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# The Effects of Gabapentin on the Firing Rates of Thermoregulatory Neurons in the Preoptic Anterior Hypothalamus in Modulation of Hot Flashes

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# THE EFFECTS OF GABAPENTIN ON THE FIRING RATES OF THERMOREGULATORY NEURONS IN THE PREOPTIC ANTERIOR HYPOTHALAMUS IN MODULATION OF HOT FLASHES

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by

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## ABSTRACT

**Introduction:**

Recent studies have identified a link between gabapentin (GP) treatment and a decrease in the incidence and severity of hot flashes (HFs) among predisposed individuals. The current research is focused on determining whether GP acts directly on neurons within the preoptic anterior hypothalamus in order to cause a change in the body's thermoregulatory set point and prevent HFs. Chapter one will review current literature, beginning with a discussion of how the human body maintains and regulates its set point temperature with a focus on thermoregulatory neurons of the preoptic anterior hypothalamus (POAH). Hot flashes and the suggested mechanisms by which hypothalamic neurons might modulate them will then be discussed. Next, GP and its potential mechanisms for action will be presented. We hypothesized that treatment with GP would decrease the firing rate of warm sensitive cells as well as temperature insensitive cells in the POAH, which would lead to an increase in set core body temperature based on the current model of neural thermoregulation.

**Methods**:

Coronal slices of the hypothalamus and surrounding brain areas of adult male Sprague-Dawley rats were prepared and recordings were made on single units. Neurons were classified as either warm sensitive or temperature insensitive and then treated with 10uM GP for ten minutes and then washed for ten minutes with artificial cerebrospinal fluid (aCSF), during which time their firing rates were recorded.

**Results:**

In response to GP, warm sensitive neurons decreased in their firing rate overall, while most temperature insensitive neurons did not change their firing rate. Twelve temperature insensitive

neurons, localized in the periventricular and paraventricular nuclei, increased their firing rate in response to GP.

**Conclusions:**

The changes in firing rate induced by GP would result in a positive shift in set point (hyperthermia) according to the current model of neural thermoregulation. This hyperthermic shift may contribute to a widened thermoneutral zone in individuals who commonly suffer from HFs and therefore a decrease in HF incidence and/or duration.

# INTRODUCTION

## I: NEURONAL MECHANISMS OF THERMOREGULATION

### 1. Maintenance of Body Temperature

Temperature maintenance in mammals is an essential homeostatic process; the body must maintain its core temperature within a narrow temperature range in order to keep constant internal conditions for proper neural and enzymatic function. In order to keep temperature constant throughout the body, heat is distributed by the cardiovascular system along with oxygen and nutrients (1). The range in which body temperature is maintained is called the thermoregulatory set point. For humans, a normal set point lies between 36-38°C (2). The range of this thermoneutral zone in which an individual can maintain body temperature without expending energy above his or her basal metabolic rate depends on many factors, including age, gender, body composition, and clothing (3). Within the thermoneutral zone, both heat loss and heat production are inactive.

As an individual's body temperature increases and surpasses the set point, neural networks within the hypothalamus activate mechanisms that promote heat loss, such as sweating, vasodilation, and panting (4,5). Hyperthermia is a condition in which the set point is elevated and can occur as a response to the environment, exercise, stress, circadian timing, or endogenous signals such as hormones. Fever is a subset of hyperthermic responses in which endogenous pyrogens, which are substances that induce fever, such as prostaglandins or cytokines elevate the thermoregulatory set point and cause an activation of the body's heat production responses, usually in response to infection. Once pyrogen activation of the heat production pathway ceases, the set point is lowered, and heat loss mechanisms are employed to reduce the body back to its normal thermoneutral zone. Conversely, hypothermia results when the set point is decreased which causes

activation of heat-production and conservation mechanisms, such as shivering, constriction of peripheral blood vessels, and increasing the body's metabolic rate.

**2. Role of the Hypothalamus in Thermoregulation**

At the center of neural control of thermoregulation is the hypothalamus, which is known to contain neural networks important for integrating sensory information and controlling homeostatic processes. Early lesion studies and direct stimulation studies have identified the preoptic anterior hypothalamus (POAH) as a critical area involved in thermoregulation (4,6,7). Neural networks within the POAH integrate sensory information about brain, body, and skin temperature and are at the center of temperature control, which spans from the hypothalamus down to effector areas in the brain stem and spinal cord. Because only activation in the POAH produces a complete set of heat loss mechanisms, this area is thought of as the thermoregulatory system's "central thermostat" (1,4).

**3. Classification of POAH Neurons**

Two groups of neurons in the POAH have been classified based on their inherent patterns of firing rates in response to local changes in temperature as assessed by in vitro and in vivo studies: warm sensitive neurons, which make up approximately 30% of the neurons in the POAH, and temperature insensitive neurons (8). Warm sensitive neurons increase their firing rates during warming and decrease their firing rates during cooling. Temperature insensitive neurons show little to no change in firing rate in response to warming or cooling. In order to determine whether a neuron is classified as warm sensitive or temperature insensitive, its thermal coefficient is calculated by plotting its firing rate in impulses (action potentials) per second as a function of temperature. Based on previous studies, a warm sensitive neuron is characterized by a thermal coefficient of 0.8 impulses per second per degree Celsius ($imp \cdot sec^{-1} \circ \cdot C^{-1}$) or higher. The changing

firing rate patterns for these two types of neurons in response to temperature change can be seen in Figure 1. Studies suggest that warm-sensitive neurons integrate local input with afferent sensory input in order to act as the dominant control center for all thermoregulatory mechanisms, including heat loss and heat production (9,10).

There are also cold sensitive neurons present in the POAH which have a thermal coefficient of -0.6 $imp \cdot sec^{-1} \circ \cdot C^{-1}$ or lower; however, studies suggest that the firing rate of these neurons is not inherent (11). These neurons depend on synaptic input, which is greatly reduced in tissue preparation, to fire an action potential. Their cold sensitivity is a product of decreased inhibitory input from warm sensitive neurons, usually in response to a drop in local temperature, or increased excitatory input from temperature insensitive neurons. These neurons are effector neurons for the heat production pathway in Hammel's model, which will be discussed later.

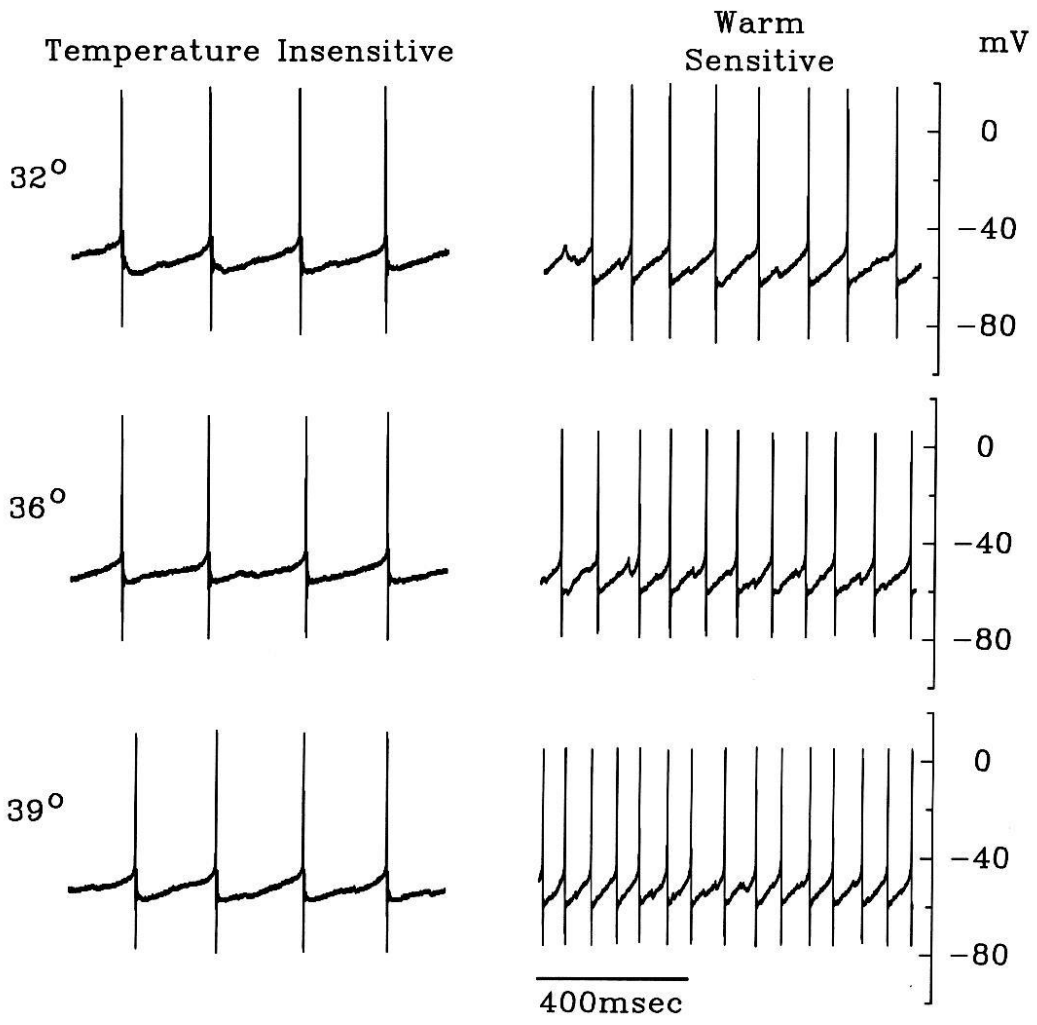


**Figure 1: The effect of temperature on firing rate of neurons of the POAH.** Left: Temperature insensitive neuron. Right: Warm sensitive neuron. (Adapted from Griffin et. al., 1996).

