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Central neural pathways for thermoregulation

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Abstract

Central neural circuits orchestrate a homeostatic repertoire to maintain body temperature during environmental temperature challenges and to alter body temperature during the inflammatory response. This review summarizes the functional organization of the neural pathways through which cutaneous thermal receptors alter thermoregulatory effectors: the cutaneous circulation for heat loss, the brown adipose tissue, skeletal muscle and heart for thermogenesis and species-dependent mechanisms (sweating, panting and saliva spreading) for evaporative heat loss. These effectors are regulated by parallel but distinct, effector-specific neural pathways that share a common peripheral thermal sensory input. The thermal afferent circuits include cutaneous thermal receptors, spinal dorsal horn neurons and lateral parabrachial nucleus neurons projecting to the preoptic area to influence warm-sensitive, inhibitory output neurons which control thermogenesis-promoting neurons in the dorsomedial hypothalamus that project to premotor neurons in the rostral ventromedial medulla, including the raphe pallidus, that descend to provide the excitation necessary to drive thermogenic thermal effectors. A distinct population of warm-sensitive preoptic neurons controls heat loss through an inhibitory input to raphe pallidus neurons controlling cutaneous vasoconstriction.

Keywords

Temperature Regulation; Physiology; Mammal; Homeostasis; Brown Adipose Tissue; Cutaneous Vasoconstriction; Shivering; Thermogenesis; Review

2. Introduction

The regulation of body temperature is one of the myriad of interrelated functions essential to the maintenance of homeostasis that are controlled primarily through dedicated pathways in the brain. Significant deviations in cellular temperature alter a variety of molecular properties, including reduced enzyme efficiency and altered diffusion capacity and membrane fluidity, which reduce critical cellular functions, including energy availability and membrane ion fluxes. The consequences of such changes in the mammalian nervous system include loss of consciousness and the inability to coordinate and execute motor activities. This is exhibited in a reversible manner in hibernating mammals where falls in brain temperature to 5°C are associated with torpor, inactivity and a dramatic fall in energy expenditure. Although recovery is possible from such seemingly large reductions in brain temperature, much less dramatic increases in brain temperature are incompatible with life.

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Hence, although not as immediately critical as the availability of oxygen and glucose, the brain is highly attentive to the potential for alterations in the temperature environment of its resident neurons and of the many tissues on which it depends for survival. This review will describe our current understanding of the core central thermoregulatory network (Figure 1): the central neural pathways that transmit ambient temperature signals, the hypothalamic networks that receive and integrate them with brain temperature information and the circuits through which the various patterns of thermal effector responses are orchestrated to sustain a homeostatic brain temperature.

The core central thermoregulatory network comprises the fundamental pathways through which cutaneous and visceral cold and warm sensation and/or reductions or elevations in brain temperature elicit changes in thermoregulatory effector tissues to counter or protect against changes in the temperature of the brain and other critical organ tissues. The effector mechanisms for cold defense, recruited in order of increasing energy costs, include thermoregulatory behavior to reduce heat loss, cutaneous vasoconstriction (CVC) to conserve heat in the body core, piloerection, non-shivering thermogenesis in brown adipose tissue (BAT) and shivering thermogenesis in skeletal muscle. For example, a functional neuroanatomical model of the core pathways providing the thermoregulatory control of CVC, BAT and shivering thermogenesis is illustrated in Figure 2. Mechanisms for heat defense include thermoregulatory behavior to increase heat loss, cutaneous vasodilation to facilitate heat loss by conducting heat from the body core to the body surface and evaporative cooling through sweating, saliva spreading or panting, employed to differing degrees by different species. Although the experimental basis to be described for the model in Figure 2 has been determined largely from studies in rodents and often under anesthetized conditions, the fundamental neural circuits elucidated through this work are expected to exist across a wide variety of species in which the above-mentioned thermoeffector mechanisms play significant roles in body temperature regulation. It will not be surprising to find, however, that the level of the central representation and the neurotransmitter modulation of the control of different thermoeffectors will vary among species according to the evolutionarily-determined significance of a particular effector system in their thermoregulatory repertoire. For example, although the fundamental pathways controlling sweating and panting are expected to be conserved in the relatively hairless human and in the dog or rodent, their relative levels of neural representation and of modulation by different transmitters should be appropriate for their respective importance in heat dissipation in these species.

Many of the tissues recruited in patterns of thermoregulatory responses also serve other functions – as examples, BAT plays a role in the metabolic regulation of energy stores, skeletal muscle is primarily involved in posture and movement and the salivary glands participate in digestion – and organ systems such as the cardiovascular and respiratory systems, principally involved in nutrient and oxygen delivery and in waste removal, although indispensible for many aspects of thermoregulation, are more directly responsive to non-thermoregulatory demands. Indeed, one of the most intriguing aspects of central homeostatic regulation is the resolution of the ‘conflicts’ implicit in treating the regulation of each homeostatic variable independently – for instance, how is the ‘decision’ made to sacrifice heat defense in favor of blood volume during prolonged exposure to a hot environment. This review will be concerned primarily with the fundamental neural pathways underlying the thermoregulatory responses of the principal thermal effector mechanisms mentioned above, with only brief comments on the often-critical interactions within the web of thermoregulatory and “non-thermoregulatory” control systems that are interleaved to achieve homeostasis.

3. Temperature Sensation

3.1. Cutaneous thermal receptor afferent pathway

3.1.1. Cutaneous thermoreception—To defend the thermal homeostasis of the brain and body from a variety of environmental thermal challenges, the thermoregulatory system must initiate defensive thermoregulatory responses before changes in environmental temperature affect body core temperature. When the skin is cooled in rats, for example, sympathetic thermogenesis in BAT and a tachycardia are elicited rapidly before the core or brain temperatures fall (Figure 3) (1). In unanesthetized animals, the core and brain temperatures are not affected or are slightly increased during exposure to a cold environment (2, 3). Such rapid thermo-defensive responses are elicited by sensing changes in environmental temperature through thermoreceptors in primary sensory nerve endings distributed in the skin and by transmitting the sensory information in a feedforward manner to the preoptic area (POA), a sensori-motor integration site for thermoregulation located rostral to the hypothalamus.

