cage-switch stress. However, the high concentrations of kynurenate in the injectate necessary to achieve blockade of glutamate receptors allows the possibility of non-specific receptor activity and possibly neurotoxicity (Koh et al., 1990). Because of their relative potency as antagonists of either non-NMDA or NMDA receptors, effective blockade could be achieved at concentrations of NBQX and APV that were 100 times smaller than that of kynurenate. The increased potency and lower concentration of NBQX and APV required to achieve may produce a more selective effect. I suggest that the decrease in temperature from baseline seen in the control animals following pretreatment with kynurenate was a consequence of a non-selective effect of the high concentration of this agent, and that greater selectivity of NBQX and APV may account for the lack of this effect in the control animals pretreated with the combination of these drugs.

Interestingly, microinjections of NBQX and APV in unstressed animals prevented the increase in temperature observed in the control (vehicle treated and unstressed) animals. These results are similar to those reported for the effect of pretreatment with kynurenate (see Figure 3).

Overall, the findings suggest that blockade of ionotropic glutamate receptors in the DMH abolishes the increase in temperature produced by cage-switch stress. One of the advantages of using NBQX and APV in combination is

that these antagonists can also be administered singly to help distinguish the roles of NMDA versus non-NMDA subclasses of glutamate receptors.

C. The effect of microinjection of APV in the DMH on increases in body temperature induced by cage-switch differs from that of NBQX

The role of NMDA versus non-NMDA subtypes of ionotropic glutamate receptors was examined by assessing the effects of microinjection of either NBQX or APV alone into the DMH. Bilateral microinjection of APV into the DMH did not significantly attenuate the increase in temperature produced by cage-switch stress as was seen when NBQX and APV were administered together (Figure 5). Pretreatment with APV also did not significantly attenuate the increase in temperature produced by the experimental procedure under control (unstressed) conditions as discussed above (Figure 5). However, a definite trend in the data suggested that such an effect might have been demonstrated with a larger sample size. One possible interpretation of these results is that activity at NMDA receptors in the DMH is not solely responsible for the increase in temperature produced by cage-switch stress. Interestingly, microinjection of a similar dose of APV (100pmol/100nL) into the DMH attenuated the increase in heart rate induced by air-jet stress (Soltis and DiMicco, 1992b). Microinjection of this same dose of APV also attenuated the increase in heart rate induced by microinjections of NMDA in the DMH and was shown to act selectively at NMDA receptors versus non-NMDA receptors (Soltis and DiMicco, 1992b). The increase in heart rate and the increase in body temperature are well-documented responses to stress that are thought to be mediated through activation of neurons in the DMH. However, the failure of the APV pretreatment to attenuate

the increase in temperature induced by cage-switch stress suggests that the increase in body temperature and the increase in heart rate are not mediated through the same populations of glutamate receptors in the DMH. Therefore, blockade of both NMDA and non-NMDA receptors appears to be required to attenuate the increase in temperature produced by cage-switch stress. Blockade of NMDA receptors tended to attenuate the increase in temperature observed in control animals although the difference was not statistically significant. The fact that NBQX alone only attenuates the increase in temperature induced by cage-switch but the addition of APV abolishes this response strongly suggests that NMDA receptors play a role in mediating some of the components of the cage-switch stress, specifically the increase in temperature induced by manipulation stress.

Unlike pretreatment with APV, microinjections of NBQX significantly attenuated the increase in temperature produced by cage-switch stress, but did not significantly attenuate the increase in temperature produce by manipulation. Careful examination of the increase in temperature of animals exposed to cage-switch (see Figure 6) suggests that the difference between the temperature response of vehicle treated animals and the response of the NBQX treated animals is about the same as the increase seen in the vehicle-treated control (unstressed) group. The results suggest the possibility that non-NMDA receptors do not mediate the response to manipulation stress but do mediate the effects evoked by cage-switch stress. Therefore, microinjection of a combination of

NBQX and APV was the only treatment that abolished the increase in temperature induced by cage-switch stress.

The data from the cage-switch studies demonstrates that activation of ionotropic glutamate receptors in the DMH is responsible for the increase in temperature produced by cage-switch stress. A similar observation was made with respect to the increases in heart rate seen in air-jet stress when APV and 6-cyano-7-nitroquino-xaline-2,3-dione (CNQX), a non-NMDA receptor antagonist, were administered as pretreatments alone or in combination (Soltis and DiMicco, 1991). When either agent was microinjected into the DMH alone the increase in heart rate induced by stress was reduced by 30%, but when the antagonists were administered in combination, the tachycardia was reduced by 60%. Thus, as with the increases in heart rate evoked by air-jet stress, the increases in body temperature induced by cage-switch stress are mediated through neuronal activity at NMDA and non-NMDA subtypes of glutamate receptors.

One possibility that accounts for why inhibition of both NMDA and non-NMDA receptors is required for the increase in temperature evoked by cage-switch stress is that both glutamate receptor subtypes participate in synaptic transmission at the same critical synapses in the DMH that are involved in the response. Early studies pointed to synaptic excitation mediated by both NMDA and non-NMDA receptors at other brain sites. In 1985, Dale and Roberts using *Xenopus* embryos demonstrated that the excitatory post-synaptic potentials (EPSP) induced in motoneurons by focal stimulation of longitudinal axons could be classified as a fast EPSP, slow EPSP or a mixed EPSP (Dale and Roberts,

1985). Blockade of either receptor subtype on a neuron exhibiting a mixed EPSP left either a pure fast EPSP or a pure slow EPSP. Dale and Roberts demonstrated that one neuron could release a transmitter that activated at both NMDA and non-NMDA receptors on the same post-synaptic neuron generating fast and slow post-synaptic potentials (Dale and Roberts, 1985). Pharmacological experiments proved that the fast EPSPs were mediated by the non-NMDA ionotropic glutamate receptors and that the slow EPSPs were mediated by NMDA ionotropic glutamate receptors (Dale and Roberts, 1985). Similar findings were reported in various hippocampal preparations (Blake et al., 1988; Collingridge and Lester, 1989; Dale and Grillner, 1986). Therefore, inhibition of just one receptor subtype, either NMDA or non-NMDA, is not sufficient to prevent the increase in temperature induced by cage-switch. After blockade of one receptor subtype, activation of the other is still capable of exciting the post-synaptic neuron, thus activating effectors to elicit an increase in body temperature.

Although it is not possible to discriminate how neurons in the DMH are activated by stress I suggest there are several possibilities. Neurons in the DMH could either be activated by glutamatergic afferent projections or by the withdrawal of tonic inhibition by GABAergic afferent projections. Neuronal excitability in the region of the DMH may be modulated by a balance between synaptic inhibition (mediated by GABA receptors) and synaptic excitation (mediated by ionotropic glutamate receptors) (Bailey et al., 2003; de Menezes et al., 2008; DiMicco et al., 2006b; Jardim and Guimaraes, 2004). This interaction

between GABAergic and glutamate receptors has been studied in the DMH and in other regions of the brain such as the hippocampus (Davies et al., 1990; Soltis and DiMicco, 1991a; Soltis and DiMicco, 1991b; Thompson and Gahwiler, 1989a). This model of synaptic circuitry suggests that both glutamatergic and GABAergic projections synapse on the same post-synaptic neuron to modulate that cell's activity. For example, under control conditions GABAergic synaptic inhibition may be sufficient to suppress activity caused by tonic stimulation of post-synaptic glutamate receptors. However, in stress, disinhibition of neurons in the DMH occurs and this allows the ionotropic glutamate receptor-mediated depolarization and subsequent activation of the post-synaptic neuron (Davies et al., 1990; Davies et al., 1982; Thompson and Gahwiler, 1989a; Thompson and Gahwiler, 1989b). GABAergic and glutamatergic neurons account for the majority of all afferents to the hypothalamus and may play a primary role in regulating output from this area (Headley and Grillner, 1990; van den Pol et al., 1990; van den Pol et al., 1994). The data presented does not allow discrimination between direct activation of relevant neurons in the DMH by glutamatergic neurons and indirect activation that may occur through disinhibition of the DMH neurons that would unmask the excitatory effect of tonic glutamatergic input. However, microinjection of either kynurenate or a combination of NBQX and APV in the region of the DMH did not induce significant changes in baseline body temperature. Although the present studies suggest a glutamatergic input to neurons in the DMH may exist, the source of this excitatory drive remains to be determined. However compelling evidence

suggests that one such region may be the periaqueductal gray region of the brain which has been shown to play a role in the stress responses mediated by glutamate receptors (de Menezes et al., 2009).

Other synaptic models may also be possible. Ionotropic glutamate receptors may be involved in feedback inhibition in which a glutamatergic neuron may synapse with both a neighboring glutamatergic neuron and a GABAergic inhibitory interneuron. Activation of the GABAergic interneuron by ionotropic glutamate agonists will induce release of GABA and in turn inhibit activity at glutamate receptors. Results from my studies only show that NMDA and non-NDMA receptors are involved in the increase in temperature induced by stress. The synaptic circuitry can't be deduced from my results but future studies could address the neural circuitry involved in the thermogenic response to stress.

In summary, the data presented suggest that the increase in temperature induced by exteroceptive stresses is dependent on the activation of both NMDA and non-NMDA ionotropic glutamate receptors in the DMH.

II. Role of ionotropic glutamate receptors in the temperature response to several interoceptive stresses

A. Disinhibition of neurons in the DMH induces increases in temperature that are mediated by NMDA and non-NMDA receptors in the DMH

Previous evidence has suggested that disinhibition of neurons or activation of neurons in the region of the DMH may be required for the generation of the physiological responses seen in stress regardless of whether the stress is interoceptive or exteroceptive. The similarity between the physiological responses to disinhibition of neurons in the DMH and the physiological

responses to emotional stress suggests that the same mechanism may be involved. Therefore, disinhibition of DMH neurons with BMI activates a common signaling pathway that is crucial for all stresses investigated in the studies presented.

Disinhibition of neurons in the DMH by microinjections of BMI induced marked increases in heart rate and modest pressor responses (DiMicco et al., 1986; DiMicco et al., 1987; Wible et al., 1988; Wible et al., 1989). Most pertinent to this thesis, disinhibition of neurons in the DMH by microinjections of BMI resulted in increases in core body temperature that were preceded by much more dramatic and rapid increases in local temperature in BAT of both anesthetized and conscious animals (Cao et al., 2004b; Zaretskaia et al., 2002).

Inhibition of neurons in the DMH by microinjections of muscimol abolished the increase in heart rate seen in air-jet stress and also attenuated the accompanying increase in pACTH (Lisa et al., 1989a; Lisa et al., 1989c; Stotz-Potter et al., 1996a; Stotz-Potter et al., 1996b). Microinjection of PGE_2 into the region of the POA, a model for fever, produced increase in heart rate and core temperature that were also attenuated by microinjections of muscimol in the DMH (Zaretskaia et al., 2003). Likewise, Madden and Morrison found that inhibition of neurons in the DMH abolished the increases in heart rate, IBAT temperature and IBAT sympathetic nerve activity evoked by microinjection of PGE_2 into the POA (Madden and Morrison, 2004; Morrison et al., 2008). The evidence suggests that neurons in the region of the DMH responsible for the increase in heart rate, pACTH levels and temperature induced by stress are

tonically inhibited by GABA (DiMicco et al., 1996; Stotz-Potter et al., 1996a; Stotz-Potter et al., 1996b; Zaretskaia et al., 2002). This GABAergic inhibitory input to the thermogenic neurons within the DMH may originate from the POA (Nakamura et al., 2005b; Zaretsky et al., 2003c). Inhibition of neurons in the POA by muscimol evokes increases in body temperature, heart rate, blood pressure, and plasma levels of ACTH, similar to the physiological responses seen following disinhibition of neurons in the DMH (Zaretsky et al., 2006).

In the current experiment, a single, unilateral microinjection of BMI into the DMH produced increases in body temperature of 0.88±0.09°C as previously reported (Zaretskaia et al., 2002; Zaretsky et al., 2003a). To examine the role of NMDA and non-NMDA subtypes of ionotropic glutamate receptors in the increase in temperature induced by BMI in the DMH, animals were pretreated with unilateral microinjection of combination of NBQX and APV or single microinjection of NBQX or APV into the DMH prior to microinjection of BMI at the same site. As described in the results, this increase in temperature was significantly attenuated but not abolished following unilateral pretreatment with a combination of NBQX and APV into the DMH. In contrast, similar bilateral microinjections of NBQX and APV completely abolished the increase in temperature produced by cage-switch stress. A possible reason for the difference in the degree of attenuation of the response seen with microinjection of NBQX and APV following microinjection of BMI in the DMH is that only one side of the DMH was inhibited by the antagonists in these experiments leaving the other side of the DMH intact. Thus, the uninhibited side of the DMH could

still signal effectors to generate an increase in body temperature in response to the stress induced by manipulation and microinjection as was seen in Figure 2. Microinjections of BMI are used as a model for stress and have been shown to produce similar responses as those seen with stress, but microinjections of BMI act by disinhibiting GABAergic neurons only. The responses to stress are due to activation of neurons and other receptors in the whole region of the DMH, which may also play a role in the responses to stress. A possibility arises from the fact that ionotropic glutamate receptors are the only receptors being inhibited in any of the studies described which is most likely not the case during the response to stress. However, it has been shown that metabotropic glutamate receptors in the DMH when activated also evoke increases in heart rate (Stotz-Potter et al., 1996b). Unlike with the responses to stress, microinjections of BMI into a single side of the DMH are only able to activate neurons on the site of injection. Therefore, it is possible that metabotropic glutamate receptors in the DMH could be the reason why combination of NBQX+APV was not able to abolish the increase in temperature evoked by BMI in the DMH or by the manipulation effect.