**4. Properties of POAH Neurons**

The change in firing rate that warm sensitive neurons exhibit in response to temperature is not attributable to a change in resting potential, but rather to a faster rise to the threshold for production of an action potential (12). The rise to threshold is also called the depolarizing prepotential, as it is the depolarizing potential that precedes an action potential. A faster depolarizing prepotential therefore causes a shortened interspike interval, which is the interval between action potentials, and leads to an overall increase in firing rate. Both warm sensitive and temperature insensitive neurons have depolarizing prepotentials but those in temperature insensitive cells show little to no change in response to changing temperature. The interspike interval of temperature insensitive neurons remains relatively constant as temperature changes. The differential effect of temperature on the depolarizing prepotentials of warm sensitive and temperature insensitive neurons is shown in Figure 2.

The change in rate of depolarizing prepotentials in warm sensitive neurons is caused by a change in membrane conductance, which may be induced by either increasing inward depolarizing currents or decreasing outward hyperpolarizing currents. To determine this effect, -50pA current was applied on insensitive and warm sensitive neurons in order to determine how conductance (as measured as resistance$^{-1}$) changed during the interspike interval (13). Resistance in temperature insensitive neurons remained constant during the interspike interval, while resistance increased in warm sensitive neurons in the same period.

The lack of change in firing rate of insensitive neurons likely indicates either a constant conductance or a combination of increasing depolarizing conductances and decreasing hyperpolarizing conductances. The increase in resistance of warm sensitive neurons is likely a product of inactivation of hyperpolarizing currents such as the outward potassium current ($I_A$).

This current controls the refractory period after an action potential, in which a neuron's membrane potential is hyperpolarized, and as such it is less excitable. It is possible that insensitive neurons utilize their sodium-potassium pumps to maintain hyperpolarization as temperature increases, which would explain why their firing rates do not increase as temperature rises (14). When hypothalamic tissue slices are treated with ouabain, which blocks action at the sodium potassium pump, temperature insensitive neurons replicate the same patterns as warm sensitive neurons in terms of changing excitability as temperature changes (15).

Warm sensitive neurons and temperature insensitive neurons also tend to have different patterns of dendritic projections, shown in Figure 3, which suggest that they receive synaptic input from different sets of neurons (11). Integrative warm sensitive neurons orient their projections medially and laterally (as seen in a coronal slice) so as to receive sensory input from afferent pathways. Insensitive neurons tend to have dendrites oriented dorsally and ventrally and may provide a constant reference for integrative warm sensitive neurons.

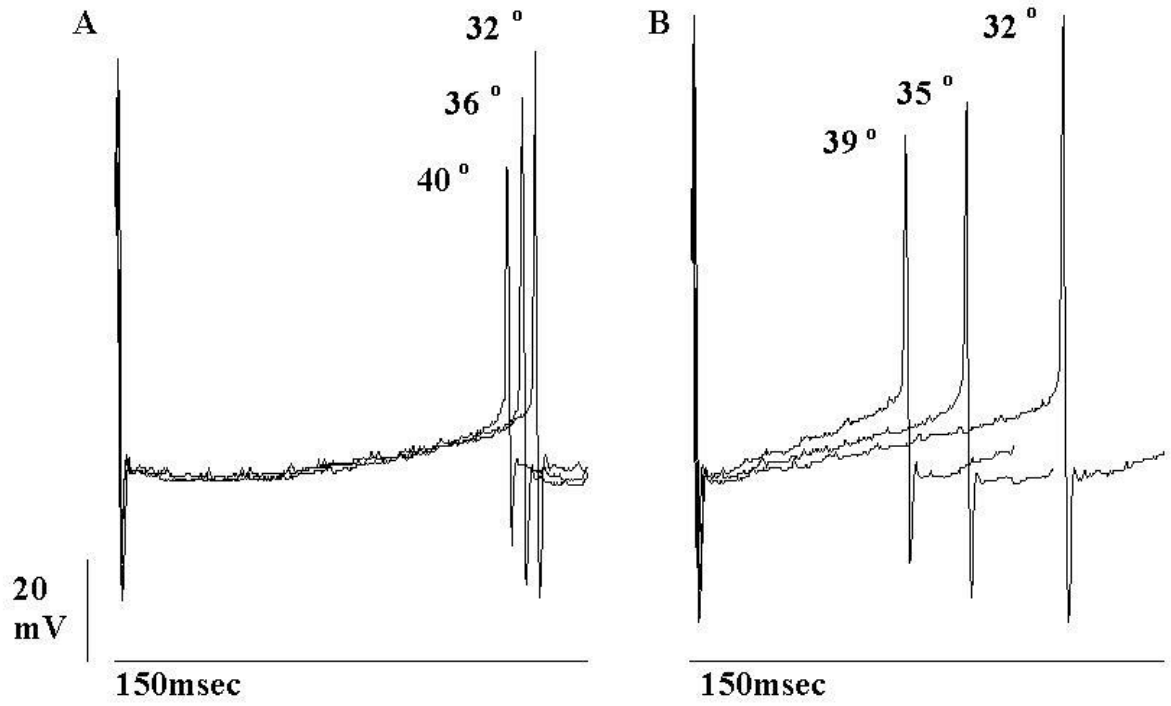


**Figure 2: Differential response of depolarizing prepotential to change in temperature of a low slope temperature insensitive neuron (A) and a warm sensitive neuron (B).** For each neuron, action potentials are shown, followed by depolarizing prepotentials, consequent action potentials, and hyperpolarizing after potentials. Warm sensitive neurons have a faster depolarizing prepotential and a shorter interspike interval as temperature increases. For both warm sensitive and temperature insensitive neurons increased temperatures cause a decrease in action potential amplitude and amplitude of hyperpolarizing after potentials. (Adapted from Griffin et. al. 1996).

