# Hypothalamic Neurons

## Mechanisms of Sensitivity to Temperature[a]

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**ABSTRACT: Rostral hypothalamic neurons are influenced by endogenous factors that affect thermoregulation and fever. Intracellular recordings reveal the synaptic and intrinsic mechanisms responsible for neuronal thermosensitivity. Many temperature-sensitive and temperature-insensitive neurons display a depolarizing prepotential that precedes action potentials. Temperature has little effect on the prepotential of insensitive neurons; however, in warm-sensitive neurons, the prepotential's depolarization is elevated by warming, and this increases the firing rate. Intracellular cAMP can increase neuronal thermosensitivity by enhancing the thermal response of the prepotential, most likely by thermosensitive ionic conductances. Warm-sensitive neurons also receive inhibitory synaptic input (IPSPs) from temperature-insensitive neurons, enhancing the thermosensitivity of some neurons, because cooling increases IPSP amplitude and duration. Therefore, even though IPSP frequencies do not change, cooling can decrease firing rates by increasing IPSP amplitudes. Because endogenous factors change neuronal firing rate and thermosensitivity, these changes likely occur both post- and presynaptically as well as by ionic conductances that determine the time interval between action potentials.**

Regulation of body temperature and fever is partially controlled by the preoptic-anterior hypothalamus. Some preoptic neurons not only sense changes in deep body temperature, but also are affected, directly or indirectly, by pyrogenic substances. The effect of temperature or pyrogens on these preoptic neurons results in changes in a variety of physiological and behavioral thermoregulatory responses. This chapter describes basic cellular mechanisms of neuronal thermosensitivity, mechanisms that are both inherent to individual neurons and synaptically derived. These cellular mechanisms can also be modified. Temperature-sensitive neurons are greatly affected by cyclic AMP-associated changes in ionic conductances, whereas temperature-insensitive neurons are strongly dependent on the sodium-potassium pump. Accordingly, endogenous substances, such as pyrogens, have ample opportunity to modify inherent and synaptic components that affect the activity of both temperature-sensitive and temperature-insensitive neurons.

### PREOPTIC NEURONS AND THERMOREGULATORY RESPONSES

The preoptic-anterior hypothalamus is extremely important in thermoregulation. As reviewed by Boulant,[1] several different animal studies have employed implanted thermodes to locally warm or cool this small area of the brain. Depending on the species,

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preoptic warming elicits a variety of responses that promote dissipation of heat from the body. These heat loss responses include panting, sweating, and increased skin blood flow, as well as behavioral responses (e.g., wetting the skin or assuming a stretched out posture to increase the body's effective surface area). Also depending on the species, preoptic cooling evokes responses that produce heat and promote heat retention within the body core. Heat production responses include shivering and nonshivering thermogenesis, and heat retention responses include cutaneous vasoconstriction and behavioral responses (e.g., assuming a curled-up posture or moving to a warmer environment).