In summary, this study provides evidence that the increase in temperature induced by removal of GABAergic inhibition in the DMH of the rat is dependent on activation of local NMDA and non-NMDA glutamate receptor subtypes.

B. Combination of NBQX and APV attenuates the increase in temperature produced by microinjections of PGE_2 in the POA

Ionotropic glutamate receptor subtypes and their roles in the thermogenic response to stress were also studied in an interoceptive stress paradigm. The interoceptive stress paradigm used was microinjection of PGE_2 in the POA, an

established model for fever. A previous study examined the role of ionotropic glutamate receptors in the DMH in the thermogenic response to microinjection of PGE_2 in the POA in anesthetized animals. Microinjection of PGE_2 in the POA produced a significant increase in body temperature, IBAT temperature and IBAT SNA, all responses which were attenuated by the microinjection of kynurenate in the DMH (Madden and Morrison, 2004). However, interpretation of the results of studies of body temperature in anesthetized animals is confounded by the fact that, because the animal cannot regulate its body temperature and becomes "poikilothermic-like" (Malkinson et al., 1988; Malkinson et al., 1993), body temperature is generally artificially supported by external means. To study the effects on body temperature without this confound induced by anesthesia, animals used in the studies described were conscious and freely moving.

In my study conscious rats were pretreated with bilateral microinjections of either aCSF or a combination of NBQX+APV into the DMH prior to microinjection of either vehicle or PGE_2 in the POA. After injection of vehicle into the DMH, microinjection of PGE_2 in the POA elevated body temperature above baseline by a mean maximum of +1.81±0.36°C (Figure 12). Injection of vehicle into the POA also evoked an increase in temperature that was significantly different from baseline (+0.44±0.08°C, Figure 12). Again, microinjection of the combination of NBQX and APV bilaterally into the DMH abolished the increase in temperature induced by experimental manipulation and almost completely abolished the increase in temperature induced by microinjection of PGE_2 into the POA. These results provide clear evidence that the increase in temperature produced by

microinjection of PGE_2 in the POA is mediated by ionotropic glutamate receptors in the DMH of conscious animals. Pretreatment with the combination of NBQX and APV showed responses similar to baseline values. Therefore, these data suggest that neuronal activity dependent on tone at ionotropic glutamate receptors in the DMH did not contribute to baseline body temperature in our study. As can be seen from Figure 11, microinjection of PGE_2 into POA caused a maximal increases in body temperature at +40–50 min. According to the results from a previous study, 30 minutes after the microinjection of PGE_2 in POA, a microinjection of muscimol in the DMH attenuated the increase in body temperature suggesting that at 30min after microinjection of PGE_2 in the POA, disinhibition of neurons in the DMH may still be occurring (Zaretskaia et al., 2003). Therefore, the apparent and prolonged increase in temperature observed following PGE_2 in the POA may be simply due to a long duration of action and the animal begins to thermoregulate at a higher body temperature or “set-point”. Therefore, thermoeffectors would be working to maintain body temperature at the higher set point. The effects of PGE_2 in the POA appear to be long lasting as temperature remained elevated 95 minutes after microinjection of PGE_2. A possible explanation for the effect of PGE_2 in the POA may also be related to the fact that tissue damage occur during the insertion of the microinjectors. Unlike any of the other paradigms described, rats for these studies have three guide cannulas implanted in the brain. The microinjectors targeting the POA may induce an inflammatory response that results in the further release of endogenous PGE_2 into the POA. This continuous release of PGE_2 in the POA

could account for the long lasting increase in temperature observed in these studies following PGE_2 in the POA. Furthermore, in the animals treated with aCSF in the POA, an increase in body temperature significantly different from baseline is also observed. Again, this increase in temperature may be evoked by the exacerbation of any tissue damage done by the implanted microinjectors.

Thus, neurons in the region of the DMH, a hypothalamic area where chemical stimulation increases heart rate and core and BAT temperature (Zaretskaia et al., 2002), play a role in the thermogenic response to PGE_2 acting in the POA. Microinjection of PGE_2 into POA elicits marked increases in body temperature in conscious and anesthetized animals, and has been employed as a model for the fever associated with bacterial infection (Aronoff and Neilson, 2001; Madden and Morrison, 2004; Milton, 1998; Nakamura et al., 2002; Zaretskaia et al., 2003). In fact, the POA is the greatest source of hypothalamic afferents to the DMH (Thompson and Swanson, 1998). Furthermore, neurons in the region of the DMH are activated by systemic administration lipopolysaccharide and by exposure to cold, both of which trigger increases in temperature induced by the POA (Elmquist and Saper, 1996; Kiyohara et al., 1995). Neurons in the POA exert tonic inhibition on downstream mechanisms capable of increasing the sympathetic thermogenic activity (Chen et al., 1998; Morrison, 2004). It is thought that microinjection of PGE_2 in the POA evokes disinhibition of neurons in the DMH (DiMicco and Zaretsky, 2007; Morrison, 2004; Nakamura et al., 2005b; Zaretskaia et al., 2002; Zaretskaia et al., 2003). As was observed in this study and others, disinhibition of neurons in the DMH by BMI

evokes an increase in temperature, heart rate, blood pressure, and pACTH levels, responses also seen with microinjection of PGE_2 into the POA (Bailey and Dimicco, 2001; DiMicco et al., 1996; Samuels et al., 2004; Zaretskaia et al., 2002; Zaretskaia et al., 2003; Zaretsky et al., 2006). Furthermore, the increase in temperature induced by PGE_2 into the POA is a response attenuated by microinjection of muscimol, a GABA agonist and general neuronal inhibitor, into DMH (Zaretskaia et al., 2003). The data from the present study suggests that the increase in temperature requires activation of local ionotropic glutamate receptors.

In summary, the increase in temperature induced by PGE_2 in the POA may involve a balance between the synaptic inhibition by GABA receptors and synaptic excitation by the ionotropic glutamate receptor subtypes in the DMH.

C. Microinjection of APV and NBQX have differing effects on increases in temperature evoked by microinjections of PGE_2 in POA

In order to characterize the roles of specific subtypes of ionotropic glutamate receptors in the DMH in the response to microinjection of PGE_2 into the POA, I studied the effect of microinjections of NBQX and APV individually into the DMH. Unlike microinjection of the combination of NBQX and APV, microinjection of APV alone did not attenuate the increase in temperature evoked by PGE_2 in the POA (Figure 12). It did however abolish the increase in temperature evident in the vehicle-treated animals, an increase most likely due to manipulation stress as discussed above. In the cage-switch studies (see Figure 5), pretreatment with APV in the unstressed animals did not significantly attenuate the increase in temperature seen in the control group although a trend

for this effect was clearly evident. The ability of APV to abolish the increase in temperature induced by the manipulation associated with the injection of vehicle suggests that activation of NMDA receptors may mediate this increase in temperature. One component of the increase in temperature seen with manipulation stress is the effect from the acute tissue damage at the injection site. It is possible that tissue damage by either the cannula or insertion of injectors results in an increase in temperature that is ultimately mediated through activation of NMDA receptors. Therefore, blockade of NMDA receptors by APV abolishes the increase in temperature seen in the control and unstressed animals. On the other hand, microinjections of NBQX into DMH did not prevent the increase in temperature seen after injection of vehicle or the increase in temperature evoked by PGE_2 into POA. In fact, after pretreatment with NBQX responses evoked by microinjection of PGE_2 were identical to those seen after pretreatment with vehicle. Therefore, non-NMDA receptors may be responsible for the increase in temperature induced by microinjections of PGE_2 in the POA.

These data suggest that the thermogenic response to PGE_2 in the POA may be composed of two components, one mediated by NMDA receptors and the other by the non-NMDA receptors. The only unexpected result from this study is the complete abolishment by pretreatment with APV of the increase in temperature seen after microinjection of vehicle, an effect presumably resulting from both the stress of the experimental manipulation and the physical effect of placing the microinjection cannula in tissue and injecting vehicle. In all the experiments described thus far, microinjections of APV failed to attenuate the

increase in temperature seen after injection of vehicle. However, this stress paradigm differed from all other stress paradigms discussed thus far in that it involved placement of a cannula in the POA region. It is possible that delivery of vehicle into the POA region may lead to excitation of a synaptic mechanism that is mediated solely by NMDA receptors in the DMH and does not require activation of the non-NMDA ionotropic glutamate receptors. One possibility is that tissue damage from the cannulas may induce an inflammatory response within the region of the DMH inducing release of interlukin 1 beta which has been shown to not only cause activation of NMDA receptors but may lead to NMDA excitotoxicity (Hagan et al., 1996a; Hagan et al., 1996b). From the data, it is not possible to determine what synaptic mechanism could be responsible for this increase in temperature evoked by the manipulations used in this experimental paradigm.

III. Future Studies

Air-jet stress, cage-switch stress, and manipulation-induced stress are considered exteroceptive stresses. However, when analyzing the manipulation-induced stress, there is a possibility that tissue damage caused by the guide cannula in the region of the DMH may also evoke an inflammatory response which may be considered a type of interoceptive stress. The data presented so far suggests that ionotropic glutamate receptors in the DMH play a role in the increase in temperature produced by cage-switch stress. Unexpectedly in the case of air-jet stress, data suggests that ionotropic glutamate receptors also play a role in thermoregulation, the ability to maintain a normal body temp in an

environment outside the TNZ (Carlson and Cottle, 1956; Foster and Frydman, 1979; Fuller et al., 1975; Golozoubova et al., 2006; Hart and Jansky, 1963; Morrison, 2004; Morrison et al., 2008). It is possible that ionotropic glutamate receptors may play a role in activating thermogenic effectors stimulated by factors other than stress such as exposure to cold ambient temperatures or behavioral changes such as those occurring during exercise. Recently our laboratory proposed that the DMH may not only mediate stress/defense responses, but that DMH neurons may also integrate thermoregulatory responses to cold and fever (DiMicco and Zaretsky, 2007). We know that disinhibition of neurons in the DMH by microinjections of BMI produces activation of sympathetic nerves to IBAT and also induce increases in IBAT temperature (Zaretskaia et al., 2002; Cao et al., 2004). Key studies showed that inhibition of neurons by microinjections of muscimol in the DMH prevented shivering in animals exposed to cold (Tanaka et al., 2001) and also reversed the activation of sympathetic nerve activity to IBAT in response to cold exposure (Nakamura and Morrison, 2007). IBAT is responsible for nonshivering thermogenesis, a major component of facultative thermogenesis in many mammals. It would be interesting to see if inhibition of ionotropic glutamate receptors in the DMH prevents nonshivering thermogenesis in animals exposed to cold temperatures. Furthermore, with the possibility that these ionotropic glutamate receptors may play a role in mediating increases in body temperature cause by exposure to cold, it would also be of interest to determine which specific thermogenic effectors

might be stimulated by these receptors (such as cutaneous vasoconstriction, IBAT thermogenesis, or metabolic effectors).

The more recent availability of selective agonists that can differentiate between the AMPA and KA receptor-mediated responses (Blair et al., 2004; Giardina and Beart, 2001; Matzen et al., 1997) may provide further insight into the synaptic circuitry and pharmacology involved in the hypothalamic mechanisms and of other regions in the CNS in the physiological responses to stress. In order to better understand the role of ionotropic glutamate receptors in the DMH, it would be useful to examine the origin of the glutamatergic projections to the DMH. There is evidence the caudal PAG region may be involved in both cold and stress-induced increases in body temperature. Recently, de Menezes and colleagues showed that microinjection of a combination of the glutamate receptor antagonists APV and NBQX at a concentration of 1mM into the caudal lateral/dorsal lateral PAG decreased stress-induced increases in heart rate (de Menezes et al., 2008). In addition to the DMH as a site involved in mediating stress-induced responses, other areas, such as the paraventricular (PVH), ventromedial (VMH) nuclei, and the posterior (PH) and lateral (LH) hypothalamic areas and most recently the periaqueductal gray (PAG), are also thought to participate in thermoregulation (Cano et al., 2003; de Menezes et al., 2006; Pattij et al., 2001; Rathner and Morrison, 2006; Thornhill et al., 1994; Thornhill and Halvorson, 1994; Yoshida et al., 2005). However, studies from our lab have called into question the anatomical specificity of the observations from studies involving the PVH, VMH and PH due to the large volumes of injectate used as

well as the lack of appropriate anatomical control injections (for review see DiMicco and Zaretsky, 2007). In my studies, injection of any of the antagonists at sites 0.5-1.0mm lateral, posterior, or anterior to the region of the DMH did not attenuate the increase in temperature response induced by stress as shown in the Figures 14A and B. Figures 14A and B show that microinjection of the combination of NBQX and APV at sites outside the region of the DMH did not have any effect on the increase in temperature induced by PGE_2 in the POA. This suggests that the effects of the antagonists are localized to neurons in the region of the DMH. Determining which areas might be involved in the responses to stress could further help us target these areas for pharmacological studies.