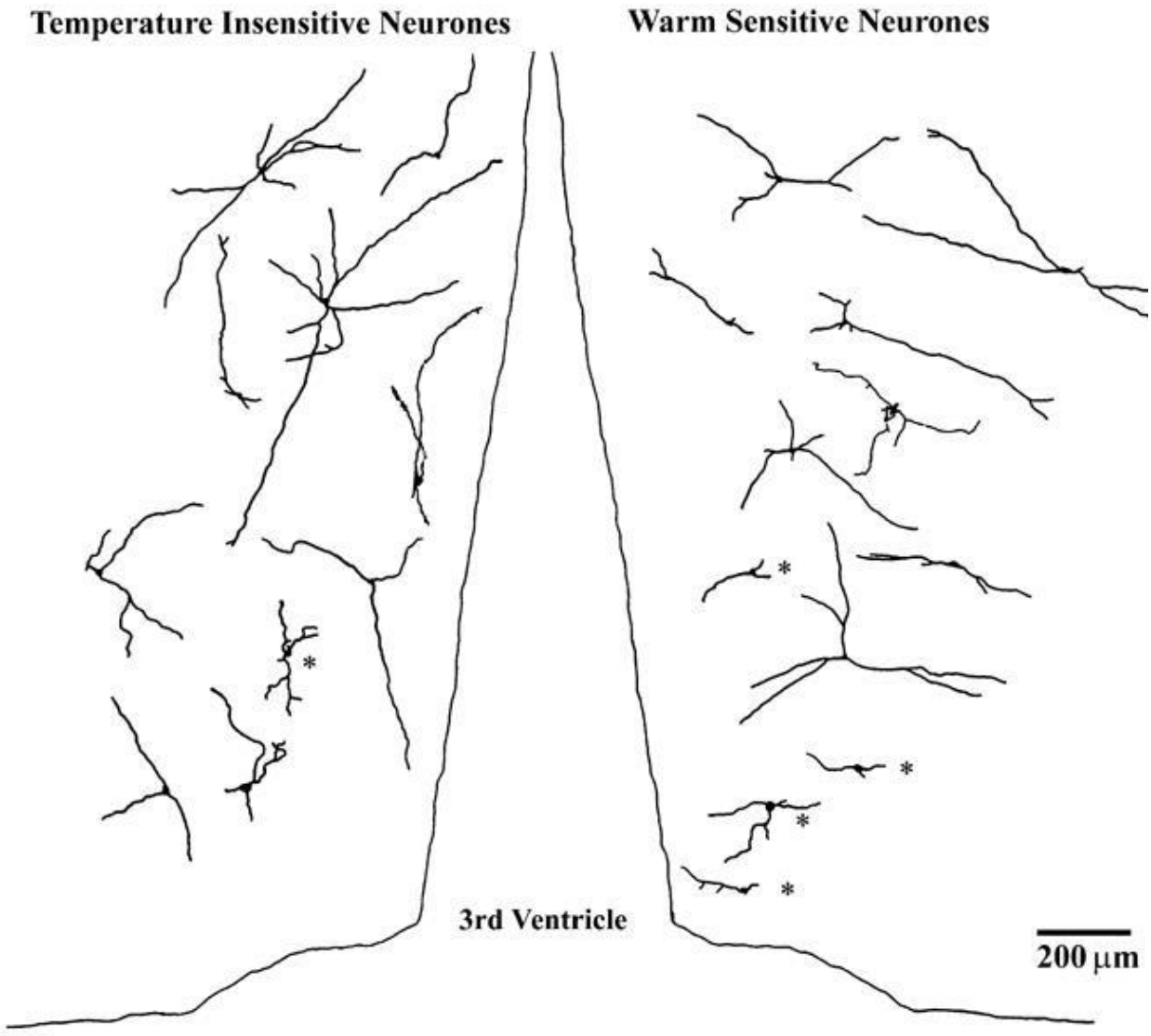


**Figure 3:** Camera lucida drawings of projections of warm sensitive neurons versus temperature insensitive neurons in coronal tissue slices. Neurons were identified at different locations on the rostral-caudal axis. The top of the figure is dorsal, and the bottom is ventral. (Adapted from Griffin, Saper, and Boulant, 2001).

**5. Hammel's Model of Thermoregulation**

In 1965, H. T. Hammel proposed a model for thermoregulation in which the activity of warm sensitive neurons and of temperature insensitive neurons in the POAH is integrated in order to determine a set point temperature (4). In this model, warm sensitive neurons act as integrators of local thermal information. Their inherent activity indicates local hypothalamic or set point temperature, which is compared with input from afferent sensory thermoreceptor pathways that provide information about skin, spinal cord, and core temperature. If thermal information in the body is inconsistent with that of the hypothalamus, these warm sensitive neurons change their firing rates to adjust body temperature back to the set point. These integrative neurons project to effector neural networks that control heat loss and heat production mechanisms.

Two populations of effector neurons, warm effector neurons that modulate heat loss mechanisms and cold effector neurons that modulate heat production pathways, receive input from warm sensitive and temperature insensitive neurons. These warm sensitive and temperature insensitive neurons synapse on both warm and cold effector neurons; these synapses may be excitatory or inhibitory. During fever, changes in activity lead to a positive (hyperthermic) shift in the thermoregulation and increase heat production in order to reach the new set point. Effector neurons for heat loss receive greater excitatory input from warm sensitive neurons than inhibitory input from temperature insensitive neurons. When the heat loss pathway is activated, heat loss mechanisms are induced. As body temperature decreases, the firing rate of warm sensitive neurons also decreases; eventually cold effector neurons that control the heat production pathway receive more excitatory input from insensitive neurons than inhibitory input from warm sensitive neurons, and the heat production pathway is turned on. These pathways work in tandem to maintain core body temperature. This model is shown in Figure 4.

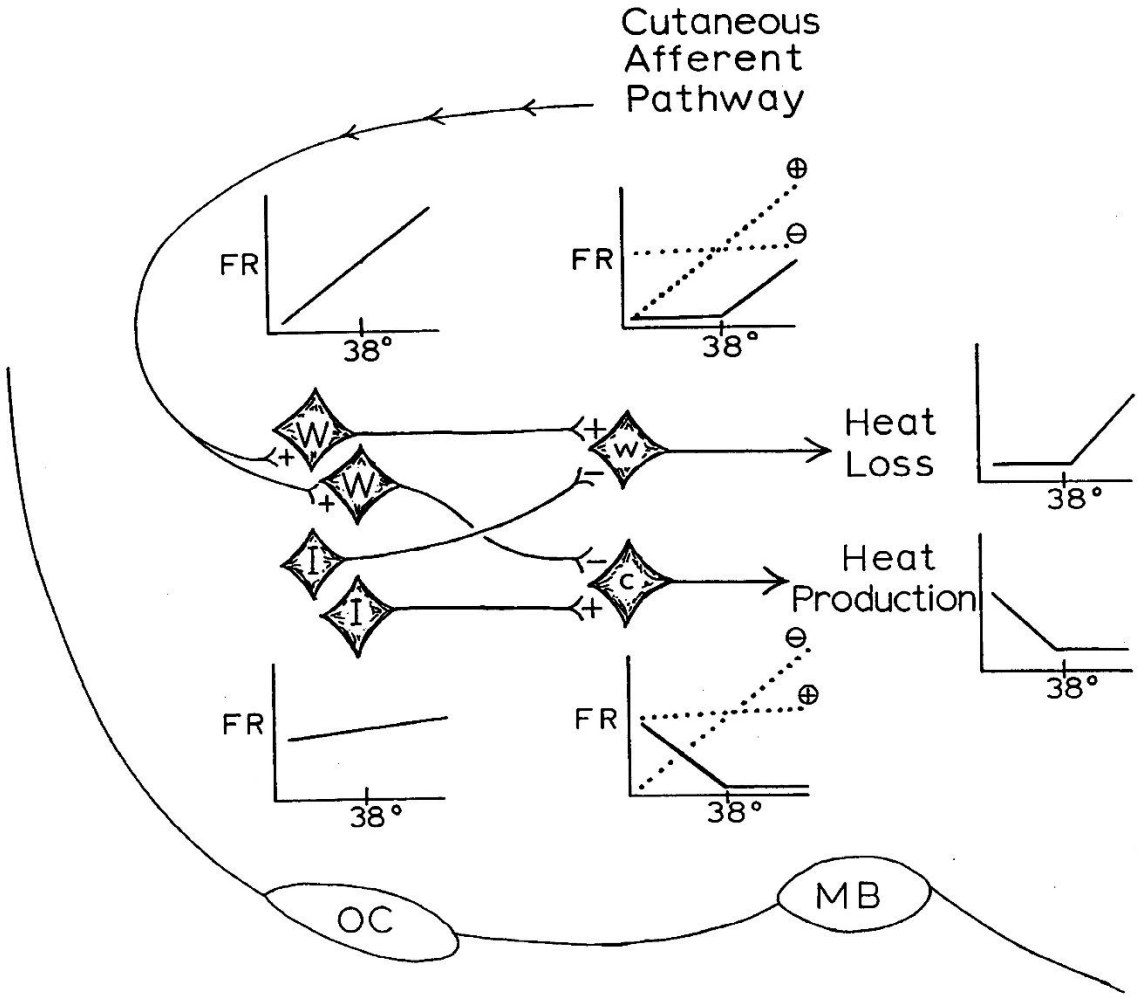


**Figure 4: Redrawn diagram based on Hammel's model of thermoregulation by hypothalamic neurons.** Heat loss and production pathways are regulated around a set point of 38°C. OC, optic chiasm; MB, mammillary body; W, warm sensitive neurons; I, temperature insensitive neurons; w, warm effector neurons controlling heat loss; c, cold effector neurons controlling heat production. Solid lines show firing rate as a function of local temperature for each neuron, and dotted lines represent excitatory (+) and inhibitory (-) input.

**6. Potential Mechanism for Fever Production by the POAH**

A fever is triggered when warm sensitive neurons respond to an increase in production of pyrogens, such as the signaling molecule prostaglandin $E_2$ (PGE2) that is released after immune stimulation; however, the mechanism by which PGE2 affects POAH neurons is still under investigation (11). Noradrenergic (NA) afferents are thought to be additional mediators of hyperthermia along with the surge in PGE2 leading up to a hyperthermic response. The effects of NA can be seen even before PGE2 levels surge. Stimulation of the α-1 subtype of NA receptors has been shown to increase core body temperature, whereas stimulation of α-2 NA receptors decreases core body temperature; these effects suggest that norepinephrine, which induces PGE2-related hyperthermia, may have a direct effect on temperature sensitive neurons in the POAH and produce changes to the thermoregulatory set point (16). Data have shown that α-1 NA simulation increases the firing rates of temperature insensitive neurons and decreases the firing rates of warm sensitive neurons, which is consistent with a positive change in set point (hyperthermia) (17). In the presence of an α-2 NA agonist, firing rates of insensitive neurons decreased, while firing rates of warm sensitive neurons increased, which is consistent with a negative change in set point (hypothermia). Understanding the differential activation of these specific receptor subtypes could inform the mechanisms by which neurons in the POAH might respond to pharmaceuticals and endogenous signaling molecules.

**7. Hot Flashes**

Hot flash (HF) is the name given to a sudden sensation of heat and heat loss mechanisms, such as sweating, vasodilation, reddening of the face, and sudden change in perception of temperature, usually associated with an abrupt drop in sex steroid hormones (18). HFs are most common among menopausal and perimenopausal women, whose estrogen levels have recently declined (19).