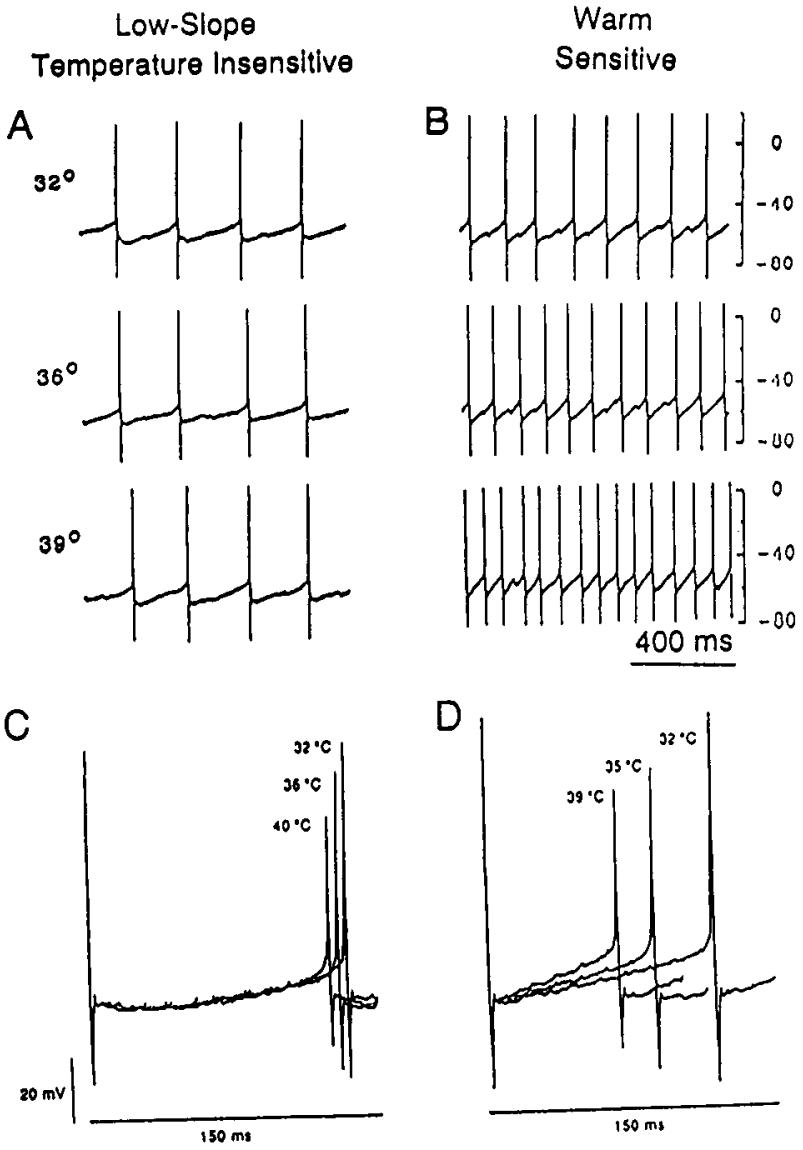


**FIGURE 1.** Effects of temperature on preoptic temperature-insensitive neuron (**A** and **C**) and preoptic warm-sensitive neuron (**B** and **D**). **A** and **B** are 1-second records at three different temperatures, and **C** and **D** are superimposed records of individual action potentials and the subsequent depolarizing prepotential and action potential. Both neurons display depolarizing prepotentials. Action potentials occur when the prepotentials reach threshold. In the warm-sensitive neuron, warming increases the prepotential's rate of depolarization which shortens the interspike interval and increases the firing rate. In both neurons, warming decreases the amplitudes of the action potentials. (From Griffin *et al.*[12])

Over the years, most *in vivo* studies of anesthetized animals and *in vitro* studies of hypothalamic tissue slices report similar proportions of temperature-sensitive and temperature-insensitive neurons in the preoptic-anterior hypothalamus.[2,3] More than 60% of the neurons are classified as temperature insensitive. As shown in FIGURE 1A, during preoptic warming and cooling, these neurons show either no change in their firing rates (i.e., low-slope temperature-insensitive neurons) or only slight changes in their firing rates (i.e., moderate-slope temperature-insensitive neurons). About 30% of preoptic neurons are considered to be warm sensitive. FIGURE 1B illustrates a warm-sensitive neuron that increases its firing rate during warming and decreases its firing rate during cooling. The criterion for neuronal thermosensitivity is based on the slope (or thermal coefficient) of firing rate (impulses/s) plotted as a function of temperature. If a neuron's thermal coefficient is at least +0.8 impulses/s/°C, the neuron is classified as warm sensitive. The temperature-insensitive neuron in FIGURE 1A has a thermal coefficient of 0.06 impulses/s/°C, and the warm-sensitive neuron in FIGURE 1B has a thermal coefficient of 1.1 impulses/s/°C.

Some neurons increase their firing rates during cooling or decrease their firing rates during warming. Cold-sensitive neurons have thermal coefficients equal to or more negative than –0.6 impulses/s/°C, but these neurons represent only a small proportion of preoptic neurons.[3] Studies suggest that many of the cold-sensitive neurons are not inherently thermosensitive but, rather, are synaptically inhibited by nearby warm-sensitive neurons. When hypothalamic tissue slices are perfused with media that block all synaptic activity, thermosensitivity is lost in most cold-sensitive neurons but retained in most warm-sensitive neurons.[4,5] Moreover, intracellular recordings of cold-sensitive neurons indicate that their firing rate activity is highly dependent on excitatory and inhibitory synaptic inputs from other preoptic neurons.[6]