The molecular mechanisms of cutaneous thermoreception have been extensively investigated and recent studies suggest that the transient receptor potential (TRP) family of cation channels mediates thermal sensation across a broad physiological range of skin temperatures. However, none of these TRP channels has been conclusively identified as a molecular thermoreceptor in primary thermal afferents that is responsible for triggering thermoregulatory responses. Potential cold receptor TRP channels are TRPM8 and TRPA1, both of which are expressed in primary somatosensory neurons (4-6). TRPM8, which is activated by modest cooling (< 27°C) (4, 5), mediates a dominant part of cold sensation since TRPM8-deficient nerve fibers show profound loss of cold sensitivity and TRPM8-deficient mice exhibit a reduced ability to avoid innocuous cold temperatures (7-9). TRPM8 can also be activated by menthol or icillin (4, 5) and application of menthol to the skin evokes warm-seeking behavior as well as cold-defensive, physiological responses including BAT and shivering thermogenesis and cutaneous vasoconstriction (10). Although the basal body core temperature of TRPM8-deficient mice in a thermoneutral environment is not different from that of wild-type mice (7), the ability of TRPM8-deficient mice to maintain their body temperatures in cold environments has not yet been tested. TRPA1 is activated by colder temperatures (< 17°C) than TRPM8 (6). However, the contribution of TRPA1 to cold sensation *in vivo* is still controversial. Kwan *et al* (11) showed that TRPA1-deficient mice displayed a reduced sensitivity to a cold temperature (0°C), while Bautista *et al* (12) found no such a deficit in the response to even lower temperatures (about −10°C) in TRPA1-deficient mice but did observe a delayed onset of shivering when these mice were exposed to cold temperatures.

TRPV3 and TRPV4 are warm-sensitive TRP channels that are activated by innocuous warm temperatures with thresholds of 33–39°C and 25–34°C, respectively (13-17). TRPV3 is increasingly activated by repeated heating (14, 17) and intriguingly, shows different sensitivity to the direction of temperature change, resulting in an hysteresis across thermal activation-deactivation cycles (17) that is similar to the thermosensitive responses exhibited by primary warm afferent fibers (18-20). Expression of either TRPV3 or TRPV4 is prominent in keratinocytes in skin epidermis, but low in somatosensory ganglia (13, 14, 21). Release and diffusion of factors such as interleukin-1 alpha (21) from stimulated keratinocytes to adjacent sensory nerve endings have been proposed as a mechanism through which thermal information detected by these TRP channels in keratinocytes could be transmitted to sensory nerves. Compared to wild-type mice, mice lacking either TRPV3 or TRPV4 show altered behaviors in discriminating innocuous warm temperatures (22, 23). However, TRPV4-deficient mice exhibit intact circadian changes in body temperature and can defend their core temperature in a cold (4°C) or hot (35°C) environment (22, 24). The

effect of TRPV3 deficiency on thermoregulatory responses to environmental thermal challenges remains to be investigated.

3.1.2. Dorsal horn—Primary somatosensory fibers deliver thermal information detected by cutaneous thermoreceptors to the spinal (or trigeminal) dorsal horn (Figure 2), in which lamina I neurons receive most cutaneous thermal signals (25). The best-known thermosensory ascending pathway from lamina I neurons is the spinothalamocortical pathway, in which lamina I neurons directly synapse on neurons in the thalamus that project to the primary somatosensory cortex, leading to perception and discrimination of cutaneous temperature (25, 26). However, the spinothalamocortical pathway does not play a significant role in the thermal afferent pathway that triggers involuntary thermoregulatory responses to environmental cold challenges. This is demonstrated by the maintenance of sympathetic thermogenic responses to skin cooling following elimination of the skin cooling-evoked changes in primary somatosensory cortex EEG activity with lesions of the thalamic regions that receive thermal somatosensory signals from the spinal cord (27). Nonetheless, spinothalamic and trigeminothalamic lamina I neurons send collaterals to the lateral parabrachial nucleus (LPB) (28, 29), which mediates cutaneous thermosensory signals to the central thermoregulatory mechanism ((27); see the next section).

Craig and colleagues have described three main classes of spinothalamic and trigeminothalamic lamina I neurons that were categorized by their responses to cutaneous thermal and mechanical stimuli: nociceptive-specific cells responding to noxious mechanical and heat stimuli; polymodal nociceptive cells responding to noxious mechanical, heat and cold stimuli; and thermoreceptive-specific cells responding linearly to graded, innocuous cooling or warming stimuli and not being activated further in the noxious temperature range (30, 31). Considering that cutaneous thermal stimuli that trigger thermoregulatory responses are mostly in the innocuous range, thermoreceptive-specific lamina I neurons with ascending axons likely convey the dominant signals leading to body temperature control.

3.1.3. Lateral Parabrachial Nucleus—Functional neuronal tracing experiments revealed that many neurons densely clustered in the external lateral subnucleus of the LPB (LPBel) in the pons are both activated (Fos expression) following cold (4°C) exposure (32) and retrogradely labeled following tracer injections into the POA (Figure 4) (27), indicating that LPBel neurons directly transmit cutaneous cold signals to the POA (Figure 2). The greatest number of double-labeled LPBel neurons were found when the tracer injection was centered on the midline subregion of the POA, including the median preoptic nucleus (MnPO) (27), suggesting that the cool sensory signals from LPBel neurons are transmitted mainly to the MnPO rather than the medial (MPO) or lateral (LPO) POA. The LPB receives numerous projections from the dorsal horn (33-35) and axonal swellings of dorsal horn neurons were found to closely associate with postsynaptic structures of POA-projecting LPB neurons (27), providing further support for the view that POA-projecting LPB neurons are activated by direct somatosensory inputs from dorsal horn lamina I neurons (Figure 2).

In strong support of these anatomical observations, electrophysiological recordings from single LPB cells *in vivo* revealed that the firing rate of most neurons in the LPBel that were antidromically identified as projecting to the MnPO, increased markedly in response to skin cooling and then returned to the basal level following skin re-warming – responses that paralleled the skin cooling-evoked changes in BAT sympathetic nerve activity (SNA) (Figure 5) (27). Most cold-sensitive LPBel neurons are not activated in response to noxious mechanical stimulus such as tail pinch (27), providing further support for the idea that LPB neurons involved in thermoregulatory afferent signaling receive thermal information predominantly from thermoreceptive-specific lamina I neurons rather than from other lamina I neurons mediating nociception.