Occurrence of HFs peaks about one year after cessation of menstruation and remains elevated for 8 years on average. HFs are also typically experienced by women undergoing treatment for breast cancer (20). Most women over age 45 experience some discomfort due to HFs, including hyperthermia; though there is a range of severity and incidence among women who experience HFs, it is widely agreed that HFs have an adverse effect on quality of life (21). The subjective feeling associated with HFs tends to last only several minutes in women, but a rise in body temperature can be observed as long as 20 minutes in advance of the HF sensation (22).

Just as HFs in women correlate with a decrease in the sex steroid estrogen, men tend to experience HFs after a sharp decrease in testosterone, like the decrease in testosterone associated with surgical castration of androgen deprivation therapy given for treatment of prostate cancer (23). Approximately 75% of men treated for prostate cancer experience HFs. Previous studies suggest that men, like women, experience a similar increase in core body temperature preceding incidence of HFs (24,25).

**8. A Potential Hypothalamic Mechanism for Hot Flashes**

It has been assumed that the mechanism behind HF involves disruption of the thermoregulatory system, because common symptoms of flashes include heat-loss mechanisms, which indicate a fever-like response (19,26). Despite this link, how thermoregulatory neurons change their activity in order to promote HFs is not known (18,19). There is evidence to support a mechanism involving hypothalamic production of NA leading up to HFs (27). When measuring concentrations of NA metabolites in blood serum during HFs, samples showed heightened levels of the brain NA metabolite 3-methoxy-4-hydroxiphenyl-glycol (MHPG) in the periphery, while concentrations of the peripherally-produced NA metabolite vanilmendelic acid (VMA) did not change (28). Furthermore, treatment with yohimbine, an α-adrenergic antagonist, both increased NA

concentrations in the hypothalamus and induced HFs in susceptible women (29). Studies have also found that the thermoneutral zone of women experiencing HFs is smaller than in non-symptomatic women, which may explain why small changes in ambient temperature can induce HFs (30,31). An increase of NA has been found to correlate with a narrowed thermoregulatory zone, which may suggest NA involvement in HF modulation (32).

## II: GABAPENTIN

### 1. Chemical Structure and Properties

Gabapentin [GP; 1-(aminomethyl) cyclohexane acetic acid)], also known by its brand names Neurontin and Horizant, is a structural analog of γ-aminobutyric acid (GABA); its structure is shown in Figure 4 (33). The molecular weight of GP is 171.34. GP is a zwitterion, meaning it has a positive and a negative charge at physiological pH, and as such does not easily cross plasma membranes. GP is not metabolized by the body and has a short half-life of approximately 5-7 hours; its bioavailability varies inversely with dosage. It has a favorable drug interaction profile and is generally well tolerated with few side effects (34).

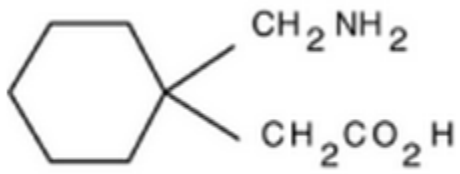


**Figure 5:** Structure of GP (from Rose & Kam, 2002).

### 2. Receptors and Mechanism

Though GP is an analog of GABA, it does not bind to any known GABA receptors (33,35). However, GP may act as a GABA agonist in the brain, as it may mediate increases in production of glutamate decarboxylase (GAD) and branched chain amino acid transaminase (BCAT), two enzymes involved in the GABA production pathway (36).

The mechanisms of GP's actions are not fully known, but several theories attempt to explain

its anticonvulsant and analgesic effects. The anticonvulsant effect associated with GP is thought to be delayed, as maximal concentrations are not reached in the brain until 60 minutes after drug administration (36,37). The leading theory for GP's ability as an anticonvulsant implicates an as yet unknown receptor within the L-system transporter amino acid protein; GP competes with L-amino acids to bind on this transport protein in order to cross plasma membranes (38). GP also has been shown to reduce the release of dopamine, serotonin, NA, and other monoamine neurotransmitters (39). Finally, it has been suggested that GP might prevent formation of new synapses by antagonizing the binding of thrombospondin, a synaptogenesis factor, to its binding site on its $\alpha_2\delta$ receptor (40). GP's mechanism is unique, as it has not been shown to interact with any common channels targeted by other anticonvulsants (41).

When used as an analgesic, GP appears to have a different mechanism of action than that of its anticonvulsant effect, as injection of GP into the subarachnoid space in the mouse brain caused nearly immediate amelioration of pain (42,43). Studies surveying GP's mechanism of pain modulation showed significant high affinity binding to voltage-dependent calcium channels (VG-$Ca^{2+}$) at their $\alpha_2\delta$ subunits in the periphery (44). Blocking neural VG-$Ca^{2+}$ channels causes inhibition of action potentials by deactivating the AMPA reception pathway, which mediates fast ionotropic synapses (36). Studies have indicated that GP does not have a binding site directly on AMPA or NMDA receptors, both of which mediate $Ca^{2+}$ influx leading up to an action potential (36,45).

**3. GP in Treatment of Hot Flashes**

Recent studies have indicated a link between GP consumption in moderate to high doses and decline in hot flash incidence, duration, and severity in perimenopausal women (20,21,35,46). HFs are often associated with a sudden drop in estrogen levels, and as such estrogen has been used

to treat HF for more than 60 years; the mechanism of its action in HF modulation is not fully understood (18). Studies comparing the efficacy of GP versus traditional estrogen therapy show that GP treatment is useful in HF treatment, especially for cancer patients and others for whom hormone replacement therapy is not advised (20,35,46). GP treatment in surgically castrated men and those undergoing androgen deprivation therapy proved to be at least moderately efficacious in reducing hot flash incidence and severity (23,25,47). The mechanism by which GP mediates HFs in both men and women is not known; this study aims to determine how GP might directly alter ion channel activity of POAH neurons that control thermoregulation.

## III: SPECIFIC AIM AND HYPOTHESIS

**Specific Aim:** To determine the effects of GP on firing rates of thermoregulatory POAH neurons and to use this information to present a mechanistic theory by which GP might affect thermoregulatory modulation during HFs.

**Hypothesis:** Treatment with GP will decrease the firing rate of warm sensitive cells as well as temperature insensitive cells in the POAH, which would lead to an increase in set core body temperature.

# THE EFFECTS OF GABAPENTIN ON FIRING RATES OF THERMOREGULATORY NEURONS IN THE PREOPTIC ANTERIOR HYPOTHALAMUS IN MODULATION OF HOT FLASHES

The following chapter is being prepared for publication.

## I. Abstract

Studies have identified a link between gabapentin (GP) treatment and a decrease in the incidence and severity of hot flashes (HFs) in predisposed individuals. The mechanism of GP's modulation of HFs is not known. It has been suggested that GP acts on voltage-gated calcium (VG-$Ca^{2+}$) channels in order to prevent fast ionotropic synapses in modulation of neuropathic pain. This study will determine whether GP acts directly on neurons within the preoptic anterior hypothalamus (POAH) in order to cause a change in the body's thermoregulatory set point in order to prevent HFs. We hypothesize that treatment with GP will decrease the firing rate of warm sensitive neurons as well as temperature insensitive neurons in the POAH, which would lead to an increase in set core body temperature according to current models of thermoregulation by POAH neurons. Coronal slices of the hypothalamus and surrounding brain areas of adult male Sprague-Dawley rats were prepared and recordings were made of single unit activity. Neurons were classified as either warm sensitive or temperature insensitive based on the slope of their firing rate plotted as a function of temperature. Neurons were treated with 10μM GP for ten minutes and then washed for ten minutes with artificial cerebrospinal fluid (aCSF), during which time their firing rates were recorded. In response to GP, warm sensitive neurons decreased their firing rates, while most temperature insensitive neurons showed little response. Twelve temperature insensitive neurons, localized in the periventricular and paraventricular nuclei, increased their firing rate in response to GP. The changes in firing rate induced by GP would indeed result in a positive shift in set point (hyperthermia) according to current models. This hyperthermic shift may contribute to a widened thermoneutral zone in individuals who commonly suffer from HFs and therefore a decrease in HF incidence and/or duration.

## II. Introduction

Homeostatic processes, including the regulation of body temperature, are controlled largely by neural networks within the hypothalamus, where information from many sensory systems is integrated with the activity of neurons that are inherently sensitive to temperature and responsive to endogenous mediators of body temperature. In particular, the preoptic anterior hypothalamus (POAH) has been identified as a focal point for control of body temperature, as determined by Hammel (4) and others (7,8). The POAH is also integral to production of fever, which is defined as a positive change in body temperature (hyperthermia) linked to change in firing rate of POAH neurons. The thermoregulatory role of the POAH relies on sensory information from its two identified neural groups, those that are insensitive to environmental temperature changes (~70%) and those that increase their firing rate in response to increasing temperature (~30%), as well as afferent inputs from peripheral sensory neurons (1). A sub-population of warm-sensitive cells are thought to integrate sensory information, and control efferent thermoregulatory pathways (11).