IV. Summary

The purpose of these experiments was to investigate the role of ionotropic glutamate receptors in the DMH in the increases in temperature induced by various stress paradigms, including both exteroceptive and interoceptive stressors. The results demonstrate that inhibition of ionotropic glutamate receptors by either kynurenate or a combination of NBQX and APV into the DMH either attenuated or prevented the increase in temperature induced by the various stress paradigms employed. The increases in temperature resulting from either exteroceptive or interoceptive stresses are mediated by activation of ionotropic glutamate receptors in the DMH, and both NMDA and non-NMDA subtypes of ionotropic glutamate receptors are involved. This suggests the possibility that the same synaptic mechanism in the hypothalamus is responsible for the increase in temperature induced by stress, regardless of whether it is

interoceptive or exteroceptive. This is important because it is thought that the increase in temperature induced by interoceptive stress, a stress usually exemplified by a bacterial infection, requires the "resetting" of the body's temperature set-point to a higher temperature. On the other hand, debate still exists regarding whether exteroceptive stress induces increases in body temperature by mechanisms that do not require resetting of the body's set-point. From the present studies, it is clear that at least in the hypothalamus, these two classes of stresses evoke increases in body temperature through the similar mechanisms that involve activation of local ionotropic glutamate receptors. Most recently in our lab, it was also shown that blockade of ionotropic glutamate receptors in the DMH attenuates the increase in pACTH, neuroendocrine marker for stress, induced by PGE_2 into POA (unpublished data). Overall, the evidence is clear that neurons in the region of the DMH play a key role in the physiological responses induced by stress and more specifically, we now know that ionotropic glutamate receptor subtypes are involved in this mechanism. Therefore, the results from all the studies presented have improved our understanding of the hypothalamic mechanisms involved in the stress response by demonstrating that

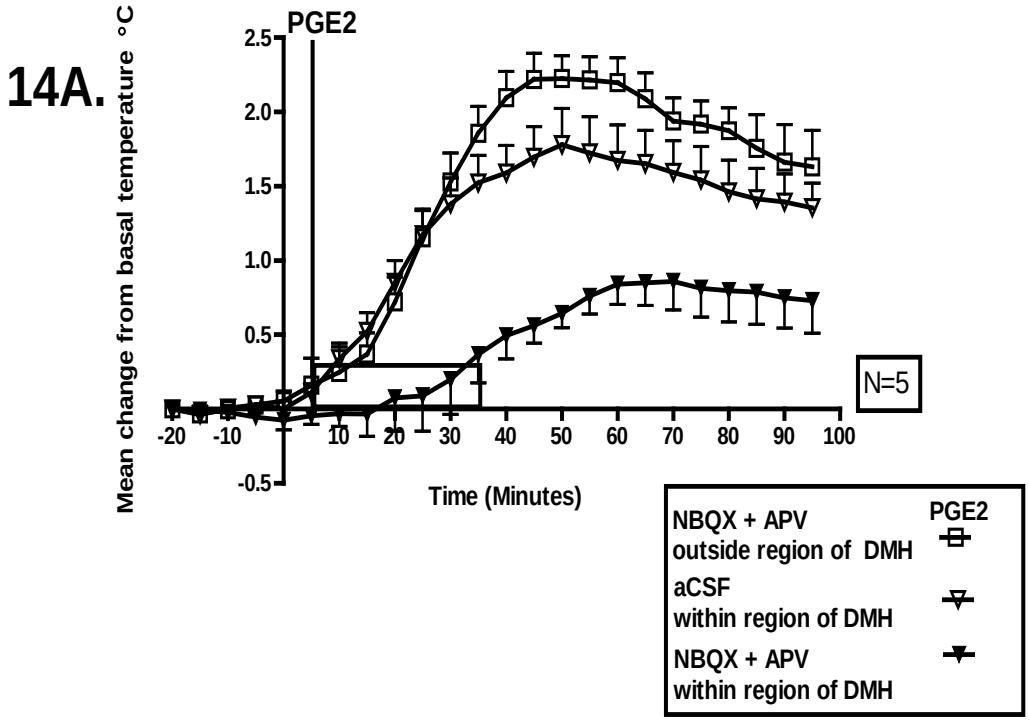


FIGURE 14A. Real time temperature response of rats pretreated with either vehicle or NBQX+APV bilaterally in the DMH followed by a microinjection of PGE $_2$ in the POA. Time zero indicates point of microinjections into the DMH. Solid line depicts microinjection of vehicle or PGE $_2$ in the POA. Light gray box is the 30 minute time period that was analyzed statistically.

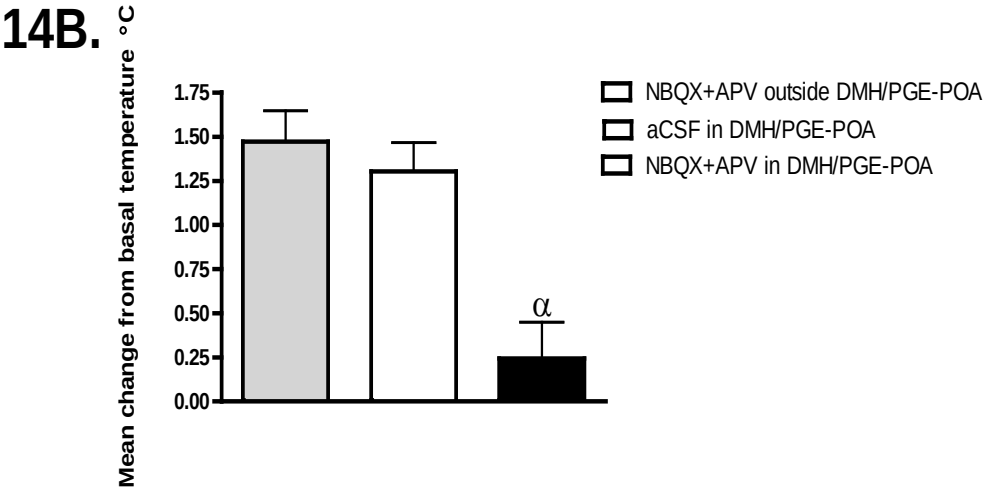


FIGURE 14B. Anatomical control group for the effects of NBQX(100pmol/side) and APV(200pmol/side) in DMH on fever evoked by microinjection of PGE $_2$ (150pmol) in the POA. Results are expressed as means ± S.E.M of the 30 minute period following treatment (gray box). (δ) Significant difference between Vehicle (DMH)/ PGE $_2$ (POA) and NBQX+APV (DMH)/ PGE $_2$ (POA). For anatomical control of the DMH target site, a set of animals were treated with NBQX+APV in areas near the region of the DMH (shaded bar). Significant differences determined using One-Way ANOVA and Fisher's LSD post-hoc test. Limits of probability considered significant were 5%.

ionotropic glutamate receptors in the DMH are key players in the thermogenic response to stress.

The DMH is likely to be one of the more important of the many CNS sites involved in the physiological response to stress. The work presented here can lead to further examination of the hypothalamic signaling pathways involved in the central control of not just the increase in body temperature but of the other physiological responses to stress. It is important to continue our quest to understand how the brain mediates stress and the stress response because stress and the repercussions of continuous exposure to stress can lead to many other negative conditions. Unlike our ancestors, our stressors are not those of being hunted by a lion for example, but it seems that we have more things that stress us. There are many techniques that allegedly can be helpful in dealing with stress including practicing yoga, meditation, acupuncture, and exercise. Drugs including benzodiazepines and barbiturates are used to treat stress and stress disorders. However, these drugs carry the risk of overuse and dependency. A better understanding of the central control of these responses can lead to new drug discovery and perhaps better treatments or prevention of stress-related illnesses. The research presented here provides another piece of the puzzle that could prove useful in our discovery of new drug targets.

REFERENCES

Akutsu, H., Kikusui, T., Takeuchi, Y., Sano, K., Hatanaka, A., Mori, Y., 2002. Alleviating effects of plant-derived fragrances on stress-induced hyperthermia in rats. Physiol Behav. 75**,** 355-60.

Alexander, G., 1979. Cold thermogenesis. Int Rev Physiol. 20**,** 43-155.

Amico, J.A., Mantella, R.C., Vollmer, R.R., Li, X., 2004. Anxiety and stress responses in female oxytocin deficient mice. J Neuroendocrinol. 16**,** 319-24.

Anis, N.A., Clark, R.B., Gration, K.A., Usherwood, P.N., 1981. Influence of agonists on desensitization of glutamate receptors on locust muscle. J Physiol. 312**,** 345-64.

Argyropoulos, G., Harper, M.-E., 2002. Molecular Biology of Thermoregulation: Invited Review: Uncoupling proteins and thermoregulation. J Appl Physiol. 92**,** 2187-2198.

Aronoff, D.M., Neilson, E.G., 2001. Antipyretics: mechanisms of action and clinical use in fever suppression. Am J Med. 111**,** 304-15.

Ascher, P., Bregestovski, P., Nowak, L., 1988. N-methyl-D-aspartate-activated channels of mouse central neurones in magnesium-free solutions. J Physiol. 399**,** 207-26.

Ascher, P., Nowak, L., 1988. The role of divalent cations in the N-methyl-D-aspartate responses of mouse central neurones in culture. J Physiol. 399**,** 247-66.

Ault, B., Evans, R.H., Francis, A.A., Oakes, D.J., Watkins, J.C., 1980. Selective depression of excitatory amino acid induced depolarizations by magnesium ions in isolated spinal cord preparations. J Physiol. 307**,** 413-28.

Bailey, T.W., Dimicco, J.A., 2001. Chemical stimulation of the dorsomedial hypothalamus elevates plasma ACTH in conscious rats. Am J Physiol Regul Integr Comp Physiol. 280**,** R8-15.

Bailey, T.W., Nicol, G.D., Schild, J.H., DiMicco, J.A., 2003. Synaptic and membrane properties of neurons in the dorsomedial hypothalamus. Brain Res. 985**,** 150-62.

Balcar, V.J., Johnston, G.A., 1972. The structural specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices. J Neurochem. 19**,** 2657-66.

Bamshad, M., Song, C.K., Bartness, T.J., 1999. CNS origins of the sympathetic nervous system outflow to brown adipose tissue. Am J Physiol. 276**,** R1569-78.

Barrera, N.P., Henderson, R.M., Edwardson, J.M., 2008. Determination of the architecture of ionotropic receptors using AFM imaging. Pflugers Arch. 456**,** 199-209.

Bellocchio, E.E., Reimer, R.J., Fremeau, R.T., Jr., Edwards, R.H., 2000. Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. Science. 289**,** 957-60.

Benavides, J., Rivy, J.P., Carter, C., Scatton, B., 1988. Differential modulation of [3H]TCP binding to the NMDA receptor by L-glutamate and glycine. Eur J Pharmacol. 149**,** 67-72.

Bennett, J.P., Jr., Logan, W.J., Snyder, S.H., 1973. Amino acids as central nervous transmitters: the influence of ions, amino acid analogues, and ontogeny on transport systems for L-glutamic and L-aspartic acids and glycine into central nervous synaptosomes of the rat. J Neurochem. 21**,** 1533-50.

Beotra, A., Sanyal, A.K., 1982. Immobilization stress induced hyperthermia in rats: possible role of noradrenaline. Indian J Exp Biol. 20**,** 759-60.

Bergink, V., van Megen, H.J., Westenberg, H.G., 2004. Glutamate and anxiety. Eur Neuropsychopharmacol. 14**,** 175-83.

Bernardis, L.L., Bellinger, L.L., 1987. The dorsomedial hypothalamic nucleus revisited: 1986 update. Brain Res. 434**,** 321-81.

Bertolino, M., Vicini, S., Costa, E., 1989. Kynurenic acid inhibits the activation of kainic and N-methyl-D-aspartic acid-sensitive ionotropic receptors by a different mechanism. Neuropharmacology. 28**,** 453-7.

Birch, P.J., Grossman, C.J., Hayes, A.G., 1988a. Kynurenate and FG9041 have both competitive and non-competitive antagonist actions at excitatory amino acid receptors. Eur J Pharmacol. 151**,** 313-5.

Birch, P.J., Grossman, C.J., Hayes, A.G., 1988b. Kynurenic acid antagonises responses to NMDA via an action at the strychnine-insensitive glycine receptor. Eur J Pharmacol. 154**,** 85-7.

Biscoe, T.J., Davies, J., Dray, A., Evans, R.H., Francis, A.A., Martin, M.R., Watkins, J.C., 1977a. Depression of synaptic excitation and of amino acid induced excitatory responses of spinal neurones by D-alpha-aminoadipate, alpha,epsilon-diaminopimelic acid and HA-966. Eur J Pharmacol. 45**,** 315-6.

Biscoe, T.J., Evans, R.H., Francis, A.A., Martin, M.R., Watkins, J.C., Davies, J., Dray, A., 1977b. D-alpha-Aminoadipate as a selective antagonist of amino acid-induced and synaptic excitation of mammalian spinal neurones. Nature. 270**,** 743-5.

Blair, R.H., Timothy, W., Ian, M., Kerr, D.S., 2004. Kainate receptor agonists and antagonists mediate tolerance to kainic acid and reduce high-affinity GTPase activity in young, but not aged, rat hippocampus. J Neurochem. 90**,** 70-79.

Blake, J.F., Brown, M.W., Collingridge, G.L., 1988. A quantitative study of the actions of excitatory amino acids and antagonists in rat hippocampal slices. Br J Pharmacol. 95**,** 291-9.

Blanc, J., Grichois, M.L., Elghozi, J.L., 1991. Effects of clonidine on blood pressure and heart rate responses to an emotional stress in the rat: a spectral study. Clin Exp Pharmacol Physiol. 18**,** 711-7.

Blatteis, C.M., 1992. Role of the OVLT in the febrile response to circulating pyrogens. Prog Brain Res. 91**,** 409-12.

Blatteis, C.M., Sehic, E., 1998. Cytokines and Fever. Ann NY Acad Sci. 840**,** 608-618.

Blessing, W.W., Yu, Y.H., Nalivaiko, E., 1999. Raphe pallidus and parapyramidal neurons regulate ear pinna vascular conductance in the rabbit. Neurosci Lett. 270**,** 33-6.

Blessing, W.W., Nalivaiko, E., 2001. Raphe magnus/pallidus neurons regulate tail but not mesenteric arterial blood flow in rats. Neuroscience. 105**,** 923-9.