In vivo physiological studies revealed that either inhibition of local neurons or blockade of their glutamate receptors in the LPBel completely suppresses skin cooling-evoked cold-defense responses, including BAT and shivering thermogenesis and increases in metabolism and heart rate (Figure 6) (27). Thus, activation of LPBel neurons, likely by glutamatergic inputs from lamina I neurons driven by cutaneous cool signals, is essential for transmission of the cold thermal afferent stimulus to initiate cold defense responses (Figure 2). Consistently, rats that have bilateral lesions of the LPB fail to maintain body temperature in a cool environment (17°C) (36). Glutamatergic stimulation of LPBel neurons with NMDA evokes increases in BAT thermogenesis, metabolism and heart rate that mimic skin cooling-evoked physiological responses (27). Such responses triggered by LPBel stimulation are blocked by antagonizing glutamate receptors in the MnPO (27), suggesting that cutaneous cool signaling to the POA is mediated by glutamatergic inputs from LPB neurons to the MnPO (Figure 2). The idea that the MnPO receives glutamatergic inputs for eliciting cold-defensive responses to environmental cooling is also supported by the finding that glutamatergic stimulation of MnPO neurons with NMDA evokes thermogenic, metabolic and tachycardic responses similar to those evoked during cold-defense (37).

Neurons in the dorsal subnucleus of the LPB (LPBd) (32), in particular those that directly project to the MnPO are activated in response to skin warming and provide the pathway that mediates heat-defensive physiological responses to skin warming (Figure 2) (38). Thus, both cool and warm cutaneous thermosensory signals that are transmitted to the POA by separate populations of LPB neurons are essential for eliciting rapid thermoregulatory responses to defend body temperature from a variety of thermal challenges.

3.2. Visceral and spinal thermal receptor afferents

In addition to cutaneous thermoreception, thermoreceptive mechanisms exist in body core structures including the brain, spinal cord and abdomen. The POA contains abundant neurons that sense changes in local brain temperature and these are described below. The afferent fibers from cold and warm receptors in the abdominal viscera are included among the splanchnic and vagus nerve afferent fibers and their responses to temperature changes are similar to those of cutaneous thermoreceptors (39, 40). However, little is known about the pathway through which abdominal thermal information is transmitted to the POA. Since a wide variety of visceral sensory information, related not only to temperature but also to gastric tension, satiety, taste, thirst and blood pressure, is transmitted to the LPB through the nucleus of the solitary tract (41, 42), it is possible that the LPB is a site at which thermal somatosensory signals are modified by those from the viscera to provide an integrated signal to the POA, a central site controlling a variety of homeostatic functions.

Temperature changes in the spinal cord can affect the activity of thermoregulatory neurons in the POA (43). Although this finding implies the existence of thermoreceptive mechanisms, such as thermosensation by spinal neurons, inherent to the spinal cord, TRP channels that are located in the central endings of primary somatosensory fibers in the spinal dorsal horn (7, 44) are also likely to sense spinal temperature and could underlie an integration of spinal thermal signals with cutaneous thermal signals at the spinal cord level. Since temperatures in deep body core structures including the brain are far less susceptible to changes in environmental temperature than are skin temperatures (2, 3, 45, 46), it seems likely that thermal information derived from these core body structures would enhance thermoregulatory responses principally evoked by cutaneous thermosensory signals only *in situ*ations of extreme thermal environments when the feedforward thermoregulatory responses driven by changes in skin temperature have proven inadequate to prevent a change in brain or body core (i.e., visceral organ) temperatures. Nonetheless, it remains of interest to understand how thermosensory signals from the skin and from the body core are integrated.

3.3. Temperature sensation within the Preoptic Area (POA)

Although neurons whose spontaneous discharge frequency is altered by changing the temperature of their local environment can be found in a variety of locations throughout the CNS, those in the POA and anterior hypothalamus have been most intensely studied because thermoregulatory responses, perhaps with the exception of certain thermoregulatory behaviors (47), are dependent on the integrity of POA neurons. Nakayama and colleagues made the first single-cell recording from thermosensitive neurons in the POA *in vivo* and found neurons with spontaneous discharge at thermoneutral temperatures that increased their discharge during local hypothalamic warming (warm-sensitive neurons) (48, 49). The POA contains warm-sensitive neurons whose tonic discharge is reduced by skin cooling and whose thermosensitivity to preoptic temperature is increased when the skin is cooled (50). Neurons sensitive to changes in bath temperature were found in subsequent recordings in the POA in hypothalamic slices and the majority of thermosensitive neurons were warm-sensitive neurons (51). In combination with these properties of warm-sensitive POA neurons, the findings that either skin cooling or direct cooling of the local environment of POA neurons evokes sympathetic thermogenesis in BAT as well as shivering thermogenesis (52, 53), and that transections immediately caudal to the POA elicit large increases in BAT temperature (54) are consistent with a model (Figure 2) in which warm-sensitive POA neurons integrate cutaneous and local thermal information and function as inhibitory projection neurons in the MPO that are tonically active at thermoneutral temperatures to suppress both shivering and non-shivering thermogenesis. Whether warm-sensitive neurons project axons outside of the POA remains unknown.

The neurophysiological mechanism underlying the thermosensitivity of warm-sensitive neurons in the POA continues to be investigated. The suggestion that a heat-induced membrane depolarization allows warm-sensitive neurons to reach their discharge threshold potential and then determines their discharge frequency (55) contrasts with the concept that a warming-dependent facilitation of the rate of rise of a depolarizing (pacemaker) prepotential in warm-sensitive neurons shortens the intervals between action potentials and thereby increases their firing rates (56). In the latter case, a transient, outward hyperpolarizing K^+ current (A-type potassium current) helps maintain a hyperpolarized membrane for a brief time after an action potential and the heat-induced increase in the inactivation rate of the A-type potassium current allows the prepotential to depolarize at a faster rate (56). Although TRPV4 channels have not been found in POA neuronal cell bodies, other ion channels that could contribute to the thermosensitivity of warm-sensitive neurons, such as hyperpolarization-activated cyclic nucleotide-gated channels and background potassium leak channels are localized in the cell bodies of many POA neurons, but are also distributed ubiquitously in the brain (13, 57). Identification of the molecule(s) responsible for the thermosensitivity of POA neurons await further investigation and the identification of specific anatomical markers for thermosensitive neurons would be a major discovery in this field.