The preeminent model for thermoregulatory control by the hypothalamus was modeled in 1965 by H. T. Hammel (4). This model suggests that activity of warm sensitive neurons is integrated with that of temperature insensitive neurons in order to establish a set point temperature. Integrator neurons can act in excitation or inhibition; these neurons synapse on two populations of effector neurons that control heat loss and heat production, respectively. This model provides the framework by which one can determine in what way endogenous modulators or chemical modifiers may alter the neuronal mechanisms for thermoregulation (17).

Warm sensitive neurons in the POAH respond to an increased production of the signal molecule prostaglandin E2 (PGE2) when the immune system is stimulated during infection. Ranels and Griffin showed in 2003 that PGE2 affects both warm sensitive neurons in the POAH

via direct inhibition and stimulates activity of some insensitive neurons, either of which would induce hyperthermia (48). However, body temperature may not solely be dependent on PGE2 production, as temperature may rise even before PGE2 is present (11). Noradrenergic (NA) afferents may be an additional cause of hyperthermia in addition to the surge in PGE2 leading up to a hyperthermic response. Stimulation of the α-1 subtype of NA receptors has been shown to increase core body temperature, whereas stimulation of α-2 NA receptors has been shown to decrease core body temperature (hypothermia); these effects suggest that norepinephrine, which induces PGE2-related hyperthermia, may directly affect temperature sensitive neurons in the POAH and thus produce changes to the thermoregulatory set point (16). It is known that fever-like symptoms including hyperthermia accompany many hot flashes, but it is unknown whether this pathway has a neuronal basis in the POAH populations that control temperature regulation.

It has been assumed that HFs have a mechanism that involves disruption of the thermoregulatory system, because common symptoms of flashes include heat-loss mechanisms, such as sweating, vasodilation, reddening of the face, and sudden change in perception of temperature, all of which indicate a fever-like response (19,26). Despite this knowledge, the molecular mechanism related to the thermoregulatory responses of hot flashes remain unknown (18,19). There is evidence to support a hypothalamic NA-related mechanism for HFs (27). High levels of the brain NA metabolite 3-methoxy-4-hydroxiphenyl-glycol (MHPG) were found in peripheral sera during hot flashes, while the peripherally-produced NA metabolite vanilmendelic acid (VMA) showed no changes in concentration (28). Furthermore, treatment with yohimbine, which is an α-adrenergic antagonist, both induced HFs and increased NA concentrations in the hypothalamus in women prone to HFs (29). Studies have also found that patients experiencing HFs have a smaller thermoneutral zone due to a decline in estrogen levels, which would explain

why small changes in ambient temperature may induce HFs (30,31). It has also been determined that an increase of NA correlates with a narrowed thermoregulatory zone, whereas an increase of serotonin or dopamine contribute to a widening of this zone (32).

Gabapentin (GP, 1-(aminomethyl) cyclohexane acetic acid) is an anticonvulsant and neural analgesic drug that is similar in structure to the neurotransmitter gamma aminobutyric acid (GABA) (33). It appears to act similarly to a GABA-B receptor activated system but potential mechanisms suggested are completely independent of GABA receptors (49). GP is thought to act as a GABA agonist in the brain, but it does not act on a known GABA receptor (33,35). As a pain modulator, GP is thought to act by binding on voltage-dependent calcium channels with high affinity, which deactivates the pathway that activates AMPA receptors and causes NA release (33). Whether or not the proposed mechanism for GP in pain is consistent with its mechanism in hyperthermic response, i.e. whether or not a noradrenergic receptor is activated during HFs, has not been determined.

Recent studies have indicated a link between GP consumption in moderate to high doses and decline in hot flash incidence, duration, and severity in perimenopausal women (20,21,35,46). Hot flashes (HFs) are most common in women in the perimenopausal period; occurrence peaks about one year after menstruation ceases and remains elevated for 8 years on average (19). HFs are among the most typical symptoms for women undergoing treatment for breast cancer (20). Between 50 and 80 percent of women above the age of 45 experience some varying level of discomfort, including hyperthermia during hot flashes; severity and incidence vary from among women, but it is widely agreed that HFs have an adverse effect on quality of life (21). HFs are often associated with a sudden drop in estrogen levels, and as such estrogen has been used to treat HFs for more than 60 years; its mechanism is for its action is not known (18). Comparative studies

show that GP treatment is also useful in HF treatment, especially for cancer patients and others for whom hormone replacement therapy is not advised (20,35,46).

This study aims to determine how GP alters the firing rates of temperature sensitive and/or insensitive cells in the POAH in order to produce heat loss-like responses. In determining the effect of GP has on the POAH, we may make inferences about the receptor types GP is affiliated with and therefore how it interacts with the brain. This information may also prove useful in HF treatment and our understanding of the HF mechanism.

**We hypothesize that treatment with GP will decrease firing rate of warm sensitive cells as well as temperature insensitive cells in the POAH, which would lead to an increase in set core body temperature according to current models of hypothalamic thermoregulation.**

**III. Materials and Methods:**

Male Sprague-Dawley rats (Harlan Inc.; 100-150g) were housed under standard conditions and provided unlimited food and water in a university-regulated animal care facility. Each recording session, one rat was brought to the laboratory where it was anesthetized with isoflurane and decapitated in accordance with procedures approved by the Animal Care and Use Committee at the College of William & Mary. The brain was removed, and a block of tissue including the hypothalamus was excised using a razor blade. A vibrotome was used in making 400 μM coronal tissues slices containing the POAH. Slices were transferred to an interface recording chamber and allowed to equilibrate. Slices were continuously perfused with artificial cerebral spinal fluid (aCSF), which is composed of (in mM) 124 NaCl, 26 $NaHCO_3$, 10 glucose, 5 KCl, 2.4 $CaCl_2$,1.3 $MgSO_4$ and 1.24 $KH_2PO_4$. The aCSF was heated to approximately 36-37°C using a thermoelectric peltier assembly and oxygenated (95% $O_2$ – 5% $CO_2$). Temperature was monitored by a

thermocouple located in close proximity to tissue slices within the recording chamber.

Extracellular single unit recordings were made with glass microelectrodes of tip diameters < 1μm that were filled with 3M NaCl. Electrical signals were recorded and firing rate was determined using an Xcell-3 amplifier and APM digital recording system (FHC Inc.). Action potential firing rate and temperature were recorded simultaneously using Axoscope Software (Molecular Devices). Once the activity of a neuron had been isolated (signal to noise ratio of ≥ 3:1), temperature within the chamber was varied 2-3°C above and below baseline temperature. Thermosensitivity (impulses $\cdot$ $s^{-1.o}C^{-1}$) was determined by plotting firing rate as a function of temperature. Previous studies have indicated warm sensitivity is defined by a regression coefficient of ≥ 0.8 impulses $\cdot$ $s^{-1.o}C^{-1}$ and cold sensitivity is defined by ≤ -0.6 impulses $\cdot$ $s^{-1.o}C^{-1}$; all other neurons are considered to be temperature insensitive (11,50).

Following determination of thermosensitivity, neurons were perfused with aCSF containing 10μM GP for 10 minutes (treatment) and then washed with normal aCSF for another 10 minutes (washout). Firing rate was monitored continuously throughout the experiment. After an experiment was completed, neuronal firing rates were digitized for quantitative determination of its response to GP treatment using Axoscope Software. Three separate one-minute representative samples were taken from each recording: one just before treatment (baseline), one during GP treatment, and one during washout. For each sample, a mean and standard error was calculated; responses to GP treatment were determined via comparison to pre-treatment firing rates using a standard T-test with $P \leq 0.05$.

Recordings were all made in the POAH and locations of each recording were noted visually using the ventral edge of the third ventricle, as a reference for lateral-medial and dorsal-ventral location. Slices were prepared with more tissue remaining on the left side of the third ventricle to

aid in left-right orientation. Coronal location was estimated be recording the depth of the electrode under the surface of the slice.

**IV. Results**

The firing rates of sixty-one hypothalamic neurons were recorded. Thermosensitivity for each neuron was determined as described in the Methods; five neurons were found to be warm sensitive (regression coefficient $\geq$ 0.8 impulses $\cdot$ $s^{-1.o}C^{-1}$), and the remaining fifty-six were temperature insensitive (regression coefficient between -0.6 and 0.8 impulses $\cdot$ $s^{-1.o}C^{-1}$). No cold sensitive neurons (regression coefficient $\leq$ -0.6) were recorded, but studies suggest that these cells do not fire without synaptic input, which is largely eliminated in tissue preparation (11).

After determination of thermosensitivity, the firing rate of each neuron was recorded during treatment with GP. Changes in firing rate that were considered statistically significant had $P \leq 0.05$ (see Methods). Table 1 shows a summary of firing rate responses collected during baseline readings, during treatment, and during the washout period for insensitive and warm sensitive neurons based on their responses to GP. Figure 1 shows patterns of thermosensitivity versus percent change in firing rate for all neurons; the overall trend in firing rate was negative, with some distinct local clusters of neurons increasing their firing rates. The locations of each recorded neuron were documented and may be seen in Figure 2.