Bonaz, B., Tache, Y., 1994. Induction of Fos immunoreactivity in the rat brain after cold-restraint induced gastric lesions and fecal excretion. Brain Res. 652**,** 56-64.

Boulant, J.A., Dean, J.B., 1986. Temperature receptors in the central nervous system. Annu Rev Physiol. 48**,** 639-54.

Boulant, J.A., 2000. Role of the preoptic-anterior hypothalamus in thermoregulation and fever. Clin Infect Dis. 31 Suppl 5**,** S157-61.

Bouwknecht, A.J., Olivier, B., Paylor, R.E., 2007. The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: a review of pharmacological and genetic studies in the mouse. Neurosci Biobehav Rev. 31**,** 41-59.

Bouwknecht, J.A., Hijzen, T.H., van der Gugten, J., Dirks, A., Maes, R.A., Hen, R., Geyer, M.A., Olivier, B., 2000. Startle responses, heart rate, and temperature in 5-HT1B receptor knockout mice. Neuroreport. 11**,** 4097-102.

Bouwknecht, J.A., Hijzen, T.H., van der Gugten, J., Maes, R.A., Hen, R., Olivier, B., 2001. Absence of 5-HT(1B) receptors is associated with impaired impulse control in male 5-HT(1B) knockout mice. Biol Psychiatry. 49**,** 557-68.

Bratincsak, A., Palkovits, M., 2005. Evidence that peripheral rather than intracranial thermal signals induce thermoregulation. Neuroscience. 135**,** 525-32.

Briese, E., De Quijada, M.G., 1970. Colonic temperature of rats during handling. Acta Physiol Lat Am. 20**,** 97-102.

Briese, E., Cabanac, M., 1991. Stress hyperthermia: physiological arguments that it is a fever. Physiol Behav. 49**,** 1153-7.

Briese, E., 1995. Emotional hyperthermia and performance in humans. Physiology & Behavior. 58**,** 615-618.

Briese, E., 1998. Normal body temperature of rats: the setpoint controversy. Neuroscience & Biobehavioral Reviews. 22**,** 427-436.

Busnardo, C., Tavares, R.F., Corrêa ,F.M. A. , 2009. Role of N-methyl-D-aspartate and non-N-methyl-D-aspartate receptors in the cardiovascular effects of L-glutamate microinjection into the hypothalamic paraventricular nucleus of unanesthetized rats. Journal of Neuroscience Research. 9999**,** NA.

Cabanac, M., Gosselin, F., 1993. Emotional fever in the lizard Callopistes maculatus (Teiidae). Animal Behaviour. 46**,** 200-202.

Campeau, S., Watson, S.J., 1997. Neuroendocrine and behavioral responses and brain pattern of c-fos induction associated with audiogenic stress. J Neuroendocrinol. 9**,** 577-88.

Cannon, B., Houstek, J., Nedergaard, J., 1998. Brown Adipose Tissue: More Than an Effector of Thermogenesis? Ann NY Acad Sci. 856**,** 171-187.

Cano, G., Passerin, A.M., Schiltz, J.C., Card, J.P., Morrison, S.F., Sved, A.F., 2003. Anatomical substrates for the central control of sympathetic outflow to interscapular adipose tissue during cold exposure. J Comp Neurol. 460**,** 303-26.

Cao, W.-H., Morrison, S.F., 2003. Disinhibition of rostral raphe pallidus neurons increases cardiac sympathetic nerve activity and heart rate. Brain Research. 980**,** 1-10.

Cao, W.-H., Fan, W., Morrison, S.F., 2004a. Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. Neuroscience. 126**,** 229-240.

Cao, W.-H., Morrison, S.F., 2006. Glutamate receptors in the raphe pallidus mediate brown adipose tissue thermogenesis evoked by activation of dorsomedial hypothalamic neurons. Neuropharmacology. 51**,** 426-437.

Cao, W.H., Fan, W., Morrison, S.F., 2004b. Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. Neuroscience. 126**,** 229-240.

Carlson, L.D., Cottle, W.H., 1956. Regulation of heat production in cold-adapted rats. Proc Soc Exp Biol Med. 92**,** 845-9.

Chen, X.M., Hosono, T., Yoda, T., Fukuda, Y., Kanosue, K., 1998. Efferent projection from the preoptic area for the control of non-shivering thermogenesis in rats. J Physiol. 512 (Pt 3)**,** 883-92.

Chiba, T., Murata, Y., 1985. Afferent and efferent connections of the medial preoptic area in the rat: a WGA-HRP study. Brain Res Bull. 14**,** 261-72.

Chittajallu, R., Braithwaite, S.P., Clarke, V.R., Henley, J.M., 1999. Kainate receptors: subunits, synaptic localization and function. Trends Pharmacol Sci. 20**,** 26-35.

Chung, K.K., Martinez, M., Herbert, J., 1999. Central serotonin depletion modulates the behavioural, endocrine and physiological responses to repeated social stress and subsequent c-fos expression in the brains of male rats. Neuroscience. 92**,** 613-25.

Chung, K.K., Martinez, M., Herbert, J., 2000. c-fos expression, behavioural, endocrine and autonomic responses to acute social stress in male rats after chronic restraint: modulation by serotonin. Neuroscience. 95**,** 453-63.

Collingridge, G.L., Lester, R.A., 1989. Excitatory amino acid receptors in the vertebrate central nervous system. Pharmacol Rev. 41**,** 143-210.

Conti, B., Tabarean, I., Andrei, C., Bartfai, T., 2004. Cytokines and fever. Front Biosci. 9**,** 1433-49.

Cooper, J., Floyd E Bloom, Robert H. Roth, 2003. The Biochemical Basis of Neuropharmacology, Vol., Oxford University Press, New York.

Cotman, C.W., Monaghan, D.T., 1986. Anatomical organization of excitatory amino acid receptors and their properties. Adv Exp Med Biol. 203**,** 237-52.

Cotman, C.W., Kahle, J.S., Miller, S.E., Ulas, J., and Bridges, R.J., 1995. Excitatory amino acid neurotransmission. In: Psychopharmacology: The Fourth Generation of Progress. Vol., F.E.a.K. Bloom, D.J, ed.^eds. Raven, New York, pp. 75-85.

Cull-Candy, S.G., 2002. Ionotropic glutamate receptors: Functional and pharmacological properties in relation to constituent subunits. In: Glutamate and GABA Receptors and Transporters: Structure, function and pharamacology. Vol., J. Egebjerg, Schousboe, A., and Krogsgaard-Larsen, P., ed.^eds. Taylor and Francis, Inc, London and New York, pp. 3-55.

Cullinan, W.E., Helmreich, D.L., Watson, S.J., 1996. Fos expression in forebrain afferents to the hypothalamic paraventricular nucleus following swim stress. J Comp Neurol. 368**,** 88-99.

Curry, K., Magnuson, D.S., McLennan, H., Peet, M.J., 1986. Acridinic acid: a new antagonist of amino acid-induced excitations of central neurones. Neurosci Lett. 66**,** 101-5.

Curtis, D.R., Eccles, J.C., 1960. Synaptic action during and after repetitive stimulation. J Physiol. 150**,** 374-98.

Curtis, D.R., Phillis, J.W., 1960. The action of procaine and atropine on spinal neurones. J Physiol. 153**,** 17-34.

Curtis, D.R., Watkins, J.C., 1960. The excitation and depression of spinal neurones by structurally related amino acids. J Neurochem. 6**,** 117-41.

Curtis, D.R., Phillis, J.W., Watkins, J.C., 1961. Actions of aminoacids on the isolated hemisected spinal cord of the toad. Br J Pharmacol Chemother. 16**,** 262-83.

Curtis, D.R., Watkins, J.C., 1961. Analogues of glutamic and gamma-amino-n-butyric acids having potent actions on mammalian neurones. Nature. 191**,** 1010-1.

Curtis, D.R., Duggan, A.W., Felix, D., Johnston, G.A., Teb ecis, A.K., Watkins, J.C., 1972. Excitation of mammalian central neurones by acidic amino acids. Brain Res. 41**,** 283-301.

Curtis, D.R., 1974. Amino acid neurotransmitters and the brain. Beattie Smith lecture, March 28, 1974. Med J Aust. 2**,** 723-31.

Dale, N., Roberts, A., 1985. Dual-component amino-acid-mediated synaptic potentials: excitatory drive for swimming in Xenopus embryos. J Physiol. 363**,** 35-59.

Dale, N., Grillner, S., 1986. Dual-component synaptic potentials in the lamprey mediated by excitatory amino acid receptors. J Neurosci. 6**,** 2653-61.

Danbolt, N.C., 2001. Glutamate uptake. Prog Neurobiol. 65**,** 1-105.

Davies, C.H., Davies, S.N., Collingridge, G.L., 1990. Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J Physiol. 424**,** 513-31.

Davies, J., Watkins, J.C., 1979. Selective antagonism of amino acid-induced and synaptic excitation in the cat spinal cord. J Physiol. 297**,** 621-35.

Davies, J., Evans, R.H., Francis, A.A., Jones, A.W., Watkins, J.C., 1981a. Antagonism of excitatory amino acid-induced and synaptic excitation of spinal neurones by cis-2,3-piperidine dicarboxylate. J Neurochem. 36**,** 1305-7.

Davies, J., Francis, A.A., Jones, A.W., Watkins, J.C., 1981b. 2-Amino-5-phosphonovalerate (2APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. Neurosci Lett. 21**,** 77-81.

Davies, J., Watkins, J.C., 1981. Differentiation of kainate and quisqualate receptors in the cat spinal cord by selective antagonism with gamma-D(and L)-glutamylglycine. Brain Res. 206**,** 172-7.

Davies, J., Evans, R.H., Jones, A.W., Smith, D.A., Watkins, J.C., 1982. Differential activation and blockade of excitatory amino acid receptors in the mammalian and amphibian central nervous systems. Comp Biochem Physiol C. 72**,** 211-24.

De Belleroche, J.S., Bradford, H.F., 1972. Metabolism of beds of mammalian cortical synaptosomes: response to depolarizing influences. J Neurochem. 19**,** 585-602.

de Menezes, R.C., Zaretsky, D.V., Fontes, M.A., DiMicco, J.A., 2006. Microinjection of muscimol into caudal periaqueductal gray lowers body temperature and attenuates increases in temperature and activity evoked from the dorsomedial hypothalamus. Brain Res. 1092**,** 129-37.

de Menezes, R.C., Zaretsky, D.V., Sarkar, S., Fontes, M.A., Dimicco, J.A., 2008. Microinjection of muscimol into the periaqueductal gray suppresses cardiovascular and neuroendocrine response to air-jet stress in conscious rats. Am J Physiol Regul Integr Comp Physiol. 295**,** R881-90.

de Menezes, R.C., Zaretsky, D.V., Fontes, M.A., DiMicco, J.A., 2009. Cardiovascular and thermal responses evoked from the periaqueductal grey require neuronal activity in the hypothalamus. J Physiol. 587**,** 1201-15.

De Novellis, V., Stotz-Potter, E.H., Morin, S.M., Rossi, F., DiMicco, J.A., 1995a. Hypothalamic sites mediating cardiovascular effects of microinjected bicuculline and EAAs in rats. Am J Physiol Regul Integr Comp Physiol. 269**,** R131-140.

De Novellis, V., Stotz-Potter, E.H., Morin, S.M., Rossi, F., DiMicco, J.A., 1995b. Hypothalamic sites mediating cardiovascular effects of microinjected bicuculline and EAAs in rats. Am J Physiol. 269**,** R131-40.

Deolindo, M., Pelosi, G.G., Tavares, R.F., Aguiar Corrêa, F.M., 2008. The ventrolateral periaqueductal gray is involved in the cardiovascular response evoked by l-glutamate microinjection into the lateral hypothalamus of anesthetized rats. Neuroscience Letters. 430**,** 124-129.

DiMicco, J.A., Abshire, V.M., Hankins, K.D., Sample, R.H., Wible, J.H., Jr., 1986. Microinjection of GABA antagonists into posterior hypothalamus elevates heart rate in anesthetized rats. Neuropharmacology. 25**,** 1063-6.

DiMicco, J.A., Abshire, V.M., Shekhar, A., Wilbe, J.H., Jr., 1987. Role of GABAergic mechanisms in the central regulation of arterial pressure. Eur Heart J. 8 Suppl B**,** 133-8.

DiMicco, J.A., Stotz-Potter, E.H., Monroe, A.J., Morin, S.M., 1996. Role of the dorsomedial hypothalamus in the cardiovascular response to stress. Clin Exp Pharmacol Physiol. 23**,** 171-6.

DiMicco, J.A., Samuels, B.C., Zaretskaia, M.V., Zaretsky, D.V., 2002. The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. Pharmacol Biochem Behav. 71**,** 469-80.

DiMicco, J.A., Sarkar, S., Zaretskaia, M.V., Zaretsky, D.V., 2006a. Stress-induced cardiac stimulation and fever: Common hypothalamic origins and brainstem mechanisms. Autonomic Neuroscience. 126-127**,** 106-119.

DiMicco, J.A., Sarkar, S., Zaretskaia, M.V., Zaretsky, D.V., 2006b. Stress-induced cardiac stimulation and fever: common hypothalamic origins and brainstem mechanisms. Auton Neurosci. 126-127**,** 106-19.

DiMicco, J.A., Zaretsky, D.V., 2007. The dorsomedial hypothalamus: a new player in thermoregulation. Am J Physiol Regul Integr Comp Physiol. 292**,** R47-63.

Dinarello, C.A., 2004. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. J Endotoxin Res. 10**,** 201-22.