3.4. Sensorimotor Integration of Thermoregulation in the POA

3.4.1. Role of POA neurons in the cold-defense responses to cutaneous thermal afferents—As described above, the POA contains neurons that receive skin cooling-driven, glutamatergic inputs from cool-responsive neurons in LPBel. That the POA subregion receiving thermosensory cold signals is predominantly in the MnPO is suggested by the findings that the projections from LPB neurons activated by skin cooling terminate mainly in a median part of the POA (27) and that glutamatergic stimulation of the MnPO with nanoinjections of NMDA evokes physiological responses mimicking cold-defensive responses, while the same stimulation of the MPO or LPO does not (Figure 7A–C) (37). Furthermore, inhibition of MnPO neurons completely blocks the activation of BAT

thermogenesis and the increases in metabolism and heart rate evoked by skin cooling (Figure 7D) (37), indicating that activation of MnPO neurons is an essential process in the central mechanism for eliciting cold-defensive responses to environmental cold challenges.

In thermal environments when cold-defensive responses are not needed (i.e., when the discharge of cutaneous cool receptors and cool-responsive LPBel neurons is low), inhibitory MPO projection neurons (potentially warm-sensitive neurons) are postulated to discharge at a sufficient rate to tonically inhibit their target neurons in caudal brain regions, such as the dorsomedial hypothalamus/dorsal hypothalamic area (DMH) and the rostral ventromedial medulla, including the rostral raphe pallidus nucleus (rRPa), both of which lead to stimulation of cold-defense responses when activated (see below).

That such a tonic inhibitory mechanism originating predominantly in the MPO subregion of the POA is a fundamental aspect of the control of thermoregulatory effectors for cold defense is based on the findings (a) that a coronal transection just caudal to the POA evokes BAT thermogenesis (54), (b) that lesion of the MPO, but not that of the ventral LPO, evokes hyperthermia by increasing metabolism and by stimulating shivering thermogenesis and heat conservation through cutaneous vasoconstriction (58) and (c) that inhibition of neurons in the MPO, but not those in the MnPO or LPO, increases body core temperature, EMG activity (shivering), metabolism and heart rate (59, 60).

Furthermore, BAT and shivering thermogenesis as well as increases in metabolism and heart rate that are evoked by skin cooling are blocked by antagonizing $GABA_A$ receptors in the MPO (1, 59). Thus, skin cooling-evoked responses are postulated to require a local circuit in the POA in which cutaneous cool signals that are received by MnPO neurons provide a GABA input to the inhibitory projection neurons in the MPO to reduce their tonic activity, resulting in disinhibition of neurons in caudal brain regions whose excitation leads to stimulation of thermoregulatory effectors for cold defense (Figure 2). Consistent with this hypothesis, increases in BAT thermogenesis, metabolism and heart rate that are evoked by stimulation of MnPO neurons are all reversed completely by antagonizing $GABA_A$ receptors in the MPO (37). The existence of GABAergic interneurons in the MnPO that innervate the MPO projection neurons, although not yet directly demonstrated, is supported by the anatomical observations (a) that some MnPO neurons innervate the MPO (61), (b) that the MnPO contains many GABAergic neurons (62, 63) and (c) that many neurons in the MnPO, rather than the MPO or LPO, are activated (express Fos protein) in response to reduced environmental temperature (32).

In addition to cool signals from the skin, direct cooling of the POA using a thermode can elicit BAT and shivering thermogenesis (52, 53). The findings that warm-sensitive neurons are the predominant thermosensitive neuron in the POA (51) and that the spontaneous activity of warm-sensitive POA neurons decreases during local cooling support the hypothesis that the thermogenic responses to direct cooling of the POA are mediated by an attenuation of the tonic activity of warm-sensitive MPO projection neurons, which, in turn, leads to a disinhibition of the caudal brain sites such as the DMH or the rRPa whose increased activity then facilitates thermogenesis (Figure 2). This mechanism would be consistent with the hypothesis that the firing rates of warm-sensitive projection neurons in the MPO, potentially a major contribution to the neurophysiological substrate underlying the thermoregulatory “balance point” (64), is determined by both thermosensory afferent signals from the skin and the effect of local brain temperature.

3.4.2. Febrile response to the pyrogenic mediator, prostaglandin (PG) E_2—The POA is known as a ‘fever center’ as well as a thermoregulatory center. Fever is a defended elevation in body temperature that plays a significant role in the acute phase reaction

stimulated by endogenous pyrogens released during infection. Fever is thought to provide an optimal hyperthermic environment for mounting host defenses against invading bacteria and viruses while reducing pathogen viability. PGE_2, which is synthesized in the brain vasculature and in peripheral tissues in response to immune signals (65-67), acts as a powerful endogenous pyrogenic mediator in the POA. Injection of PGE_1 into various subcortical brain regions indicated that the POA is the sole region that can sense the E-series of PGs to produce fever (68, 69) and this localization was refined when nanoinjections (1 ng/10 nl) of PGE_2 within the POA revealed the responsive sites to be in the MPO and MnPO (70).

In these POA subregions, the EP3 subtype of PGE receptor is localized on many neuronal somata and dendrites (Figure 8A) (71, 72). Although mRNA expression for the EP1 and EP4 subtypes is also detected in the POA (73), analyses of mice lacking each of the known PGE receptor subtypes showed that only EP3 receptor-deficient mice completely failed to show a febrile response to PGE_2, interleukin-1 beta, or endotoxin (Figure 8B) (74) and EP1 receptor-deficient mice showed a partial attenuation of endotoxin-induced fever (75). Furthermore, genetic deletion of the EP3 receptor specifically in neurons distributed in the MnPO and MPO suppressed most of the febrile response to PGE_2 or endotoxin (76). These lines of evidence indicate that the EP3 receptors in somatodendritic portions of POA neurons are the principal target site of PGE_2 for its pyrogenic action and that activation of these receptors by PGE_2 triggers neuronal processes leading to fever induction.