Of insensitive neurons, 33 showed no significant change in firing rate to either GP treatment or the washout period following treatment. The average change in these neurons was $-0.001 \pm 0.02$ impulses $\cdot$ $s^{-1}$. The response of an insensitive neuron that showed no significant change is shown in Figure 3. Twelve insensitive neurons increased their firing rate in response to GP; the average increase in firing rate was $0.19 \pm 0.05$ impulses $\cdot$ $s^{-1}$. The response of an insensitive neuron that showed an increase in firing rate in response to GP is shown in Figure 3. Eleven

insensitive neurons decreased their firing rate in response to GP; the average decrease was -0.10 ± 0.03 impulses $\cdot s^{-1 \cdot o}C^{-1}$.

Of the five warm sensitive neurons recorded, three decreased their firing rates. The average decrease in firing rate during GP treatment was –6.47 ± 3.75 impulses $\cdot s^{-1}$. During washout, firing rate continued to decrease to an average of just 0.37 impulses$\cdot s^{-1}$, indicating a change of -13.29 ± 4.25 impulses $\cdot s^{-1}$ from baseline. The response of a warm sensitive neuron that decreased its firing rate in response to GP may be seen in Figure 4. The remaining two warm sensitive cells increased their firing rates. The change in firing rates of warm cells whose increased their firing rates in response to GP was 0.84 ± 0.29 impulses $\cdot s^{-1}$ above baseline. The response of a warm sensitive neuron that increased its firing rate in response to GP may be seen in Figure 5.

**V. Discussion**

GP is effective for treatment of seizures, neuropathic pain, and many other afflictions (33,36). Human trials have shown that GP is effective in the treatment of hot flashes in both women and men, which suggests that it has a thermoregulatory mechanism (20,21,22,23,24,35,46,). This study gives evidence for GP's mediation of HFs via action on the thermoregulatory neurons within the POAH.

The POAH is known to contain neural networks that act as the body's thermoregulatory control center. The thermoregulatory model proposed by Hammel in 1965 suggests that heat production and heat loss mechanisms are each modulated by a population of effector neurons that gets contrasting synaptic input from warm sensitive and temperature insensitive neurons (4). When effector neurons that modulate heat loss reach a threshold at which they receive greater excitatory input from warm sensitive neurons than the inhibitory input from temperature insensitive neurons, these effector neurons turn on heat loss mechanisms such as sweating and

vasodilation in order to cool the body's core temperature back to its set point. In contrast, when effector neurons that modulate heat production receive greater excitatory input from temperature insensitive neurons than inhibitory input from warm sensitive neurons, they turn on heat production mechanisms in order to raise the body to its set point.

GP caused a population shift in activity among recorded hypothalamic neurons, warm sensitive and temperature insensitive alike. According to Hammel's model, a decrease in activity of warm sensitive neurons causes effector neurons that promote heat production mechanisms to increase their activity, resulting in hyperthermia. The two warm sensitive that increased their firing rate in response to GP showed modest changes; though one of these warm sensitive neurons showed a +41% response, it had a significantly lower baseline firing rate than all other warm sensitive cells (2.76 impulses/sec versus an average of 11.54 impulses/sec in other warm sensitive neurons), and as such its percent increase seems dramatic. In contrast, the cells that decreased their firing rate in response to GP showed drastic changes during treatment (-87%, -96% and -47% change) and continued to decrease their firing rates during the washout period.

The results show an increase in firing rate among temperature insensitive neurons in the paraventricular and nearby periventricular nucleus. Neurons of the paraventricular nucleus receive input from NA neurons that run adjacent to the third ventricle; GP likely has an inhibitory action at NA neurons that synapse on neurons of the paraventricular nucleus, and the lessened inhibitory input results in an increase in firing rates of paraventricular neurons (51,52). The periventricular nucleus does not have a well-defined functional correlate, but it is known to contain warm sensitive and temperature insensitive thermoregulatory neurons (53,54). This increase in firing rate of temperature insensitive cells within the paraventricular and periventricular nuclei of the POAH could indicate further cause of hyperthermia in accordance with Hammel's model of neuronal

thermoregulation.

Considering the short timeframe of each experiment, it is most likely that GP acts in inhibition at VG-$Ca^{2+}$ channels by blocking synapses and increasing the interspike interval of warm sensitive cells. We propose that GP modulates α-2 NA receptors by binding voltage-dependent calcium channels of adrenergic neurons antagonistically, thus preventing the calcium cascade that leads to NA release. There is evidence to suggest that GP acts to inhibit synapse formation at terminals of warm sensitive neurons, as the firing rate of these neurons continued to decrease after treatment during the 10-minute washout period. GP may also have a similar mechanism of action on some temperature insensitive cells in the POAH; insensitive neurons that decreased their firing rate may contribute to the lessened inhibition on insensitive neurons in the paraventricular and periventricular nuclei, therefore mediating the increase in firing rate of these paraventricular and periventricular neurons.

This study provides evidence for a hypothalamic mechanism for modulation of HFs by GP. Data suggests that GP binds antagonistically to warm sensitive neurons of the POAH and to neurons that synapse on temperature insensitive neurons in the paraventricular nucleus, causing excitability in these neurons. Both of these results induce hypothermia and shift the body's set point to a higher temperature. A higher set point means a widened thermoneutral zone; a small thermoneutral zone has been linked to HF incidence (30,31). Because heat loss mechanisms associated with HFs occur when the body reaches its threshold for heat loss (e.g. 39°C), if this threshold is higher, the body can be in a hyperthermic state longer without activating the heat loss pathway. A higher thermoneutral zone allows those prone to HFs to endure small derailments in temperature, such as standing in a hot room or eating spicy food, without experiencing heat loss mechanisms associated with HFs.

In order to corroborate data collected in this study, a future study should repeat our methods while blocking spontaneous activity of POAH neurons in order to determine whether GP lengthens the interspike interval of POAH neurons inherently or if its effect is population-dependent. Determining whether paraventricular and periventricular neurons in particular have a direct or population-dependent response to GP is a priority and would provide insight about the receptor subtypes that exist on these neurons. An injection study in whole animals would also help to determine exactly how GP acts to regulate body temperature via thermoregulatory neurons in the hypothalamus.

**VI: Conclusions**

Our data suggest that GP acts on POAH neurons to produce a hyperthermic shift in set point. According to the current model of thermoregulatory control by the hypothalamus, either a decrease in firing rate of warm sensitive neurons or an increase in firing rate of temperature insensitive neurons would cause hyperthermia. GP produces both of these effects, which results in a positive shift in set point. This shift suggests that GP modulates HFs by widening the body's thermoneutral zone, which reduces the incidence of HFs by setting a higher threshold for activation of heat loss mechanisms.

**VII: Figures**

| Thermosensitivity (impulses · $s^{-1}$·$^oC^{-1}$ ) | Response | N | Firing Rate (impulses · $s^{-1}$ ± SEM) Baseline | Gabapentin | Washout |
|---|---|---|---|---|---|
| Insensitive (<0.8) | | | | | |
| | Total | 56 | 1.13±0.09 | 1.15±0.10 | 1.17±0.10 |
| | No Change | 33 | 1.14±0.12 | 1.14±.0.12 | 1.16±0.13 |
| | Increase | 12 | 1.24±0.20 | 1.43±.024 | 1.43±0.27 |
| | Decrease | 11 | 0.98±0.20 | 0.89±.017 | 0.94±0.18 |
| Warm (≥0.8) | | | | | |
| | Total | 5 | 11.27±3.22 | 7.77±3.89 | 4.31±2.76 |
| | Increase | 2 | 7.68±4.92 | 8.52±4.63 | 8.35±3.59 |
| | Decrease | 3 | 13.66±4.40 | 7.20±6.55 | 0.37±0.02 |