Dingledine, R., Borges, K., Bowie, D., Traynelis, S.F., 1999. The glutamate receptor ion channels. Pharmacol Rev. 51**,** 7-61.

Duchen, M.R., Burton, N.R., Biscoe, T.J., 1985. An intracellular study of the interactions of N-methyl-DL-aspartate with ketamine in the mouse hippocampal slice. Brain Res. 342**,** 149-53.

Eikelboom, R., 1986. Learned anticipatory rise in body temperature due to handling. Physiol Behav. 37**,** 649-53.

Elmquist, J.K., Saper, C.B., 1996. Activation of neurons projecting to the paraventricular hypothalamic nucleus by intravenous lipopolysaccharide. J Comp Neurol. 374**,** 315-31.

Evans, R.H., Francis, A.A., Hunt, K., Oakes, D.J., Watkins, J.C., 1979. Antagonism of excitatory amino acid-induced responses and of synaptic excitation in the isolated spinal cord of the frog. Br J Pharmacol. 67**,** 591-603.

Evans, R.H., Watkins, J.C., 1981. Pharmacological antagonists of excitant amino acid action. Life Sci. 28**,** 1303-8.

Evans, R.H., Francis, A.A., Jones, A.W., Smith, D.A., Watkins, J.C., 1982. The effects of a series of omega-phosphonic alpha-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. Br J Pharmacol. 75**,** 65-75.

Evans, R.H., Evans, S.J., Pook, P.C., Sunter, D.C., 1987. A comparison of excitatory amino acid antagonists acting at primary afferent C fibres and motoneurones of the isolated spinal cord of the rat. Br J Pharmacol. 91**,** 531-7.

Eyigor, O., Centers, A., Jennes, L., 2001. Distribution of ionotropic glutamate receptor subunit mRNAs in the rat hypothalamus. J Comp Neurol. 434**,** 101-24.

Fisahn, A., 2005. Kainate receptors and rhythmic activity in neuronal networks: hippocampal gamma oscillations as a tool. J Physiol. 562**,** 65-72.

Follett, P.L., Rosenberg, P.A., Volpe, J.J., Jensen, F.E., 2000. NBQX Attenuates Excitotoxic Injury in Developing White Matter. J. Neurosci. 20**,** 9235-9241.

Fonnum, F., 1984. Glutamate: a neurotransmitter in mammalian brain. J Neurochem. 42**,** 1-11.

Foster, D.O., Frydman, M.L., 1978. Brown adipose tissue: the dominant site of nonshivering thermogenesis in the rat. Experientia Suppl. 32**,** 147-51.

Foster, D.O., Frydman, M.L., 1979. Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. Can J Physiol Pharmacol. 57**,** 257-70.

Fuller, C.A., Horwitz, B.A., Horowitz, J.M., 1975. Shivering and nonshivering thermogenic responses of cold-exposed rats to hypothalamic warming. Am J Physiol. 228**,** 1519-24.

Fyda, D.M., Cooper, K.E., Veale, W.L., 1991. Contribution of brown adipose tissue to central PGE1-evoked hyperthermia in rats. Am J Physiol. 260**,** R59-66.

Ganong, A.H., Cotman, C.W., 1986. Kynurenic acid and quinolinic acid act at N-methyl-D-aspartate receptors in the rat hippocampus. J Pharmacol Exp Ther. 236**,** 293-9.

Gemmell, R.T., Hales, J.R., 1977. Cutaneous arteriovenous anastomoses present in the tail but absent from the ear of the rat. J Anat. 124**,** 355-8.

Giardina, S.F., Beart, P.M., 2001. Excitotoxic profiles of novel, low-affinity kainate receptor agonists in primary cultures of murine cerebellar granule cells. Neuropharmacology. 41**,** 421-32.

Girardier, L., Stock, M., 1983. Mammalian thermogenesis, Vol., Chapman and Hall, London ; New York.

Golozoubova, V., Cannon, B., Nedergaard, J., 2006. UCP1 is essential for adaptive adrenergic nonshivering thermogenesis. Am J Physiol Endocrinol Metab. 291**,** E350-7.

Gordon, C.J., Heath, J.E., 1986. Integration and Central Processing in Temperature Regulation. Annual Review of Physiology. 48**,** 595-612.

Gordon, C.J., 1990. Thermal biology of the laboratory rat. Physiol Behav. 47**,** 963-91.

Gordon, C.J., 1993. Temperature regulation in laboratory animals, Vol., Cambridge University Press.

Groenink, L., van Bogaert, M.J., van der Gugten, J., Oosting, R.S., Olivier, B., 2003. 5-HT1A receptor and 5-HT1B receptor knockout mice in stress and anxiety paradigms. Behav Pharmacol. 14**,** 369-83.

Hagan, P., Barks, J.D., Yabut, M., Davidson, B.L., Roessler, B., Silverstein, F.S., 1996a. Adenovirus-mediated over-expression of interleukin-1 receptor antagonist reduces susceptibility to excitotoxic brain injury in perinatal rats. Neuroscience. 75**,** 1033-45.

Hagan, P., Poole, S., Bristow, A.F., Tilders, F., Silverstein, F.S., 1996b. Intracerebral NMDA injection stimulates production of interleukin-1 beta in perinatal rat brain. J Neurochem. 67**,** 2215-8.

Hainsworth, F.R., 1967. Saliva spreading, activity, and body temperature regulation in the rat. Am J Physiol. 212**,** 1288-92.

Harris, R.L., Musher, D.M., Bloom, K., Gathe, J., Rice, L., Sugarman, B., Williams, T.W., Jr., Young, E.J., 1987. Manifestations of sepsis. Arch Intern Med. 147**,** 1895-906.

Hart, J.S., Jansky, L., 1963. Thermogenesis due to exercise and cold in warm- and cold-acclimated rats. Can J Biochem Physiol. 41**,** 629-34.

Hayashi, T., 1954. Effects of sodium glutamate on the nervous system. Keio Journal of Medicine. 3**,** 192-193.

Headley, P.M., Grillner, S., 1990. Excitatory amino acids and synaptic transmission: the evidence for a physiological function. Trends Pharmacol Sci. 11**,** 205-11.

Henn, F.A., Hamberger, A., 1971. Glial cell function: uptake of transmitter substances. Proc Natl Acad Sci U S A. 68**,** 2686-90.

Herman, J.P., Cullinan, W.E., 1997. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci. 20**,** 78-84.

Hermann, D.M., Luppi, P.H., Peyron, C., Hinckel, P., Jouvet, M., 1997. Afferent projections to the rat nuclei raphe magnus, raphe pallidus and reticularis gigantocellularis pars alpha demonstrated by iontophoretic application of choleratoxin (subunit b). J Chem Neuroanat. 13**,** 1-21.

Hertz, L., 2006. Glutamate, a neurotransmitter--And so much more: A synopsis of Wierzba III. Neurochemistry International. 48**,** 416-425.

Hertz, L., Bock, E., and Schousboe, A., 1979. GFA content, glutamate uptake and activity of glutamate metabolizing enzymes in differentating mouse astrocytes in primary cultures. Developmental Neuroscience. 1**,** 226-238.

Heyes, M.P., 1993. Metabolism and neuropathologic significance of quinolinic acid and kynurenic acid. Biochem Soc Trans. 21**,** 83-9.

Himms-Hagen, J., 1995. Role of brown adipose tissue thermogenesis in control of thermoregulatory feeding in rats: a new hypothesis that links thermostatic and glucostatic hypotheses for control of food intake. Proc Soc Exp Biol Med. 208**,** 159-69.

Hokfelt, T., Ljungdahl, A., 1972. Autoradiographic identification of cerebral and cerebellar cortical neurons accumulating labeled gamma-aminobutyric acid (3 H-GABA). Exp Brain Res. 14**,** 354-62.

Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. Annu Rev Neurosci. 17**,** 31-108.

Holt, S.J., Wheal, H.V., York, D.A., 1987. Hypothalamic control of brown adipose tissue in Zucker lean and obese rats. Effect of electrical stimulation of the ventromedial nucleus and other hypothalamic centres. Brain Res. 405**,** 227-33.

Honore, T., Davies, S.N., Drejer, J., Fletcher, E.J., Jacobsen, P., Lodge, D., Nielsen, F.E., 1988. Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. Science. 241**,** 701-3.

Honore, T., 1989. Excitatory amino acid receptor subtypes and specific antagonists. Med Res Rev. 9**,** 1-23.

Hori, T., Nakashima, T., Koga, H., Kiyohara, T., Inoue, T., 1988. Convergence of thermal, osmotic and cardiovascular signals on preoptic and anterior hypothalamic neurons in the rat. Brain Res Bull. 20**,** 879-85.

Hosoya, Y., Ito, R., Kohno, K., 1987. The topographical organization of neurons in the dorsal hypothalamic area that project to the spinal cord or to the nucleus raphe pallidus in the rat. Exp Brain Res. 66**,** 500-6.

Hosoya, Y., Sugiura, Y., Zhang, F.Z., Ito, R., Kohno, K., 1989. Direct projection from the dorsal hypothalamic area to the nucleus raphe pallidus: a study using anterograde transport with Phaseolus vulgaris leucoagglutinin in the rat. Exp Brain Res. 75**,** 40-6.

Hubbard, R.W., Matthew, C.B., Francesconi, R., 1982. Heat-stressed rat: effects of atropine, desalivation, or restraint. J Appl Physiol. 53**,** 1171-4.

Huettner, J.E., Bean, B.P., 1988. Block of N-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. Proc Natl Acad Sci U S A. 85**,** 1307-11.

Huettner, J.E., 2003. Kainate receptors and synaptic transmission. Progress in Neurobiology. 70**,** 387-407.

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

Hunter, W.S., 1997. Anteroventral third ventricle lesion suppresses fever, but not stress-induced hyperthermia in rats. Ann N Y Acad Sci. 813**,** 420-6.

Isaac, J.T., Ashby, M., McBain, C.J., 2007. The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. Neuron. 54**,** 859-71.

Ishiwata, T., Saito, T., Hasegawa, H., Yazawa, T., Kotani, Y., Otokawa, M., Aihara, Y., 2005. Changes of body temperature and thermoregulatory responses of freely moving rats during GABAergic pharmacological stimulation to the preoptic area and anterior hypothalamus in several ambient temperatures. Brain Res. 1048**,** 32-40.

IUPS Thermal Commission (Ed.) 2001. Glossary of terms for thermal physiology. Third edition. Revised by The Commission for Thermal Physiology of the International Union of Physiological Sciences (IUPS Thermal Commission).

Jane, D.E., Lodge, D., Collingridge, G.L., 2009. Kainate receptors: pharmacology, function and therapeutic potential. Neuropharmacology. 56**,** 90-113.

Jansen, A.S., Wessendorf, M.W., Loewy, A.D., 1995. Transneuronal labeling of CNS neuropeptide and monoamine neurons after pseudorabies virus injections into the stellate ganglion. Brain Res. 683**,** 1-24.

Jardim, M.C., Guimaraes, F.S., 2004. Role of glutamate ionotropic receptors in the dorsomedial hypothalamic nucleus on anxiety and locomotor behavior. Pharmacology Biochemistry and Behavior. 79**,** 541-546.

Javitt, D.C., 2004. Glutamate as a therapeutic target in psychiatric disorders. Mol Psychiatry. 9**,** 984-97, 979.

Jepson, M.M., Millward, D.J., Rothwell, N.J., Stock, M.J., 1988. Involvement of sympathetic nervous system and brown fat in endotoxin-induced fever in rats. Am J Physiol Endocrinol Metab. 255**,** E617-620.

Johnson, J.L., 1972. Glutamic acid as a synaptic transmitter in the nervous system. A review. Brain Res. 37**,** 1-19.
Johnson, J.W., Ascher, P., 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature. 325**,** 529-31.
Johnston, G.A., Curtis, D.R., De Groat, W.C., Duggan, A.W., 1968. Central actions of ibotenic acid and muscimol. Biochem Pharmacol. 17**,** 2488-9.
Kalter, S.S., Rodriguez, A.R., Cummins, L.B., Heberling, R.L., Foster, S.O., 1979. Experimental smallpox in chimpanzees. Bull World Health Organ. 57**,** 637-41.
Karjalainen, J., Viitasalo, M., 1986. Fever and cardiac rhythm. Arch Intern Med. 146**,** 1169-71.
Kehne, J.H., McCloskey, T.C., Baron, B.M., Chi, E.M., Harrison, B.L., Whitten, J.P., Palfreyman, M.G., 1991. NMDA receptor complex antagonists have potential anxiolytic effects as measured with separation-induced ultrasonic vocalizations. European Journal of Pharmacology. 193**,** 283-292.
Keim, S.R., Shekhar, A., 1996. The effects of GABAA receptor blockade in the dorsomedial hypothalamic nucleus on corticotrophin (ACTH) and corticosterone secretion in male rats. Brain Res. 739**,** 46-51.
Kelly, L., Bielajew, C., 1991. Ventromedial hypothalamic regulation of brown adipose tissue. Neuroreport. 2**,** 41-4.
Kemp, J.A., Foster, A.C., Leeson, P.D., Priestley, T., Tridgett, R., Iversen, L.L., Woodruff, G.N., 1988. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the N-methyl-D-aspartate receptor complex. Proc Natl Acad Sci U S A. 85**,** 6547-50.
Kita, H., Oomura, Y., 1982. An HRP study of the afferent connections to rat medial hypothalamic region. Brain Res Bull. 8**,** 53-62.
Kiyohara, T., Miyata, S., Nakamura, T., Shido, O., Nakashima, T., Shibata, M., 1995. Differences in Fos expression in the rat brains between cold and warm ambient exposures. Brain Res Bull. 38**,** 193-201.
Kiyokawa, Y., Kikusui, T., Takeuchi, Y., Mori, Y., 2004. Partner's stress status influences social buffering effects in rats. Behav Neurosci. 118**,** 798-804.
Kleckner, N.W., Dingledine, R., 1988. Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science. 241**,** 835-7.
Kloog, Y., Haring, R., Sokolovsky, M., 1988. Kinetic characterization of the phencyclidine-N-methyl-D-aspartate receptor interaction: evidence for a steric blockade of the channel. Biochemistry. 27**,** 843-8.
Kluger, M.J., O'Reilly, B., Shope, T.R., Vander, A.J., 1987. Further evidence that stress hyperthermia is a fever. Physiol Behav. 39**,** 763-6.
Kluger, M.J., Kozak, W., Leon, L.R., Soszynski, D., Conn, C.A., 1995. Cytokines and fever. Neuroimmunomodulation. 2**,** 216-23.
Kluger, M.J., Kozak, W., Leon, L.R., Conn, C.A., 1998. The use of knockout mice to understand the role of cytokines in fever. Clin Exp Pharmacol Physiol. 25**,** 141-4.
Koh, J.Y., Goldberg, M.P., Hartley, D.M., Choi, D.W., 1990. Non-NMDA receptor-mediated neurotoxicity in cortical culture. J. Neurosci. 10**,** 693-705.