The neuronal population expressing EP3 receptors in the POA contains subpopulations that provide direct projections either to the DMH or to the rRPa (Figure 8C, D) (63, 77), but very few EP3 receptor-expressing POA neurons send collaterals to both sites (78). Furthermore, EP3 receptor-expressing POA neurons multisynaptically innervate BAT as evidenced by pseudorabies viral tracing (79). The majority of EP3-expressing POA neurons are GABAergic (63) and antagonizing $GABA_A$ receptors in the DMH or rRPa evokes fever-like responses including BAT thermogenesis and tachycardia (80-83). Coronal transection just caudal to the POA evokes BAT thermogenesis (54) and inhibition of POA neurons with a muscimol nanoinjection elicits hyperthermic, cardiovascular and neuroendocrine responses similar to those evoked by a PGE_2 nanoinjection into the same site (60). Binding of PGE_2 to EP3 receptors can inhibit neuronal activity by coupling to inhibitory GTP-binding proteins (84) and the tonic activity of most warm-sensitive neurons in the POA is inhibited by the E-series of PGs (85, 86). In addition, intracerebroventricular (icv) PGE_2 application reduces cAMP level in the POA and icv administration of an inhibitor of phosphodiesterase, a degradation enzyme for cAMP, blunts fever evoked by intra-POA PGE_2 application (87). Further, blockade of alpha-adrenergic receptors in the POA does not affect the increase in local PGE_2 in response to systemic lipopolysaccharide (LPS), but eliminates the early phase of LPS-induced fever (88). Together, these data suggest a model (Figure 2) in which EP3 receptor-expressing POA neurons, potentially the population of warm-sensitive POA neurons described above, normally maintain a tonic GABAergic inhibition of thermogenic neurons in the DMH or the rRPa and, during infection, PGE_2, produced locally and/or systemically, binds to their EP3 receptors and attenuates their tonic firing activity through reducing the intracellular cAMP level, which, in turn, leads to disinhibition of thermogenic neurons in caudal brain regions and activation of thermoregulatory effectors to increase heat production and reduce heat loss (77, 89).

The observation that separate populations of EP3 receptor-expressing POA neurons project to the DMH or to the rRPa (78) indicates that neurons in these two caudal brain sites that are involved in febrile responses are separately controlled by these populations of POA neurons. As illustrated schematically in Figure 2, the rRPa contains sympathetic premotor neurons that multi-synaptically innervate BAT and cutaneous blood vessels (89-91) and the rRPa

mediates both BAT thermogenesis and sympathetic constriction of skin blood vessels that are evoked by either PGE_2 application into the POA or by cold challenges (1, 63, 92, 93). The DMH mediates BAT thermogenic response to injection of PGE_2 into the POA or to skin cooling (1, 77, 94), but, in contrast, does not mediate cutaneous vasoconstriction stimulated by intra-POA PGE_2 or by cooling (93). These findings are synthesized in a circuit model (Figure 2) that includes a tonic inhibition from the separate populations of EP3 receptor-expressing POA neurons that project to the DMH and to the rRPa to control BAT thermogenesis and cutaneous vasoconstriction, respectively, as well as BAT sympathoexcitatory signals that are transmitted to BAT sympathetic premotor neurons in the rRPa from those DMH neurons that are disinhibited following pyrogenic or cold cutaneous stimuli.

4. Pathways to Thermoregulatory Effectors

4.1. Thermoregulatory behavior

Thermoregulatory behaviors in animals are stereotypical somatic motor acts directed primarily toward minimizing or optimizing heat transfer from the body to the environment. In rodents, such behaviors include postural changes (huddling in the cold or spreading the limbs in the heat), movement to a “preferred” environment (cold seeking in a hot environment) or spreading of saliva in a hot environment. Thermoregulatory behaviors can also generate heat in the cold, such as increased, non-shivering movement. Thermoregulatory behaviors are included in the category of “motivated” behaviors, expected to have important relationships to the limbic emotional and the dopaminergic reward systems in the brain. The emotional distress of being too hot or too cold is commonly a significant factor in motivating human behavior to seek or produce an ambient temperature that is more “comfortable” and the establishment of a homeostatic ambient temperature (i.e., within the thermoneutral zone) is accompanied by the reward (satisfaction) of being in a pleasant thermal environment.

Since the skin receives the first indication of a potential threat to brain and core temperature homeostasis, it is not surprising that thermoregulatory behavior in animals is triggered primarily by cutaneous thermal receptors. Although skin thermal receptors can also initiate human thermoregulatory behavior, by adulthood, humans have established such a rich repertoire of experience, learned responses and options for altering their environment that a variety of non-thermal cues (hearing a weather report or seeing a weather condition or the “predicted” effect of not programming the thermostat) play a significant role in initiating thermoregulatory behaviors. It is also not surprising that the complexity of the neural circuitry required for initiating, organizing, performing and controlling even simple thermoregulatory motor acts and the absence of a disorder of thermoregulatory behavior (although disrupted or absent thermoregulatory behaviors would be expected in a variety of movement disorders) seem to have combined to result in a minimal understanding of the organization of the neural pathways underlying thermoregulatory behavior (95, 96). Lesion studies suggest that, with the exception of limb extension in a hot environment (97), a wide range of thermoregulatory behaviors can occur in the absence of the neurons in the autonomic/shivering thermoregulatory sensorimotor integration area of the POA (47, 98-100). It would be of interest to distinguish whether the cutaneous sensory information required for initiation of thermoregulatory behavior is that conveyed along the spinothalamic thermal sensory pathway or that along the spinoparabrachial autonomic/shivering thermal afferent pathway (27).