## NEURONAL INTEGRATION OF THERMAL AND NONTHERMAL INFORMATION

It has been suggested that some preoptic warm-sensitive neurons facilitate heat loss responses and inhibit heat production responses, whereas cold-sensitive neurons might have the opposite roles.[7,8] If this is true, then it might be predicted that pyrogens cause fever by inhibiting warm-sensitive neurons controlling heat loss responses and by exciting cold-sensitive neurons controlling heat production responses. Early electrophysiological studies support this hypothesis for pyrogens (reviewed by Boulant[1,3]). Furthermore, as predicted, a more recent tissue slice study found that interleukin-1 (and other fever-producing substances) tends to inhibit warm-sensitive neurons, excite cold-sensitive neurons, and have little effect on temperature-insensitive neurons.[9]

Similar neuronal distinctions have been found in preoptic neuronal responses to peripheral temperature. Preoptic temperature-insensitive neurons are rarely affected by changes in skin or spinal cord temperature, but most preoptic warm- and cold-sensitive neurons are affected by at least one of these peripheral temperatures.[10] Moreover, in preoptic thermosensitive neurons, the responses to preoptic and peripheral temperature are usually similar. For instance, a preoptic warm-sensitive neuron may increase its firing rate in response to hypothalamic (core) warming or skin warming, and the greatest firing rate increase could occur if both the core and the skin are warmed simultaneously.

Preoptic warm-sensitive neurons act as integrators of thermal information. They are inherently sensitive to their own hypothalamic temperature, and they synaptically receive afferent information from skin and spinal thermoreceptors. Supporting the phys-

iological differences between temperature-sensitive and temperature-insensitive neurons are morphological distinctions between these cell types.[3] Preoptic temperature-insensitive neurons tend to orient their dendrites rostrally and caudally, parallel to the third ventricle. Warm-sensitive neurons, however, orient their dendrites perpendicular to the third ventricle, so that one "hand" of dendrites reaches laterally, possibly to receive afferent information from ascending fibers of the median forebrain bundle. The same warm-sensitive neurons send other dendrites medially towards the third ventricle. These medial dendrites could receive afferent information from the periventricular fibers, or they may even receive chemosensitive information regarding endogenous substances in the cerebrospinal fluid of the third ventricle. In this way, preoptic warm-sensitive neurons may be viewed as important integrators of homeostatic information; they are inherently sensitive to their own local environment, and they send dendrites to receive synaptic information from at least two different sources.

## INHERENT MECHANISMS OF NEURONAL THERMOSENSITIVITY

Unlike warm-sensitive neurons, preoptic temperature-insensitive neurons are strongly dependent on the sodium-potassium pump.[11] The Na-K pump is electrogenic and therefore helps maintain membrane hyperpolarization. Because it is metabolically driven, the pump is also affected by temperature. While warming may increase a depolarizing conductance in these neurons, warming also increases the hyperpolarizing Na-K pump, and this serves to stabilize the resting membrane potential and maintain an unaltered firing rate in temperature-insensitive neurons. As shown in FIGURE 2, when the Na-K pump is inhibited with ouabain, temperature-insensitive neurons increase their firing rates and become warm sensitive.[11] If endogenous factors (e.g., pyrogens or antipyretics) change Na-K pump activity, the firing rate and thermosensitivity of temperature-insensitive neurons would change; and this, in turn, could alter the neural network controlling the thermoregulatory set point.

In the comparison of temperature-insensitive neurons and warm-sensitive neurons, FIGURE 1 indicates that both types of neurons display spontaneous firing rates. Each action potential is followed by an after-hyperpolarizing potential (AHP) and then a depolarizing prepotential which, when reaching threshold, produces the next action potential. As with all neurons, warming reduces action potential amplitudes in both cell types. More importantly, FIGURE 1C shows that warming and cooling have little or no effect on the depolarizing prepotential in temperature-insensitive neurons. Accordingly, the interspike interval between action potentials remains constant, and firing rate does not change during a change in temperature. By contrast, FIGURE 1D indicates that the depolarizing prepotential of warm-sensitive neurons is greatly affected by temperature.[12] Warming increases the prepotential's rate of depolarization, and this shortens the interspike interval and increases firing rate. Cooling slows the rate of depolarization, which lengthens the interspike interval and decreases firing rate.