Kozak, M.S., Hahn, H.H., Lennarz, W.J., Wood, W.B., Jr., 1968. Studies on the pathogenesis of fever. XVI. Purification and further chemical characterization of granulocytic pyrogen. J Exp Med. 127**,** 341-57.

Kozak, W., Conn, C.A., Klir, J.J., Wong, G.H., Kluger, M.J., 1995. TNF soluble receptor and antiserum against TNF enhance lipopolysaccharide fever in mice. Am J Physiol. 269**,** R23-9.

Kozak, W., Soszynski, D., Rudolph, K., Leon, L.R., Conn, C.A., Kluger, M.J., 1997. Soluble tumor necrosis factor alpha receptor prevents decrease of body temperature in mice treated with indomethacin and lipopolysaccharide. Ann N Y Acad Sci. 813**,** 264-71.

Kozak, W., Kluger, M.J., Soszynski, D., Conn, C.A., Rudolph, K., Leon, L.R., Zheng, H., 1998. IL-6 and IL-1 beta in fever. Studies using cytokine-deficient (knockout) mice. Ann N Y Acad Sci. 856**,** 33-47.

Kozak, W., Kluger, M.J., Tesfaigzi, J., Kozak, A., Mayfield, K.P., Wachulec, M., Dokladny, K., 2000. Molecular mechanisms of fever and endogenous antipyresis. Ann N Y Acad Sci. 917**,** 121-34.

Krogsgaard-Larsen, P., Honore, T., Hansen, J.J., Curtis, D.R., Lodge, D., 1980. New class of glutamate agonist structurally related to ibotenic acid. Nature. 284**,** 64-6.

Kuroshima, A., 1993. Brown adipose tissue thermogenesis as physiological strategy for adaptation. Jpn J Physiol. 43**,** 117-39.

Lam, W., Louis, W.J., Verberne, A.J.M., 1995. Effect of dorsal periaqueductal grey lesion on baroreflex and cardiovascular response to air-jet stress. Journal of the Autonomic Nervous System. 53**,** 35-42.

Lean, M.E., James, W.P., Jennings, G., Trayhurn, P., 1986. Brown adipose tissue in patients with phaeochromocytoma. Int J Obes. 10**,** 219-27.

Lin, M.T., Chern, Y.F., Wang, H.S., Chandra, A., 1979. Effects of gamma-hydroxybutyric acid on metabolic, respiratory and vasomotor activities and body temperature in rats. J Pharmacol Exp Ther. 211**,** 167-70.

Lipton, J.M., Clark, W.G., 1986. Neurotransmitters in Temperature Control. Annual Review of Physiology. 48**,** 613-623.

Lisa, M., Filippelli, A., Marmo, E., Wible, J.H., Jr., DiMicco, J.A., 1989a. Microinjection of muscimol into posterior hypothalamus blocks cardiovascular response to experimental stress in rats. Pharmacol Res. 21 Suppl 1**,** 9-10.

Lisa, M., Marmo, E., Wible, J.H., Jr, DiMicco, J.A., 1989b. Injection of muscimol into posterior hypothalamus blocks stress-induced tachycardia. Am J Physiol Regul Integr Comp Physiol. 257**,** R246-251.

Lisa, M., Marmo, E., Wible, J.H., Jr., DiMicco, J.A., 1989c. Injection of muscimol into posterior hypothalamus blocks stress-induced tachycardia. Am J Physiol. 257**,** R246-51.

Little, R.A., Stoner, H.B., 1968. The measurement of heat loss from the rat's tail. Q J Exp Physiol Cogn Med Sci. 53**,** 76-83.

Lodge, D., Jones, M.G., Palmer, A.J., 1991. Excitatory amino acids: new tools for old stories or pharmacological subtypes of glutamate receptors: electrophysiological studies. Can J Physiol Pharmacol. 69**,** 1123-8.

Lomax, E., 1979. Historical development of concepts of thermoregulation, Vol., Marcel Dekker, New York, Basel.

Long, N.C., Vander, A.J., Kluger, M.J., 1990a. Stress-induced rise of body temperature in rats is the same in warm and cool environments. Physiol Behav. 47**,** 773-5.

Long, N.C., Vander, A.J., Kunkel, S.L., Kluger, M.J., 1990b. Antiserum against tumor necrosis factor increases stress hyperthermia in rats. Am J Physiol. 258**,** R591-5.

Loo, P., Braunwalder, A., Lehmann, J., Williams, M., 1986. Radioligand binding to central phencyclidine recognition sites is dependent on excitatory amino acid receptor agonists. Eur J Pharmacol. 123**,** 467-8.

Mackowiak, P.A., Wasserman, S.S., Levine, M.M., 1992. A critical appraisal of 98.6 degrees F, the upper limit of the normal body temperature, and other legacies of Carl Reinhold August Wunderlich. JAMA. 268**,** 1578-80.

Madden, C.J., Morrison, S.F., 2004. Excitatory amino acid receptors in the dorsomedial hypothalamus mediate prostaglandin-evoked thermogenesis in brown adipose tissue. Am J Physiol Regul Integr Comp Physiol. 286**,** R320-325.

Malkinson, T.J., Cooper, K.E., Veale, W.L., 1988. Physiological changes during thermoregulation and fever in urethan-anesthetized rats. Am J Physiol. 255**,** R73-81.

Malkinson, T.J., Veale, W.L., Cooper, K.E., 1993. Experimental characterization and applications of an anesthetized animal model for thermoregulatory investigations. Biomed Sci Instrum. 29**,** 369-76.

Marazziti, D., Di Muro, A., Castrogiovanni, P., 1992. Psychological stress and body temperature changes in humans. Physiol Behav. 52**,** 393-5.

Martin, D., Lodge, D., 1985. Ketamine acts as a non-competitive N-methyl-D-aspartate antagonist on frog spinal cord in vitro. Neuropharmacology. 24**,** 999-1003.

Martin, D.S., Appelt, C., Rodrigo, M.C., Egland, M.C., 1996. Acute stress increases venomotor tone in conscious rats. Am J Physiol Heart Circ Physiol. 271**,** H1375-1383.

Matzen, L., Engesgaard, A., Ebert, B., Didriksen, M., Frolund, B., Krogsgaard-Larsen, P., Jaroszewski, J.W., 1997. AMPA Receptor Agonists: Synthesis, Protolytic Properties, and Pharmacology of 3-Isothiazolol Bioisosteres of Glutamic Acid. J Med Chem. 40**,** 520-527.

Mayer, M.L., Westbrook, G.L., 1984. Mixed-agonist action of excitatory amino acids on mouse spinal cord neurones under voltage clamp. J Physiol. 354**,** 29-53.

Mayer, M.L., Westbrook, G.L., 1987. Permeation and block of N-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. J Physiol. 394**,** 501-27.

Mayer, M.L., Westbrook, G.L., Vyklicky, L., Jr., 1988. Sites of antagonist action on N-methyl-D-aspartic acid receptors studied using fluctuation analysis and a rapid perfusion technique. J Neurophysiol. 60**,** 645-63.

Mayorov, D.N., Head, G.A., 2002. Ionotropic glutamate receptors in the rostral ventrolateral medulla mediate sympathetic responses to acute stress in conscious rabbits. Autonomic Neuroscience. 98**,** 20-23.

McDougall, S.J., Widdop, R.E., Lawrence, A.J., 2005. Central autonomic integration of psychological stressors: focus on cardiovascular modulation. Auton Neurosci. 123**,** 1-11.

McLennan, H., Huffman, R.D., Marshall, K.C., 1968. Patterns of excitation of thalamic neurones by amino-acids and by acetylcholine. Nature. 219**,** 387-8.

McLennan, H., 1982a. The isomers of 2-amino-5-phosphonovalerate as excitatory amino acid antagonists--a reappraisal. Eur J Pharmacol. 79**,** 135-7.

McLennan, H., 1982b. 2-amino-7-phosphonoheptanoic acid as an amino acid antagonist. Can J Physiol Pharmacol. 60**,** 91-4.

Millan, M.J., Tsang, Y.F., Prezewlocki, R., Hollt, V., Herz, A., 1981. The influence of foot-shock stress upon brain, pituitary and spinal cord pools of immunoreactive dynorphin in rats. Neurosci Lett. 24**,** 75-9.

Milton, A.S., 1998. Prostaglandins and fever. Prog Brain Res. 115**,** 129-39.

Monaghan, D.T., Cotman, C.W., 1982. The distribution of [3H]kainic acid binding sites in rat CNS as determined by autoradiography. Brain Res. 252**,** 91-100.

Monaghan, D.T., Holets, V.R., Toy, D.W., Cotman, C.W., 1983. Anatomical distributions of four pharmacologically distinct 3H-L-glutamate binding sites. Nature. 306**,** 176-9.

Monaghan, D.T., Yao, D., Cotman, C.W., 1984. Distribution of [3H]AMPA binding sites in rat brain as determined by quantitative autoradiography. Brain Res. 324**,** 160-4.

Monaghan, D.T., Cotman, C.W., 1985. Distribution of N-methyl-D-aspartate-sensitive L-[3H]glutamate-binding sites in rat brain. J. Neurosci. 5**,** 2909-2919.

Monaghan, D.T., Yao, D., Cotman, C.W., 1985. L-[3H]Glutamate binds to kainate-, NMDA- and AMPA-sensitive binding sites: an autoradiographic analysis. Brain Res. 340**,** 378-83.

Monaghan, D.T., Bridges, R.J., Cotman, C.W., 1989. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol. 29**,** 365-402.

Morimoto, A., Watanabe, T., Morimoto, K., Nakamori, T., Murakami, N., 1991. Possible involvement of prostaglandins in psychological stress-induced responses in rats. J Physiol. 443**,** 421-9.

Morin, S.M., Stotz-Potter, E.H., Dimicco, J.A., 2001. Injection of muscimol in dorsomedial hypothalamus and stress-induced Fos expression in paraventricular nucleus. Am J Physiol Regul Integr Comp Physiol. 280**,** R1276-1284.

Morrison, S.F., Sved, A.F., Passerin, A.M., 1999. GABA-mediated inhibition of raphe pallidus neurons regulates sympathetic outflow to brown adipose tissue. Am J Physiol. 276**,** R290-7.

Morrison, S.F., 2003. Raphe pallidus neurons mediate prostaglandin E2-evoked increases in brown adipose tissue thermogenesis. Neuroscience. 121**,** 17-24.

Morrison, S.F., 2004. Central pathways controlling brown adipose tissue thermogenesis. News Physiol Sci. 19**,** 67-74.

Morrison, S.F., Nakamura, K., Madden, C.J., 2008. Central control of thermogenesis in mammals. Exp Physiol. 93**,** 773-97.

Munir, M., Subramaniam, S., McGonigle, P., 1993. Polyamines modulate the neurotoxic effects of NMDA in vivo. Brain Res. 616**,** 163-70.

Nadler, J.V., White, W.F., Vaca, K.W., Redburn, D.A., Cotman, C.W., 1977. Characterization of putative amino acid transmitter release from slices of rat dentate gyrus. J Neurochem. 29**,** 279-90.

Nadler, J.V., White, W.F., Vaca, K.W., Perry, B.W., Cotman, C.W., 1978. Biochemical correlates of transmission mediated by glutamate and aspartate. J Neurochem. 31**,** 147-55.

Nakamura, K., Matsumura, K., Kaneko, T., Kobayashi, S., Katoh, H., Negishi, M., 2002. The rostral raphe pallidus nucleus mediates pyrogenic transmission from the preoptic area. J Neurosci. 22**,** 4600-10.

Nakamura, K., Matsumura, K., Hubschle, T., Nakamura, Y., Hioki, H., Fujiyama, F., Boldogkoi, Z., Konig, M., Thiel, H.J., Gerstberger, R., Kobayashi, S., Kaneko, T., 2004. Identification of sympathetic premotor neurons in medullary raphe regions mediating fever and other thermoregulatory functions. J Neurosci. 24**,** 5370-80.

Nakamura, K., Morrison, S.F., 2007. Central efferent pathways mediating skin cooling-evoked sympathetic thermogenesis in brown adipose tissue. Am J Physiol Regul Integr Comp Physiol. 292**,** R127-36.

Nakamura, Y., Nakamura, K., Matsumura, K., Kobayashi, S., Kaneko, T., Morrison, S.F., 2005a. Direct pyrogenic input from prostaglandin EP3 receptor-expressing preoptic neurons to the dorsomedial hypothalamus. Eur J Neurosci. 22**,** 3137-46.