4.2. Cutaneous vasoconstriction

Cutaneous blood flow brings metabolic heat to the body surface where it is available for transfer to the environment, particularly from regions of relatively hairless skin, such as the tail of the rat or mouse or the ear of the rabbit or elephant. Blood flow can be routed centrally, i.e., the low cutaneous blood flow during cold exposure, or peripherally, i.e., the high cutaneous blood flow during heat exposure, to control the level of heat loss to the environment as a contributing factor to the maintenance of body temperature and to the defense of an elevated body temperature during fever. In humans, increased CVC sympathetic outflow mediates the reduction in cutaneous blood flow in a cold environment, while a sympathetic vasodilator outflow is principally responsible for the increase in cutaneous blood flow in hyperthermic environments (101). Extensive studies on the mechanisms and neurochemical mediators of the human cutaneous vasodilation support an acetylcholine-mediated relaxation of vascular smooth muscle (102) that is dependent on local prostanoids, but not nitric oxide (103), while others support a role for norepinephrine acting via nitric oxide and for neuropeptide Y (104, 105). Some of these differences may arise from differences in the mechanisms mediating the cutaneous vasomotor response to local heating and that to whole-body warming. In contrast, rats do not appear to have a cholinergic sympathetic vasodilator system (106) and thus the thermoregulatory control of blood flow to the rat tail is primarily dependent on the level of CVC sympathetic outflow (107). In both humans and rodents, heating-evoked cutaneous vasodilation is accompanied by a marked increase in visceral (i.e., splanchnic and renal) vasoconstriction (108-110) which is independent of angiotensin (108). Whether this visceral vasoconstriction is a component of the POA-mediated thermoregulatory response to cutaneous and/or central warming or whether it is reflex-driven in response to the increase in blood flow to the skin is not known. In primates and in older rats, heating also induces a marked tachycardia (111, 112), mediated principally by vagal withdrawal. The elevated heart rate, which would contribute to the maintenance of arterial pressure in the face of a significant cutaneous vasodilation, may be a reflex response to a fall in central venous pressure.

Direct recordings of CVC postganglionic sympathetic nerve activity in several species, including humans, cats, rabbits and rats, indicate a moderate level of CVC sympathetic activity at normothermic body temperatures and this activity has a respiratory and a cardiac modulation, although the baroreceptor reflex influence on CVC discharge is generally described as weak. Sympathetic preganglionic neurons controlling the rat tail CVC outflow are located in T11-L2 spinal segments (89, 91, 113). Retrograde tracing studies employing PRV also indicate that premotor inputs to CVC spinal sympathetic networks arise from neurons in the ventromedial medulla, including the rRPa and the parapyramidal (PPy) region, some of which contain 5-HT and others that express the vesicular glutamate transporter 3 (VGLUT3) (89, 91, 114) and from the rostral ventrolateral medulla (RVLM), some of which are C1 neurons, as well as from the A5 noradrenergic cell group, lateral hypothalamic area and paraventricular hypothalamic area (91).

Functionally, activation of neurons in the rostral medullary raphe (or the RVLM) overcomes the inhibition of rat tail CVC elicited by warming the POA (115) (Figure 9D) and eliminates blood flow in the rabbit ear pinna (116) (Figure 9E). Inhibition of independent outputs from either the rRPa or the RVLM could prevent the cooling-evoked increases in rat tail CVC (117) (Figure 9B). Similarly, rostral raphe and PPy neuronal activity is important for the cold-evoked decrease in rabbit ear blood flow (118, 119) (Figure 9A, 9C) and the influence of these raphe-spinal pathways on CVC preganglionic neuronal discharge is dependent on an interaction of spinal serotonergic and glutamatergic inputs (120). In summary, these data indicate that thermoregulatory alterations in cutaneous blood flow mediated by CVC sympathetic outflow are determined by the activity of populations of CVC sympathetic

premotor neurons located primarily in the rostral raphe, PPy and RVLM (Figure 2) and involve release of at least glutamate and 5-HT within the CVC spinal sympathetic network.

Neurons within several brain regions influence cutaneous heat loss through their effects on the activity of CVC sympathetic premotor neurons. The following findings are consistent with the thermoregulatory control of CVC, similar to that of other thermal effectors, being mediated primarily by warm-sensitive neurons in the POA and with PGE_2-mediated inhibition of such warm-sensitive POA neurons contributing to the activation of CVC sympathetic premotor neurons during fever. L- glutamate injections into the POA, electrical stimulation of the POA and preoptic warming each elicit vasodilation in the rat paw and the rat tail (121) (Figure 10B). Injection of PGE_2 or GABA into the POA increases CVC outflow (93, 122, 123) (Figure 10C). Transection of the neuraxis immediately caudal to the POA increases CVC outflow (93) (Figure 10A). However, since subsequent transections caudal to the DMH, but rostral to the rRPa, did not significantly reduce CVC outflow and injection of muscimol into DMH did not affect either spontaneous, thermally-sensitive or PGE_2-into-POA-evoked CVC neuronal discharge (93) (Figure 10C), indicating that activation of DMH neurons is not required for thermoregulatory or febrile activation of CVC sympathetic outflow. In combination with these physiological results, the demonstration that POA contains GABAergic neurons that express EP3 receptors for PGE_2 and project to the rRPa (63) and that these are distinct from those EP3-expressing POA neurons that project to the DMH (78) supports a model (Figure 2) in which warm-sensitive POA neurons provide a direct GABAergic input to the rRPa to regulate the discharge of CVC sympathetic premotor neurons. As a corollary, the principal source of the excitatory drive to CVC sympathetic premotor neurons in the rRPa would appear to arise from neurons caudal to the DMH, likely in the brainstem (93).

Nonetheless, these data derived from transection experiments could also be consistent with a PGE_2-evoked and cooling-driven increase in CVC neuronal discharge arising from inhibition of POA neurons that excite neurons, located between the POA and the rRPa, which are inhibitory to CVC sympathetic premotor neurons in the rRPa. Several observations suggest that a population of such neurons may exist in the rostral periaqueductal gray (PAG). Neurons in the rostral PAG receive input from neurons in the POA that are activated (increased Fos expression) by environmental warming, but not cooling (124), environmental warming also increases Fos expression in the rostral PAG (125), and chemical excitation of neurons within the rostral PAG increases both tail temperature and blood flow (126), presumably due to inhibition of CVC sympathetic outflow. The roles of direct and/or indirect pathways between CVC-regulating, warm-sensitive POA neurons and CVC sympathetic premotor neurons in the ventromedial and ventrolateral medulla remain to be elucidated.