**Table 1: The effects of GP on the firing rates of POAH neurons.**

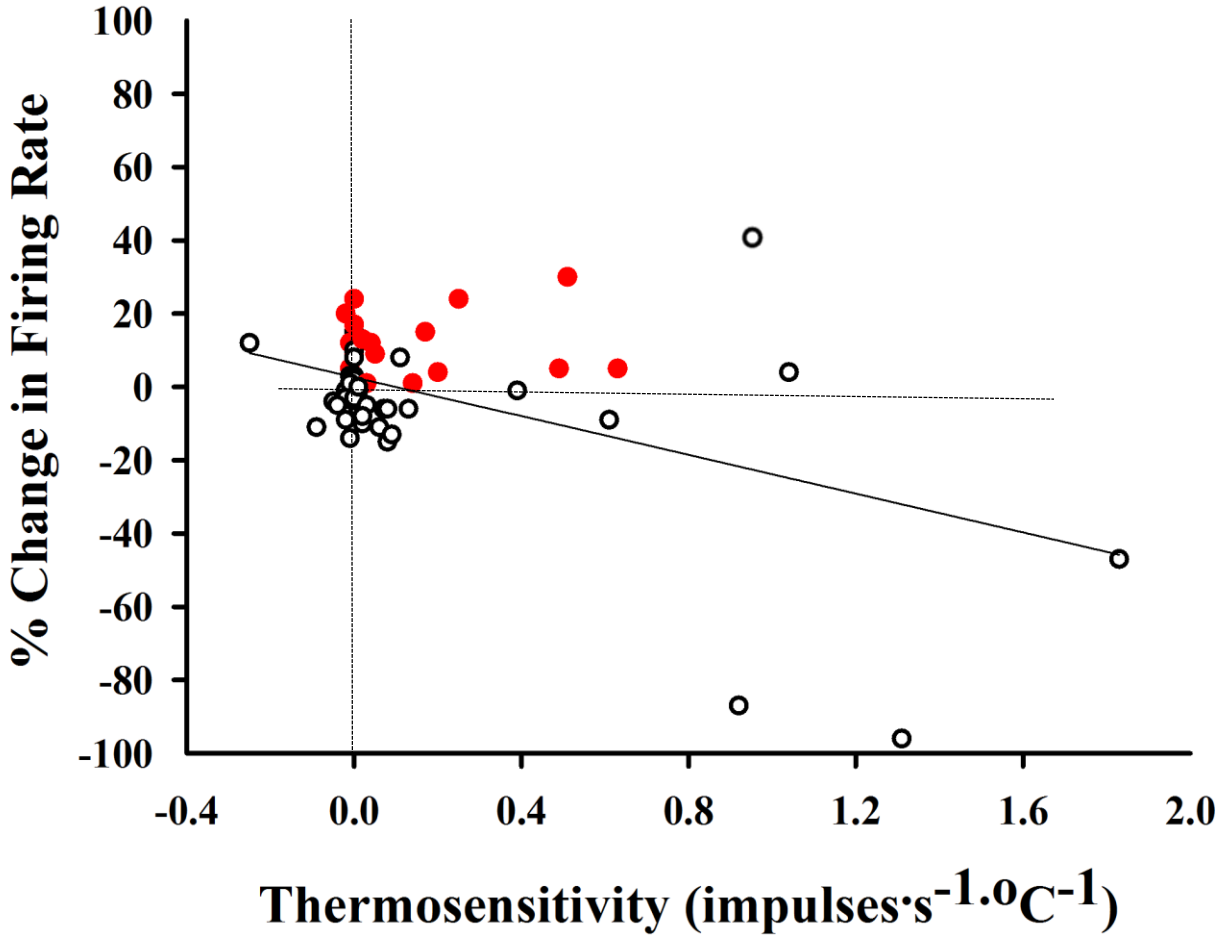


**Figure 1: Percent change in firing rate versus thermosensitivity.** Warm sensitive neurons are characterized by a thermosensitivity of ≥0.8 impulses · $s^{-1.o}C^{-1}$. Neurons colored red were found in the paraventricular or paraventricular nuclei.

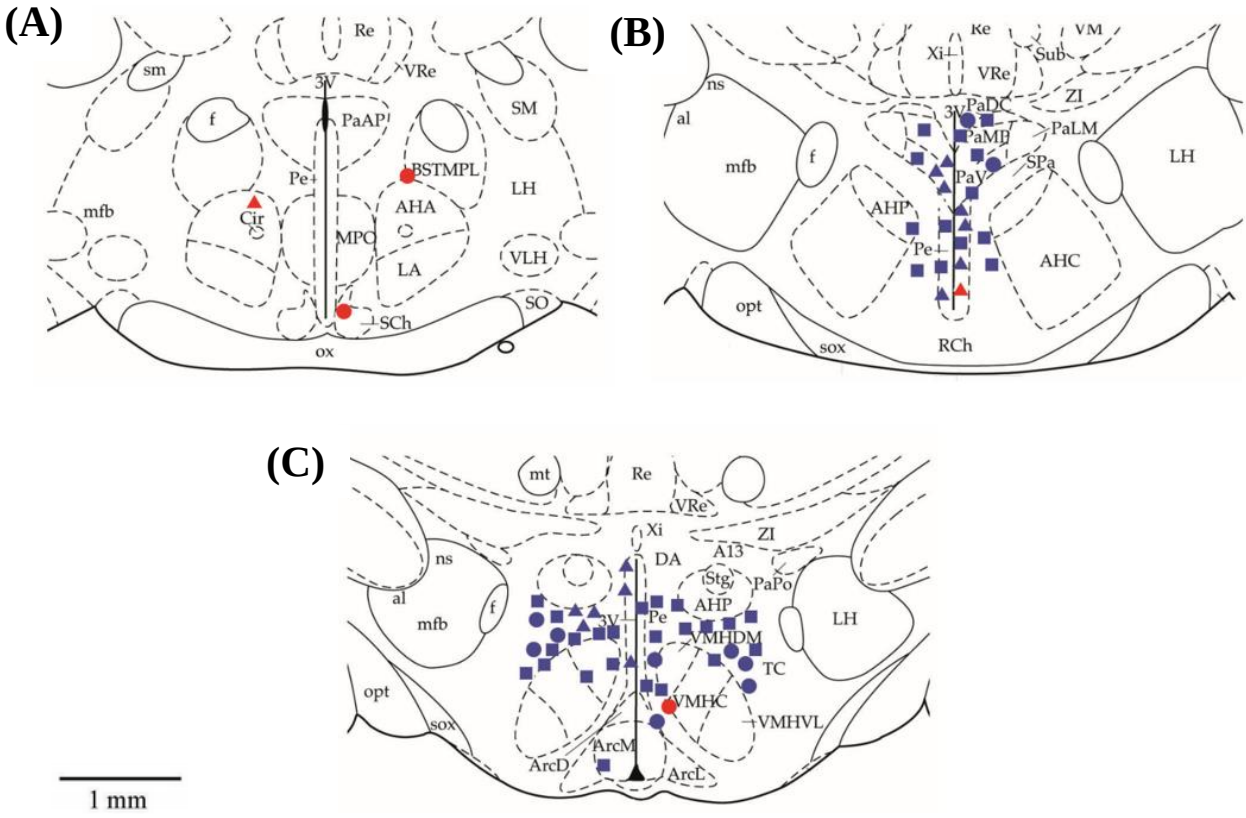


**Figure 2: Locations of POAH neurons in response to temperature and GP.** Slice maps are shown in the coronal plane, with figure (A) most rostral and figure (C) most caudal. Distances from bregma: (A) = -1.4 mm; (B) = -1.9 mm; (C) = -2.3 mm. Diagrams were adapted from Paxinos and Watson, 1998. Blue shapes indicate temperature insensitive neurons, whereas red shapes represent warm sensitive neurons. Triangles indicate an increase in firing rate in response to GP, circles represent a decrease in firing rate in response to GP, and squares represent no change in firing rate in response to GP. 3V, third ventricle; Re, reuniens thalamic nucleus; VRe, ventral reuniens thalamic nucleus; sm, stria medullaris of the thalamus; f, fornix; PaAP, paraventicular hypothalamic nucleus; SM, nucleus of the stria medullaris; BSTMPL, bed nucleus of the stria terminalis medial/posterolateral; Pe, periventricular nucleus; mfb, medial forebrain bundle; Cir, circular nucleus; MPO, median preoptic nucleus; AHA, anterior hypothalamic area anterior; LH, lateral hypothalamus; LA, nucleus laminaris; VLH, ventral lateral hypothalamus; SCh, suprachiasmactic nucleus; SO, supraoptic nucleus; ox, optic chiasm; Xi, xiphoid thalamic nucleus; Sub, submedius thalamic nucleus; PaDC, paraventricular hypothalamic nucleus dorsal cap; ZI, zona incerta; PaMP, paraventricular hypothalamic nucleus medial/parvocellular; PaLM, paraventricular hypothalamic nucleus lateral/magnocellular; Spa, subparaventricular zone of the hypothalamus; ns, nigrostriatal fibers; al, ansa lenticularis; PaV, paraventricular nucleus ventral; AHP, anterior hypothalamic area posterior; AHC, anterior hypothalamic area central; Pe, periventricular hypothalamic nucleus; RCh, retrochiasmatic nucleus; opt, optic tract; sox, supraoptic nucleus; mt, medial terminal nucleus of accessory optic tract; DA, dorsal hypothalamic area; A13, dopaminergic group 13; Stg, stigmoid hypothalamic nucleus; PaPo, paraventricular hypothalamic nucleus posterior; VMHDM, ventromedial hypothalamic nucleus dorsomedial; TC, tuber cinereum area; VMHC, ventromedial hypothalamic nucleus central; VMHVL; ventromedial hypothalamic nucleus ventrolateral; ArcD, arcuate hypothalamic nucleus dorsal; ArcM, arcuate hypothalamic nucleus medial; ArcL, arcuate hypothalamic nucleus lateral.