Nakamura, Y., Nakamura, K., Matsumura, K., Kobayashi, S., Kaneko, T., Morrison, S.F., 2005b. Direct pyrogenic input from prostaglandin EP3 receptor-expressing preoptic neurons to the dorsomedial hypothalamus. European Journal of Neuroscience. 22**,** 3137-3146.

Nalivaiko, E., Blessing, W.W., 2001. Raphe region mediates changes in cutaneous vascular tone elicited by stimulation of amygdala and hypothalamus in rabbits. Brain Res. 891**,** 130-7.

Nalivaiko, E., Blessing, W.W., 2002. Potential role of medullary raphe-spinal neurons in cutaneous vasoconstriction: an in vivo electrophysiological study. J Neurophysiol. 87**,** 901-11.

Nedergaard, J., Bengtsson, T., Cannon, B., 2007. Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab. 293**,** E444-52.

Negishi, M., Sugimoto, Y., Ichikawa, A., 1995. Prostaglandin E receptors. Journal of Lipid Mediators and Cell Signalling. 12**,** 379-391.

Nicholls, D.G., Locke, R.M., 1984. Thermogenic mechanisms in brown fat. Physiol. Rev. 64**,** 1-64.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A., 1984. Magnesium gates glutamate-activated channels in mouse central neurones. Nature. 307**,** 462-5.

Oka, K., Oka, T., Hori, T., 1997. Prostaglandin E2 may induce hyperthermia through EP1 receptor in the anterior wall of the third ventricle and neighboring preoptic regions. Brain Research. 767**,** 92-99.

Oka, T., Oka, K., Hori, T., 2001. Mechanisms and mediators of psychological stress-induced rise in core temperature. Psychosom Med. 63**,** 476-86.

Oka, T., Oka, K., Kobayashi, T., Sugimoto, Y., Ichikawa, A., Ushikubi, F., Narumiya, S., Saper, C.B., 2003. Characteristics of thermoregulatory and febrile responses in mice deficient in prostaglandin EP1 and EP3 receptors. J Physiol. 551**,** 945-954.

Oka, T., Oka, K., 2007. Age and gender differences of psychogenic fever: a review of the Japanese literature. Biopsychosoc Med. 1**,** 11.

Olivier, B., Zethof, T., Pattij, T., van Boogaert, M., van Oorschot, R., Leahy, C., Oosting, R., Bouwknecht, A., Veening, J., van der Gugten, J., Groenink, L., 2003. Stress-induced hyperthermia and anxiety: pharmacological validation. European Journal of Pharmacology. 463**,** 117-132.

Olverman, H.J., Jones, A.W., Watkins, J.C., 1984. L-glutamate has higher affinity than other amino acids for [3H]-D-AP5 binding sites in rat brain membranes. Nature. 307**,** 460-2.

Osaka, T., 2004. Cold-induced thermogenesis mediated by GABA in the preoptic area of anesthetized rats. Am J Physiol Regul Integr Comp Physiol. 287**,** R306-13.

Osborne, P.G., Kurosawa, M., 1994. Perfusion of the preoptic area with muscimol or prostaglandin E2 stimulates cardiovascular function in anesthetized rats. J Auton Nerv Syst. 46**,** 199-205.

Ottersen, O.P., Storm-Mathisen, J., 1984. Glutamate- and GABA-containing neurons in the mouse and rat brain, as demonstrated with a new immunocytochemical technique. J Comp Neurol. 229**,** 374-92.

Ottersen, O.P., Storm-Mathisen, J., 1987. Localization of amino acid neurotransmitters by immunocytochemistry. Trends in Neurosciences. 10**,** 250-255.

Pae, Y.S., Lai, H., Horita, A., 1985. Hyperthermia in the rat from handling stress blocked by naltrexone injected into the preoptic-anterior hypothalamus. Pharmacol Biochem Behav. 22**,** 337-9.

Parrott, R.F., Vellucci, S.V., Forsling, M.L., Goode, J.A., 1995. Hyperthermic and endocrine effects of intravenous prostaglandin administration in the pig. Domest Anim Endocrinol. 12**,** 197-205.

Pattij, T., Hijzen, T.H., Groenink, L., Oosting, R.S., van der Gugten, J., Maes, R.A.A., Hen, R., Olivier, B., 2001. Stress-induced hyperthermia in the 5-HT1A receptor knockout mouse is normal. Biological Psychiatry. 49**,** 569-574.

Pattij, T., Groenink, L., Hijzen, T.H., Oosting, R.S., Maes, R.A., van der Gugten, J., Olivier, B., 2002. Autonomic changes associated with enhanced anxiety in 5-HT(1A) receptor knockout mice. Neuropsychopharmacology. 27**,** 380-90.

Paxinos, G., and Watson, C. , 2007. The Rat Brain in Stereotaxic Coordinates, Vol., Elsevier/Academic Press, New York.

Pechnick, R.N., Morgan, M.J., 1987. The role of endogenous opioids in footshock-induced hyperthermia. Pharmacol Biochem Behav. 28**,** 95-100.

Peet, M.J., Curry, K., Magnuson, D.S., McLennan, H., 1986a. Ca2+-dependent depolarization and burst firing of rat CA1 pyramidal neurones induced by N-methyl-D-aspartic acid and quinolinic acid: antagonism by 2-amino-5-phosphonovaleric and kynurenic acids. Can J Physiol Pharmacol. 64**,** 163-8.

Peet, M.J., Gregersen, H., McLennan, H., 1986b. 2-Amino-5-phosphonovalerate and Co2+ selectively block depolarization and burst firing of rat hippocampal CA1 pyramidal neurones by N-methyl-D-aspartate. Neuroscience. 17**,** 635-41.

Perkins, M.N., Rothwell, N.J., Stock, M.J., Stone, T.W., 1981. Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. Nature. 289**,** 401-2.

Pinheiro, P., Mulle, C., 2006. Kainate receptors. Cell Tissue Res. 326**,** 457-82.

Poole, S., Stephenson, J.D., 1977. Core temperature: some shortcomings of rectal temperature measurements. Physiol Behav. 18**,** 203-5.

Potashner, S.J., 1978. The spontaneous and electrically evoked release, from slices of guinea-pig cerebral cortex, of endogenous amino acids labelled via metabolism of D-[U-14C]glucose. J Neurochem. 31**,** 177-86.

Pralong, E., Millar, J.D., Lodge, D., 1992. Specificity and potency of N-methyl-D-aspartate glycine site antagonists and of mephenesin on the rat spinal cord in vitro. Neurosci Lett. 136**,** 56-8.

Quan, N., Blatteis, C.M., 1989. Microdialysis: A system for localized drug delivery into the brain. Brain Res Bull. 22**,** 621-625.

Ransom, R.W., Stec, N.L., 1988. Cooperative modulation of [3H]MK-801 binding to the N-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. J Neurochem. 51**,** 830-6.

Rathner, J.A., Morrison, S.F., 2006. Rostral ventromedial periaqueductal gray: a source of inhibition of the sympathetic outflow to brown adipose tissue. Brain Res. 1077**,** 99-107.

Rivest, S., Rivier, C., 1991. Influence of the paraventricular nucleus of the hypothalamus in the alteration of neuroendocrine functions induced by intermittent footshock or interleukin. Endocrinology. 129**,** 2049-57.

Romanovsky, A.A., Simons, C.T., Szekely, M., Kulchitsky, V.A., 1997. The vagus nerve in the thermoregulatory response to systemic inflammation. Am J Physiol. 273**,** R407-13.

Romanovsky, A.A., Blatteis, C.M., 1998. Pathophysiology of opioids in hyperthermic states. Prog Brain Res. 115**,** 111-27.

Romanovsky, A.A., Szekely, M., 1998. Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. Med Hypotheses. 50**,** 219-26.

Romanovsky, A.A., Ivanov, A.I., Szekely, M., 2000. Neural route of pyrogen signaling to the brain. Clin Infect Dis. 31 Suppl 5**,** S162-7.

Romanovsky, A.A., 2007. Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. Am J Physiol Regul Integr Comp Physiol. 292**,** R37-46.

Rorick-Kehn, L., Hart, J., McKinzie, D., 2005. Pharmacological characterization of stress-induced hyperthermia in DBA/2 mice using metabotropic and ionotropic glutamate receptor ligands. Psychopharmacology. 183**,** 226-240.

Rothwell, N.J., Stock, M.J., Thexton, A.J., 1983. Decerebration activates thermogenesis in the rat. J Physiol. 342**,** 15-22.

Rowsey, P.J., Yang, Y.-L., Gordon, C.J., 2002. Peripheral cholinergic pathway modulates hyperthermia induced by stress in rats exposed to open-field stress. J Appl Physiol. 92**,** 789-794.

Saito, T., Ishiwata, T., Hasegawa, H., Nomoto, S., Kotani, Y., Otokawa, M., Aihara, Y., 2008. Effect of chronic cold exposure on noradrenergic modulation in the preoptic area of thermoregulation in freely moving rats. Life Sci. 83**,** 79-84.

Samuels, B.C., Zaretsky, D.V., DiMicco, J.A., 2002. Tachycardia evoked by disinhibition of the dorsomedial hypothalamus in rats is mediated through medullary raphe. J Physiol. 538**,** 941-6.

Samuels, B.C., Zaretsky, D.V., DiMicco, J.A., 2004. Dorsomedial hypothalamic sites where disinhibition evokes tachycardia correlate with location of raphe-projecting neurons. Am J Physiol Regul Integr Comp Physiol. 287**,** R472-8.

Saper, C.B., Breder, C.D., 1992. Endogenous pyrogens in the CNS: role in the febrile response. Prog Brain Res. 93**,** 419-28; discussion 428-9.

Sarkar, S., Zaretskaia, M.V., Zaretsky, D.V., Moreno, M., DiMicco, J.A., 2007. Stress- and lipopolysaccharide-induced c-fos expression and nNOS in hypothalamic neurons projecting to medullary raphe in rats: a triple immunofluorescent labeling study. Eur J Neurosci. 26**,** 2228-38.

Sawchenko, P.E., Li, H.Y., Ericsson, A., 2000. Circuits and mechanisms governing hypothalamic responses to stress: a tale of two paradigms. Prog Brain Res. 122**,** 61-78.

Scammell, T.E., Griffin, J.D., Elmquist, J.K., Saper, C.B., 1998. Microinjection of a cyclooxygenase inhibitor into the anteroventral preoptic region attenuates LPS fever. Am J Physiol Regul Integr Comp Physiol. 274**,** R783-789.

Selye, H., Fortier, C., 1950. Adaptive reaction to stress. Psychosom Med. 12**,** 149-57.
Shekhar, A., Hingtgen, J.N., DiMicco, J.A., 1990. GABA receptors in the posterior hypothalamus regulate experimental anxiety in rats. Brain Res. 512**,** 81-8.
Silver, R.A., Traynelis, S.F., Cull-Candy, S.G., 1992. Rapid-time-course miniature and evoked excitatory currents at cerebellar synapses in situ. Nature. 355**,** 163-6.
Simerly, R.B., Swanson, L.W., 1988. Projections of the medial preoptic nucleus: a Phaseolus vulgaris leucoagglutinin anterograde tract-tracing study in the rat. J Comp Neurol. 270**,** 209-42.
Simons, C.T., Kulchitsky, V.A., Sugimoto, N., Homer, L.D., Szekely, M., Romanovsky, A.A., 1998. Signaling the brain in systemic inflammation: which vagal branch is involved in fever genesis? Am J Physiol. 275**,** R63-8.
Singer, R., Harker, C.T., Vander, A.J., Kluger, M.J., 1986. Hyperthermia induced by open-field stress is blocked by salicylate. Physiol Behav. 36**,** 1179-82.
Smith, J.E., Jansen, A.S., Gilbey, M.P., Loewy, A.D., 1998. CNS cell groups projecting to sympathetic outflow of tail artery: neural circuits involved in heat loss in the rat. Brain Res. 786**,** 153-64.
Smith, R.E., Horwitz, B.A., 1969. Brown fat and thermogenesis. Physiol. Rev. 49**,** 330-425.
Soltis, R.P., DiMicco, J.A., 1991a. GABAA and excitatory amino acid receptors in dorsomedial hypothalamus and heart rate in rats. Am J Physiol. 260**,** R13-20.
Soltis, R.P., DiMicco, J.A., 1991b. Interaction of hypothalamic GABAA and excitatory amino acid receptors controlling heart rate in rats. Am J Physiol. 261**,** R427-33.
Soltis, R.P., DiMicco, J.A., 1992a. Hypothalamic excitatory amino acid receptors mediate stress-induced tachycardia in rats. Am J Physiol Regul Integr Comp Physiol. 262**,** R689-697.
Soltis, R.P., DiMicco, J.A., 1992b. Hypothalamic excitatory amino acid receptors mediate stress-induced tachycardia in rats. Am J Physiol. 262**,** R689-97.
Soszynski, D., Kozak, W., Conn, C.A., Rudolph, K., Kluger, M.J., 1996. Beta-adrenoceptor antagonists suppress elevation in body temperature and increase in plasma IL-6 in rats exposed to open field. Neuroendocrinology. 63**,** 459-67.
Soszynski, D., Kozak, W., Kluger, M.J., 1998. Endotoxin Tolerance Does Not Alter Open Field-Induced Fever in Rats. Physiology & Behaviour. 63**,** 689-692.
Spooren, W.P.J.M., Schoeffter, P., Gasparini, F., Kuhn, R., Gentsch, C., 2002. Pharmacological and endocrinological characterisation of stress-induced hyperthermia in singly housed mice using classical and candidate anxiolytics (LY314582, MPEP and NKP608). European Journal of Pharmacology. 435**,** 161-170.
Stallcup, W.B., Bulloch, K., Baetge, E.E., 1979. Coupled transport of glutamate and sodium in a cerebellar nerve cell line. J Neurochem. 32**,** 57-65.