4.3. Thermogenesis in brown adipose tissue (BAT)

In response to a cold environment, to a fall in core body temperature or to the presence of pyrogenic cytokines, CNS thermoregulatory networks can stimulate thermogenesis primarily in three tissues: brown adipose tissue (BAT), heart and skeletal muscle (shivering). In contrast to the ancillary nature of thermogenic shivering in skeletal muscles that are normally used to produce movement and posture, non-shivering or adaptive thermogenesis in BAT is the specific metabolic function of this tissue and is accomplished by the heat generating capacity of a significant proton leak across the extensive mitochondrial membranes of the brown adipocytes facilitated by the high expression of uncoupling protein-1 (UCP1) in BAT mitochondrial membranes (127). The level of BAT SNA and norepinephrine release and beta3-adrenergic receptor binding to brown adipocytes determine the level of thermogenesis in BAT by regulating both the activity of lipases providing the immediate fuel molecules for BAT mitochondria and the level of expression of BAT

mitochondrial UCP1 (127). BAT is an important thermoregulatory effector in rodents and other small mammals (128), but also contributes to cold defense in both infant and adult humans (129-132).

4.3.1. The rostral ventromedial medulla contains BAT sympathetic premotor neurons—Within the hierarchical organization of the central network controlling BAT thermogenesis, medullary neurons play a key role as sympathetic premotor neurons – providing the essential excitatory input to drive spinal motor neuron activity. Transynaptic retrograde transport studies involving viral tracer injections into the interscapular BAT pad indicated that BAT sympathetic premotor neurons are located in the rostral ventromedial medulla, centered in the rRPa and extending into nearby raphe magnus nucleus and over the pyramids to the PPy area (79, 90, 133, 134). Other brainstem regions with spinally-projecting neurons also became infected following virus injections into BAT and these, as well as the rRPa area, overlapped regions previously shown with conventional retrograde tracing to provide inputs to the intermediolateral (IML) cell column containing sympathetic preganglionic neurons (SPNs). A comparison of the localization of Fos induced by cold exposure, which activates BAT thermogenesis, with the locations of virally-labeled neurons following inoculations of BAT, provided function-based evidence that the rRPa and the ventromedial parvicellular subdivision of the paraventricular hypothalamic nucleus were the two potential premotor populations having a principal role in mediating the descending regulation of the spinal sympathetic circuit controlling BAT thermogenesis (90).

Anatomical studies have indicated that rostral ventromedial medullary neurons, including those in the rRPa and surrounding the pyramids, that project to the IML to influence the discharge of BAT SPNs contain one or more of the following markers: (a) the vesicular glutamate transporter 3 (VGLUT3), potentially indicative of glutamatergic neurons (89, 135); (b) serotonin (5-HT) or tryptophan hydroxylase, a synthetic enzyme for 5-HT (89, 90, 135) and (c) glutamic acid decarboxylase-67 (GAD-67), a marker for GABAergic neurons (135). That VGLUT3-expressing and serotonin-containing neurons in the rostral ventromedial medulla are functionally-related to the control of cold defense effectors is suggested by the findings that the majority of VGLUT3-containing neurons in the rRPa express Fos in response to cold exposure or icv PGE_2 (89) and that physiologically-identified, putative serotonergic neurons in the rRPa increase their firing rate in response to cold (136) (137) or PGE_2 administration (137).

Several lines of physiological evidence also support the existence of BAT sympathetic premotor neurons in the rRPa region of the rostral ventromedial medulla. Injections of agonists for either NMDA or non-NMDA glutamate receptors into the rRPa evoke intense activations of BAT SNA (Figure 11A) (92). Thus, there are neurons in the rRPa that express NMDA and non-NMDA subtypes of glutamate receptors and that are capable of increasing the sympathetic drive to BAT. Disinhibition of neurons in the restricted region of the rRPa by antagonizing local $GABA_A$ receptors also increases BAT SNA and BAT thermogenesis (Figure 11B) (82, 138) and anatomical evidence indicates that VGLUT3-expressing neurons in the rRPa receive numerous GABA inputs (139). These data indicate that in an anesthetized rat with a normothermic body temperature, the rRPa neurons controlling BAT SNA receive a dominant, tonically-active, GABAergic inhibition and that relief of this inhibition allows a potent increase in the rRPa neuronal discharge, indicating, in turn, the existence of an ongoing, or bicuculline-activated, excitatory input to these rRPa neurons or of a complement of membrane ion channels supporting intrinsic activity in these rRPa neurons. The physiological roles and the sources of the tonically-active GABAergic inhibitory input(s) and of the ongoing excitatory drive(s) to BAT sympathetic premotor neurons in the rRPa have yet to be identified. Relevant to the inhibitory control of BAT sympathetic premotor neurons in rRPa, however, is the demonstration that the POA contains

GABAergic neurons that project to the rRPa (63) and that the inhibition of BAT SNA elicited by activation of neurons in the PVH is prevented by blockade of $GABA_A$ receptors in the rRPa (140). In addition, ponto-medullary, but not hypothalamo-pontine transections of the neuraxis (141-143) or injection of procaine into the region of the retrorubral field (141, 144) elicits a large increase in BAT thermogenesis indicating the existence of pontine neurons that provide a potent, tonic inhibition of BAT SNA, likely through their effects on the discharge of BAT sympathetic premotor neurons in the rRPa.

Conversely, inhibition of the activity in neurons in the rRPa reverses the increases in BAT SNA and BAT thermogenesis and heart rate elicited by all thermogenic stimuli. The cold defense stimulus of skin cooling elicits responses (Figure 3) including increases in BAT SNA, BAT temperature (a measure of thermogenesis), expired CO_2 (reflecting a stimulation of oxidative metabolism such as in BAT and the heart) and heart rate. Inhibition of local neurons in the rRPa with nanoinjection of glycine or muscimol produces a rapid and complete reversal of the skin cooling-evoked increase in BAT SNA and an immediate waning of the accompanying metabolic and cardiac responses, despite the sustained reduction in skin temperature (1). Inhibition of rostral ventromedial medullary neurons produces dramatic falls in body temperature in the awake rat (145), consistent with the possibility that, in a sub-thermoneutral, room temperature environment, the activity of BAT sympathetic premotor neurons in the rRPa and BAT thermogenesis are actively contributing to the maintenance of core body temperature. The effects of inhibiting neurons in the rRPa lead to the conclusion that the activity of BAT sympathetic premotor neurons in the rRPa provides the supraspinal excitatory drive to spinal sympathetic circuits that is both necessary and sufficient for the thermogenic responses to thermoregulatory and febrile stimuli and to a variety of neurochemical mediators that increase body temperature. For example, other thermogenic stimuli, in addition to cold defense, whose stimulated BAT thermogenesis and tachycardia are reversed or prevented by inhibition of neural activity in the rRPa region of the rostral ventromedial medulla include the pyrogenic mediator PGE_2 (Figure 11C) (63, 92, 146, 147); disinhibition of neurons in the DMH (80) or in the lateral hypothalamus (148); activation of central mu-opioid receptors (149), central melanocortin receptors (150) or preoptic CRF receptors(151) and systemic administration of the adipose tissue hormone, leptin (152).