Differences also appear to exist in the predominant ionic conductances responsible for the depolarizing prepotentials of temperature-insensitive and warm-sensitive neurons.[12] During the prepotential depolarization in temperature-insensitive neurons, the net conductance remains constant. By contrast, in warm-sensitive neurons, the prepotential depolarization is primarily associated with a decreasing outward $K^+$ current, such as the potassium A current, $I_A$. Studies have shown that temperature has strong effects on $I_A$ inactivation.[12,13] Therefore, following an action potential in warm-sensitive neurons, the outward $I_A$ helps maintain a short period of hyperpolarization, but during $I_A$ inactivation, the membrane gradually depolarizes, and this constitutes much of

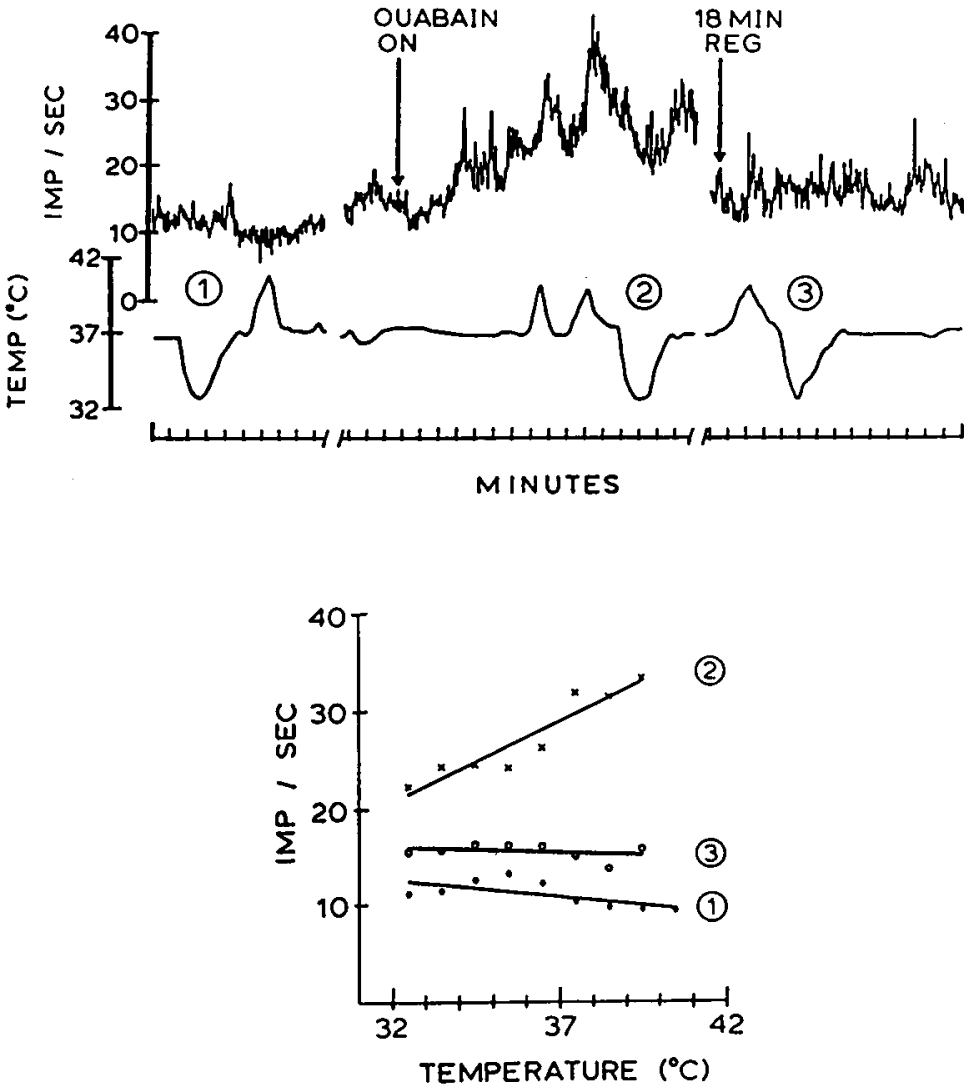


**FIGURE 2.** An extracellular recording of a temperature-insensitive neuron in a rat hypothalamic tissue slice. **(Top)** Records of firing rate and tissue temperature before (1), during (2), and after (3) perfusion with a medium containing $10^{-5}$ M ouabain to block the Na-K pump. **(Bottom)** Thermoresponse plots of firing rate as a function of temperature for the three periods shown at the top. (From Curras and Boulant.[11])

the prepotential. Warming increases the rate of $I_A$ inactivation, which concomitantly increases the rate of depolarization in the prepotential.