Stewart, J., Eikelboom, R., 1979. Stress masks the hypothermic effect of naloxone in rats. Life Sci. 25**,** 1165-72.

Stone, T.W., 1991. Kynurenine and glycine enhance neuronal sensitivity to N-methyl-D-aspartate. Life Sci. 48**,** 765-72.

Storm-Mathisen, J., Leknes, A.K., Bore, A.T., Vaaland, J.L., Edminson, P., Haug, F.M., Ottersen, O.P., 1983. First visualization of glutamate and GABA in neurones by immunocytochemistry. Nature. 301**,** 517-20.

Storm-Mathisen, J., Ottersen, O.P., 1990. Immunocytochemistry of glutamate at the synaptic level. J Histochem Cytochem. 38**,** 1733-43.

Stotz-Potter, E.H., Morin, S.M., DiMicco, J.A., 1996a. Effect of microinjection of muscimol into the dorsomedial or paraventricular hypothalamic nucleus on air stress-induced neuroendocrine and cardiovascular changes in rats. Brain Res. 742**,** 219-24.

Stotz-Potter, E.H., Willis, L.R., DiMicco, J.A., 1996b. Muscimol acts in dorsomedial but not paraventricular hypothalamic nucleus to suppress cardiovascular effects of stress. J Neurosci. 16**,** 1173-9.

Stricker, E.M., Hainsworth, F.R., 1971. Evaporative cooling in the rat: Interaction with heat loss from the tail. Q J Exp Physiol Cogn Med Sci. 56**,** 231-241.

Striker, G.E., Tenckhoff, H.A., 1971. A transcutaneous prosthesis for prolonged access to the peritoneal cavity. Surgery. 69**,** 70-4.

Szekely, M., Balasko, M., Kulchitsky, V.A., Simons, C.T., Ivanov, A.I., Romanovsky, A.A., 2000. Multiple neural mechanisms of fever. Auton Neurosci. 85**,** 78-82.

Szymusiak, R., Satinoff, E., 1982. Acute thermoregulatory effects of unilateral electrolytic lesions of the medial and lateral preoptic area in rats. Physiol Behav. 28**,** 161-70.

Takamori, S., Rhee, J.S., Rosenmund, C., Jahn, R., 2000. Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons. Nature. 407**,** 189-94.

Tanaka, M., Tonouchi, M., Hosono, T., Nagashima, K., Yanase-Fujiwara, M., Kanosue, K., 2001. Hypothalamic region facilitating shivering in rats. Jpn J Physiol. 51**,** 625-9.

ter Horst, G.J., Luiten, P.G., 1986. The projections of the dorsomedial hypothalamic nucleus in the rat. Brain Res Bull. 16**,** 231-48.

Thomas, J.W., Hood, W.F., Monahan, J.B., Contreras, P.C., O'Donohue, T.L., 1988. Glycine modulation of the phencyclidine binding site in mammalian brain. Brain Res. 442**,** 396-8.

Thompson, R.H., Swanson, L.W., 1998. Organization of inputs to the dorsomedial nucleus of the hypothalamus: a reexamination with Fluorogold and PHAL in the rat. Brain Res Brain Res Rev. 27**,** 89-118.

Thompson, S.M., Gahwiler, B.H., 1989a. Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus in vitro. J Neurophysiol. 61**,** 501-11.

Thompson, S.M., Gahwiler, B.H., 1989b. Activity-dependent disinhibition. III. Desensitization and GABAB receptor-mediated presynaptic inhibition in the hippocampus in vitro. J Neurophysiol. 61**,** 524-33.

Thornhill, J., Jugnauth, A., Halvorson, I., 1994. Brown adipose tissue thermogenesis evoked by medial preoptic stimulation is mediated via the ventromedial hypothalamic nucleus. Can J Physiol Pharmacol. 72**,** 1042-8.

Thornhill, J.A., Halvorson, I., 1994. Electrical stimulation of the posterior and ventromedial hypothalamic nuclei causes specific activation of shivering and nonshivering thermogenesis. Can J Physiol Pharmacol. 72**,** 89-96.

van den Pol, A.N., Wuarin, J.P., Dudek, F.E., 1990. Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. Science. 250**,** 1276-8.

van den Pol, A.N., Hermans-Borgmeyer, I., Hofer, M., Ghosh, P., Heinemann, S., 1994. Ionotropic glutamate-receptor gene expression in hypothalamus: localization of AMPA, kainate, and NMDA receptor RNA with in situ hybridization. J Comp Neurol. 343**,** 428-44.

Varoqui, H., Schafer, M.K., Zhu, H., Weihe, E., Erickson, J.D., 2002. Identification of the differentiation-associated Na+/PI transporter as a novel vesicular glutamate transporter expressed in a distinct set of glutamatergic synapses. J Neurosci. 22**,** 142-55.

Verdoorn, T.A., Kleckner, N.W., Dingledine, R., 1989. N-methyl-D-aspartate/glycine and quisqualate/kainate receptors expressed in Xenopus oocytes: antagonist pharmacology. Mol Pharmacol. 35**,** 360-368.

Vignon, J., Chicheportiche, R., Chicheportiche, M., Kamenka, J.M., Geneste, P., Lazdunski, M., 1983. [3H]TCP: a new tool with high affinity for the PCP receptor in rat brain. Brain Res. 280**,** 194-7.

Watanabe, T., Hashimoto, M., Okuyama, S., Inagami, T., Nakamura, S., 1999. Effects of targeted disruption of the mouse angiotensin II type 2 receptor gene on stress-induced hyperthermia. J Physiol. 515 (Pt 3)**,** 881-5.

Watkins, J.C., 1981. Pharmacology of excitatory amino acid transmitters. Adv Biochem Psychopharmacol. 29**,** 205-12.

Watkins, J.C., Davies, J., Evans, R.H., Francis, A.A., Jones, A.W., 1981. Pharmacology of receptors for excitatory amino acids. Adv Biochem Psychopharmacol. 27**,** 263-73.

Watkins, J.C., Evans, R.H., 1981. Excitatory amino acid transmitters. Annu Rev Pharmacol Toxicol. 21**,** 165-204.

Watkins, J.C., Jane, D.E., 2006. The glutamate story. Br J Pharmacol. 147 Suppl 1**,** S100-8.

Whyte, D.G., Johnson, A.K., 2005. Lesions of the anteroventral third ventricle region (AV3V) disrupt cardiovascular responses to an elevation in core temperature. Am J Physiol Regul Integr Comp Physiol. 288**,** R1783-90.

Wible, J.H., Jr, Luft, F.C., DiMicco, J.A., 1988. Hypothalamic GABA suppresses sympathetic outflow to the cardiovascular system. Am J Physiol Regul Integr Comp Physiol. 254**,** R680-687.

Wible, J.H., Jr., DiMicco, J.A., Luft, F.C., 1989. Hypothalamic GABA and sympathetic regulation in spontaneously hypertensive rats. Hypertension. 14**,** 623-8.

Williams, K., Romano, C., Molinoff, P.B., 1989. Effects of polyamines on the binding of [3H]MK-801 to the N-methyl-D-aspartate receptor: pharmacological evidence for the existence of a polyamine recognition site. Mol Pharmacol. 36**,** 575-81.

Wong, E.H., Kemp, J.A., Priestley, T., Knight, A.R., Woodruff, G.N., Iversen, L.L., 1986. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. Proc Natl Acad Sci U S A. 83**,** 7104-8.

Woodruff, G.N., Foster, A.C., Gill, R., Kemp, J.A., Wong, E.H., Iversen, L.L., 1987. The interaction between MK-801 and receptors for N-methyl-D-aspartate: functional consequences. Neuropharmacology. 26**,** 903-9.

Xia, Y., Krukoff, T.L., 2001. Cardiovascular responses to subseptic doses of endotoxin contribute to differential neuronal activation in rat brain. Brain Res Mol Brain Res. 89**,** 71-85.

Xia, Y., Krukoff, T.L., 2003. Differential neuronal activation in the hypothalamic paraventricular nucleus and autonomic/neuroendocrine responses to I.C.V. endotoxin. Neuroscience. 121**,** 219-31.

Yoshida, K., Konishi, M., Nagashima, K., Saper, C.B., Kanosue, K., 2005. Fos activation in hypothalamic neurons during cold or warm exposure: projections to periaqueductal gray matter. Neuroscience. 133**,** 1039-46.

Young, A.A., Dawson, N.J., 1982. Evidence for on-off control of heat dissipation from the tail of the rat. Can J Physiol Pharmacol. 60**,** 392-8.

Zaretskaia, M.V., Zaretsky, D.V., Shekhar, A., DiMicco, J.A., 2002. Chemical stimulation of the dorsomedial hypothalamus evokes non-shivering thermogenesis in anesthetized rats. Brain Res. 928**,** 113-25.

Zaretskaia, M.V., Zaretsky, D.V., DiMicco, J.A., 2003. Role of the dorsomedial hypothalamus in thermogenesis and tachycardia caused by microinjection of prostaglandin E2 into the preoptic area in anesthetized rats. Neurosci Lett. 340**,** 1-4.

Zaretsky, D.V., Zaretskaia, M.V., DiMicco, J.A., 2003a. Stimulation and blockade of GABA(A) receptors in the raphe pallidus: effects on body temperature, heart rate, and blood pressure in conscious rats. Am J Physiol Regul Integr Comp Physiol. 285**,** R110-6.

Zaretsky, D.V., Zaretskaia, M.V., DiMicco, J.A., 2003b. Stimulation and blockade of GABAA receptors in the raphe pallidus: effects on body temperature, heart rate, and blood pressure in conscious rats. Am J Physiol Regul Integr Comp Physiol. 285**,** R110-116.

Zaretsky, D.V., Zaretskaia, M.V., Samuels, B.C., Cluxton, L.K., DiMicco, J.A., 2003c. Microinjection of muscimol into raphe pallidus suppresses tachycardia associated with air stress in conscious rats. J Physiol (Lond). 546**,** 243-250.

Zaretsky, D.V., Hunt, J.L., Zaretskaia, M.V., DiMicco, J.A., 2006. Microinjection of prostaglandin E2 and muscimol into the preoptic area in conscious rats: Comparison of effects on plasma adrenocorticotrophic hormone (ACTH), body temperature, locomotor activity, and cardiovascular function. Neuroscience Letters. 397**,** 291-296.

CURRICULUM VITAE

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Ph.D., Pharmacology
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AWARDS and FELLOWSHIPS

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TEACHING EXPERIENCE

Chemistry Professor**,** Department of Chemistry, Our Lady of the Lake University, 2007-present
Courses taught or teaching include Introduction to Physical Science I and II, General Chemistry I and II, Organic Chemistry I and II, Selected Topics in Chemistry, Biochemistry (one semester course) and the complementary lab for each course.

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PUBLICATIONS

Sarkar S, Zaretskaia MV, Zaretsky DV, Moreno M, DiMicco JA (2007). Stress- and lipopolysaccharide-induced c-fos expression and nNOS in hypothalamic neurons projecting to medullary raphe in rats: a triple immunofluorescent labeling study. European Journal of Neuroscience, 26 (8): 2228-38.

MANUSCRIPTS IN PREPARATION

Cervantes, M. Y, DiMicco, J.A, Zaretsky, D.V. "Role of glutamatergic transmission in the DMH in the temperature responses to stress."

Cervantes, M. Y, DiMicco, J.A, Zaretsky, D.V. "Different roles for hypothalamic non-NMDA and NMDA receptors in the adrenergic response to stress."

CONFERENCE ABSTRACTS

M.Y. Moreno, S.Sarkar, D.V Zaretsky, J.A. DiMicco. "LPS induces c-Fos expression in neurons in the dorsomedial hypothalamus that project to the raphe pallidus." Society of Neuroscience, 2005.

M.Y. Moreno, D.V Zaretsky, J.A. DiMicco. "Acute hypothermic and hypotensive responses induced by systemic lipopolysaccharide (LPS) in conscious rats are closely correlated and subject to marked tolerance." Society for Neuroscience, 2005.

CONFERENCES and ACADEMIC PRESENTATIONS

M.Y. Moreno. "Role of the dorsomedial hypothalamus in LPS-induced fever." Pharmacology and Toxicology Seminar Series, Indianapolis, IN December 2006.

M.Y. Moreno, S. Sarkar, D.V Zaretsky, J.A. DiMicco. "LPS induces c-Fos expression in neurons in the dorsomedial hypothalamus that project to the raphe pallidus." Society for Neuroscience Conference, Washington, DC, November 2005.

M.Y. Moreno. "LPS induces c-fos expression in neurons in the dorsomedial hypothalamus that project to the raphe pallidus." Sigma Xi Student Research Competition, Indianapolis, IN, May 2005. *3rd Place*

M.Y. Moreno. "Taking the Heat: Role of DMH in LPS-induced Fever." Pharmacology and Toxicology Seminar Series, Indianapolis, IN, February 2005.

M.Y. Moreno, D.V Zaretsky, J.A. DiMicco. "Acute hypothermic and hypotensive responses induced by systemic lipopolysaccharide (LPS) in conscious rats are closely correlated and subject to marked tolerance." Society of Neuroscience Conference, San Diego, CA, October 2004.

M.Y. Moreno. "Lipopolysaccharide (LPS) and Thermoregulation." Pharmacology and Toxicology Seminar Series, Indianapolis, IN, June 2004.

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