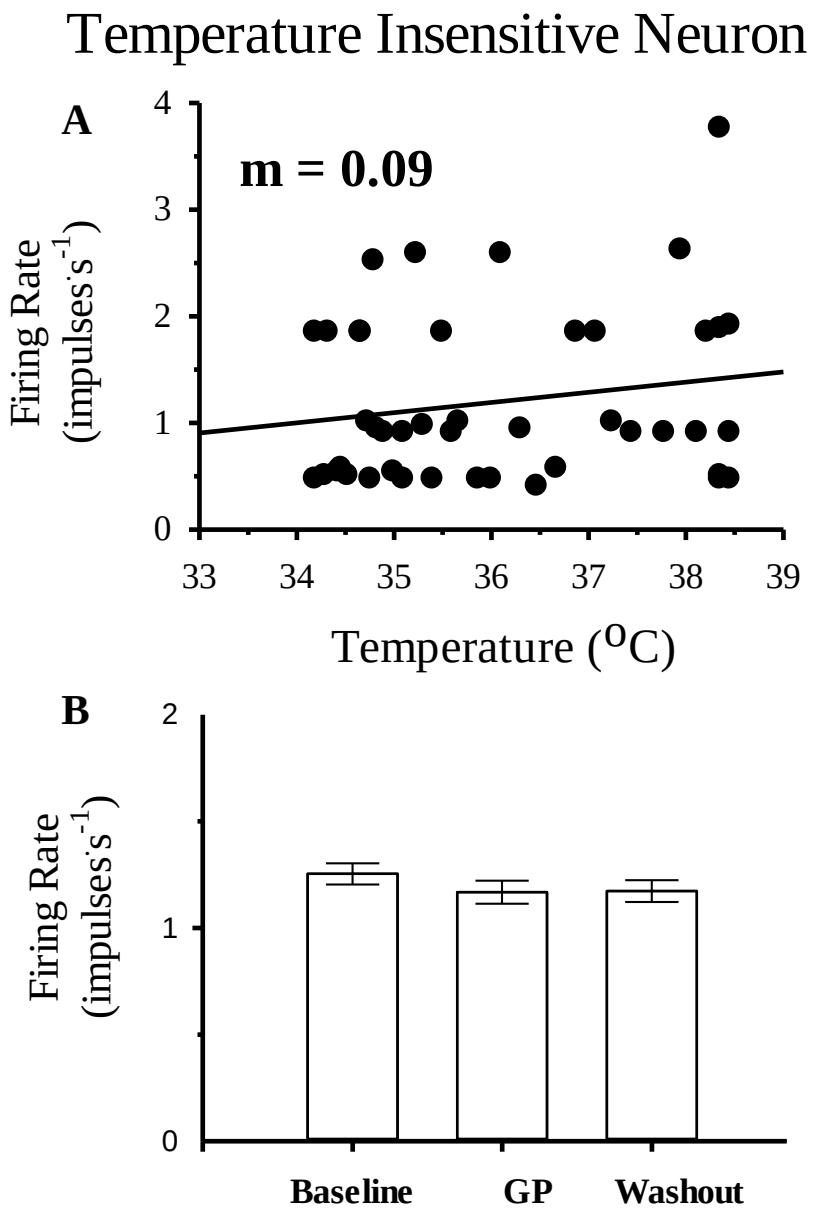


**Figure 3: Response of a temperature insensitive neuron that showed no change in firing rate in response to to GP.** (A): Firing rate as a function of temperature; regression coefficient = 0.09 impulses · $s^{-1.o}C^{-1}$. (B): Average firing rates and standard errors for three separate one minute intervals during basement, GP treatment, and washout periods.

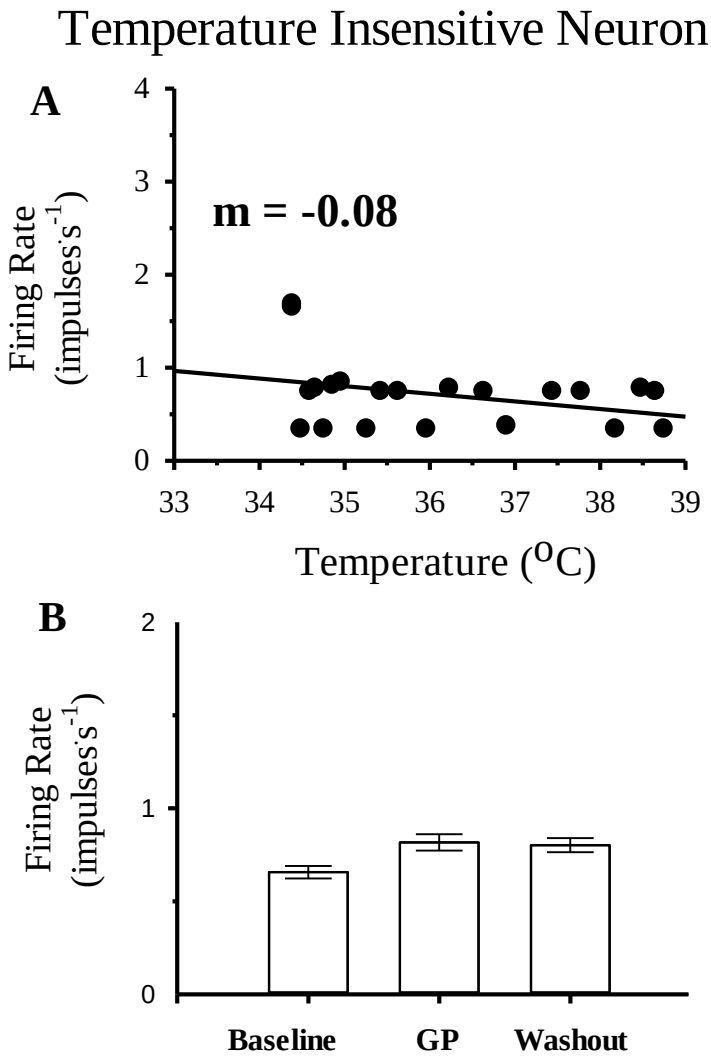


**Figure 4: Response of a temperature insensitive neuron that increased firing rate in response to GP.** (A): Firing rate as a function of temperature; regression coefficient = -0.08 impulses · $s^{-1}$·$^{o}C^{-1}$. (B): Average firing rates and standard errors for three separate one minute intervals during basement, GP treatment, and washout periods.

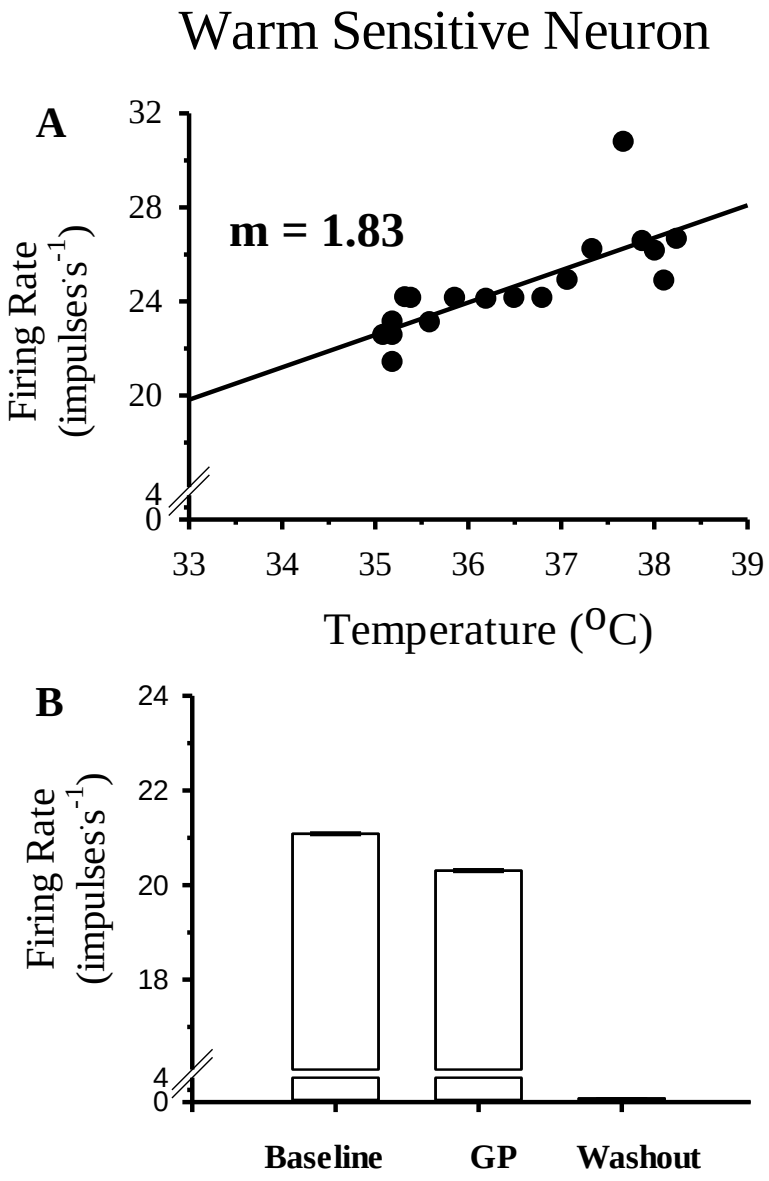


**Figure 5: Response of a warm sensitive neuron that decreased firing rate in response to GP.** (A): Firing rate as a function of temperature; regression coefficient = 1.83 impulses · $s^{-1.o}C^{-1}$. (B): Average firing rates and standard errors for three separate one minute intervals during basement, GP treatment, and washout periods.

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