Preoptic neuronal temperature sensitivity is not due to thermally induced changes in the resting membrane potential.[14] In some neurons, however, in addition to the depolarizing prepotential, warm sensitivity is associated with the AHP that immediately follows an action potential.[15] In this case, warming can reduce the AHP amplitude and allow the subsequent prepotential to start from a more depolarized level. This also reduces the interspike interval and increases the firing rate.

Drugs that increase intracellular cyclic AMP also increase thermosensitivity, especially in warm-sensitive neurons. These drugs, however, have little effect on the low-slope temperature-insensitive neurons.[16] Cyclic AMP appears to increase thermosensi-

tivity by its actions on both the AHP and the depolarizing prepotential.[15] When intracellular cyclic AMP concentrations are high, warming causes greater increases in the prepotential's rate of depolarization, increasing neuronal firing rate and thermosensitivity. This is another instance that may permit endogenous factors (e.g., pyrogens or antipyretics) to influence neuronal activity. By either increasing or decreasing cyclic AMP, these substances can potentially alter the activity of neurons that control thermoregulatory responses.

## SYNAPTIC MECHANISMS OF NEURONAL THERMOSENSITIVITY

Even though preoptic warm-sensitive neurons are inherently thermosensitive, they receive much inhibitory synaptic input from nearby temperature-insensitive neurons. This synaptic input produces inhibitory postsynaptic potentials (IPSPs) that can enhance the thermosensitivity of warm-sensitive neurons.[6] FIGURE 3A shows depolarizing prepotentials and action potentials in a warm-sensitive neuron at two different

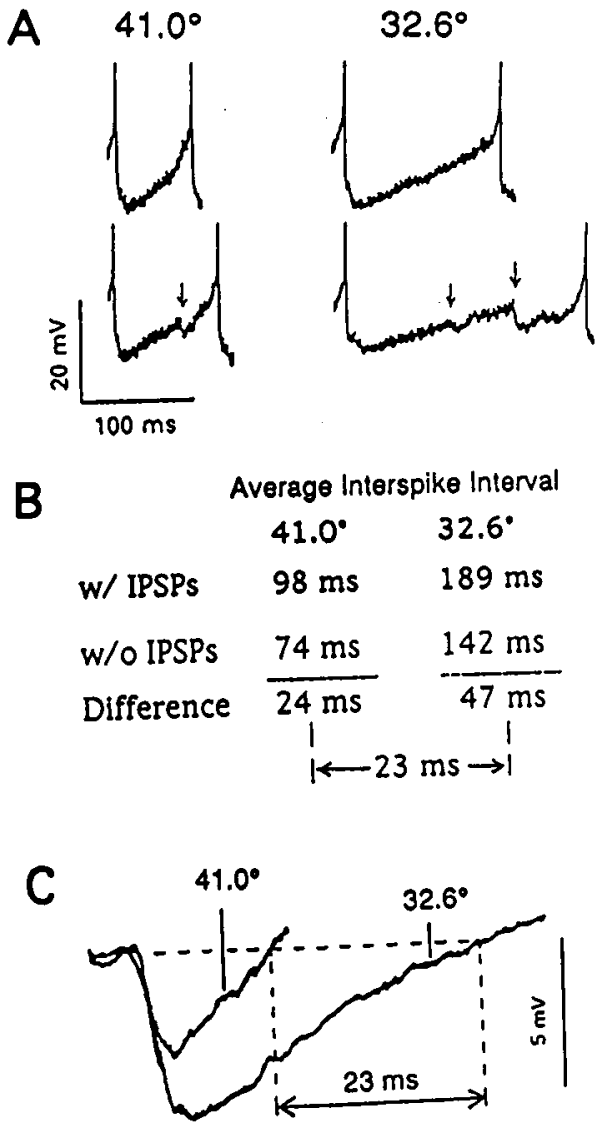


**FIGURE 3.** Effect of temperature on the ability of IPSPs to lengthen the interspike interval. **(A)** For two different temperatures, records show interspike intervals between action potentials. Top records do not contain IPSPs. Bottom records contain IPSPs (indicated by *arrows*). **(B)** For the two temperatures, average interspike intervals either with or without IPSPs during the interval. Cooling to 32.6° enhances (by 23 ms) the ability of an IPSP to lengthen the average interval. **(C)** Computer-averaged IPSPs ($n$ = 16–17) recorded at the two temperatures. Cooling to 32.6° lengthens (by 23 ms) the duration of the averaged IPSP. (From Curras *et al.*[6])

temperatures. Cooling to 32.6°C decreases the rate of rise of the depolarizing prepotential, thereby increasing the interspike interval and slowing the firing rate. At both warm and cool temperatures, the top two traces in FIGURE 3A show interspike intervals without IPSPs, and the bottom two traces in FIGURE 3A show interspike intervals when IPSPs are present. At both temperatures, IPSPs lengthen the interspike interval; however, this lengthening is enhanced during cooling.

As shown in FIGURE 3B, at 41 degrees the average interspike interval is 24 ms longer when IPSPs are present, but at 32.6 degrees, the interspike interval is 47 ms longer when IPSPs are present. Therefore, cooling increases, by 23 ms (i.e., 47–24 ms), the effectiveness of an IPSP to lengthen the interspike interval. Similarly, for the average IPSPs shown in FIGURE 3C, cooling increases not only IPSP amplitude, but also its duration, again by 23 ms. Since most temperature-insensitive neurons have slow firing rates (i.e., 1–2 impulses/s), a 23-ms change in the interspike has no significant effect on their firing rates. Warm-sensitive neurons, however, have higher firing rates with shorter interspike intervals. Accordingly, a cold-induced increase in IPSP duration could slow the firing rate enough to contribute to the thermosensitivity of a neuron that is already warm sensitive. As an example, if a warm-sensitive neuron fires at 10 impulses/s, its interspike interval is 100 ms. A cold-induced increase of 23 ms represents a quarter of this interval, and this would slow the firing rate enough to increase the neuron's thermal coefficient. Therefore, the effect of temperature on IPSP effectiveness can contribute to the sensitivity of warm-sensitive neurons.

FIGURE 3A illustrates another way in which cooling enhances IPSP effectiveness. At 41°C, the depolarizing prepotential is rapid and only allows time for one IPSP to occur during the interspike interval. At 32.6°C, however, the slowed rate of the prepotential permits enough time for two IPSPs to occur, causing substantial lengthening of the interspike interval. Thus, during cooling, the slower firing rates allow additional opportunities for IPSPs to slow the firing rates even more.

The cooling-induced increase in IPSP effectiveness is also due to the effect of temperature on the input resistance of warm-sensitive neurons. As with most neurons, cooling causes an increase in the warm-sensitive neuron's resistance. As shown by Ohm's law, the amplitude of the IPSP voltage change is equal to the inhibitory current times the membrane resistance. Therefore, because cooling increases the resistance, cooling also increases IPSP amplitude (and duration).

## SUMMARY

In the regulation of body temperature and fever, preoptic neurons respond to changes in core temperature and endogenous substances. Unlike temperature-insensitive neurons, preoptic warm-sensitive neurons act as integrators of thermal information. Warm-sensitive neurons send dendrites medially and laterally to receive synaptic input from pathways relaying peripheral thermoreceptive information. In warm-sensitive neurons, a primary mechanism of thermosensitivity involves the prepotential whose rate of depolarization increases during warming. Inactivation of the potassium A current appears to be an important component of this prepotential. A secondary influence on neuronal thermosensitivity is the effect of temperature on local inhibitory postsynaptic potentials. Thermosensitivity in warm-sensitive neurons is enhanced by cyclic AMP, which may be a mechanism for endogenous substances to modify neuronal firing rate and thermosensitivity. By contrast, temperature-insensitive neurons are strongly dependent on the sodium-potassium pump, and endogenous substances that change pump activity may alter the firing rate and thermosensitivity of these neurons.

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