Blockade of glutamate receptors in the rRPa is also effective in reversing cold-evoked and pyrogen-mediated stimulations of BAT thermogenesis and of heart rate (1, 92), as well as those elicited by disinhibition of neurons in the DMH (153). These findings implicate glutamate receptor activation in the tonic excitation of BAT and cardiac sympathetic premotor neurons in the rRPa that is revealed by reducing their tonic inhibitory inputs and in their excitation from rostral inputs such as neurons in the DMH (Figure 2). Together with the anatomical evidence for direct projections from the rRPa region to IML neurons, including the SPNs, that control BAT thermogenesis, these functional data indicate that the rRPa region contains the principal population of BAT sympathetic premotor neurons providing the final common bulbospinal pathway for the sympathoexcitatory drive to the spinal network controlling BAT SNA (Figure 2).

The brainstem also contains the pathways mediating the inhibition of BAT thermogenesis in response to arterial hypoxia, a reflex to restrict oxygen consumption in the face of reduced oxygen availability or compromised oxygen diffusion and transport in the blood. Systemic hypoxia or bolus systemic injections of sodium cyanide produce a prompt and complete reversal of the BAT SNA activations evoked by hypothermia and by PGE_2 in the POA and this response to hypoxia is eliminated by section of the carotid sinus nerves or by inhibition of second-order arterial chemoreceptor sensory neurons in the commissural region of the nucleus of the tractus solitarius (NTS) (154). Interestingly, hypoxia also eliminates the BAT

SNA activation resulting from bicuculline nanoinjection into the rRPa, suggesting that activation of a GABAergic input to BAT sympathetic premotor neurons in the rRPa is unlikely to mediate the hypoxic inhibition of BAT thermogenesis. Indirect evidence points to a possible role for a spinal inhibitory mechanism. Similar to arterial hypoxia, disinhibition of neurons in the RVLM reduces the BAT SNA activation following bicuculline into the rRPa (81) and both anatomical (155) and electrophysiological (156) studies support the existence of a bulbospinal inhibitory pathway to SPNs from the RVLM. The pathway between the NTS and the BAT SPNs mediating the hypoxic inhibition of BAT metabolism remains to be elucidated.

4.3.2. Dorsomedial hypothalamus neurons drive BAT thermogenesis—The observation that transection of the neuraxis immediately caudal to the POA (approximately 1.3-1.8 mm caudal to bregma) increases BAT SNA and BAT thermogenesis (54) suggests that the POA projection neurons are inhibitory to BAT thermogenesis. In contrast, transections made in the midbrain, just caudal to the hypothalamus (approximately 4-4.5 mm caudal to bregma), do not increase basal levels of BAT thermogenesis in normothermic animals (142) and, in fact, reverse PGE_2-evoked increases in BAT SNA and thermogenesis (157, 158). The findings suggest that, although a long inhibitory pathway from the POA neurons to BAT sympathetic premotor neurons in the rRPa may contribute to the regulation of BAT thermogenesis, a source of excitatory drive to BAT thermogenesis must exist between the POA and the rostral midbrain. Several areas of the hypothalamus caudal to the POA, including the PVH, the ventromedial hypothalamus, the posterior hypothalamus and the DMH have been implicated in the control of BAT thermogenesis, however the anatomical specificity of the observations from studies has been called into question (159) due to the large injection volumes used, as well as the lack of appropriate anatomical control injections, or the use of electrical stimulation, electrolytic lesion or other methodological approaches that affect not only cell bodies but also fibers of passage.

There is strong evidence supporting a role for DMH neurons in the control of BAT thermogenesis. Administration of endotoxin or cold exposure increases the expression of Fos in neurons of the DMH (90, 125, 160, 161). Blockade of $GABA_A$ receptors in the DMH increases BAT SNA (80) and BAT thermogenesis and heart rate (Figure 12A) (83), suggesting a tonic GABAergic inhibitory input to BAT thermogenic neurons within the DMH (Figure 2). This tonic GABAergic input to neurons within the DMH may originate in the POA as evidenced by the observation that POA-derived GABAergic axon swellings make close appositions with DMH neurons, including those that project to the rRPa (Figure 12B)(77). In addition, inhibition of neurons in the DMH blocks febrile (Figure 12D, 12E) (77, 94, 157, 162) and cold-evoked (1) excitation of BAT SNA and thermogenesis, as well as shivering thermogenesis (163). Blockade of ionotropic glutamate receptors within the DMH also blocks the increase in BAT SNA and thermogenesis evoked by PGE_2 within the POA (Figure 12C) (94), indicating that a glutamatergic input to neurons within the DMH is essential for these febrile responses, though the source of this input has yet to be determined. Taken together these data support a model (Figure 2) in which a GABAergic inhibitory input from warm-sensitive neurons in the POA to BAT sympathoexcitatory neurons in the DMH is reduced by skin or core cooling or by PGE_2 acting on EP3 receptors within the POA, thereby allowing an increased discharge of DMH neurons that promote BAT thermogenesis.

Since neurons in the DMH do not project directly to sympathetic preganglionic neurons, these neurons likely contribute to BAT thermogenic sympathetic outflow by influencing the activity of the BAT sympathetic premotor neurons in the rRPa. The DMH contains neurons that project directly to the rRPa (77, 164-166), some of which express Fos in response to thermogenic stimuli such as cold (166), endotoxin administration or stress (160) and some

of which receive GABAergic putative synapses from neurons in the MPO (77). Indeed, glutamate receptor activation within the rRPa is necessary for the increase in BAT SNA and thermogenesis evoked by disinhibition of neurons within the DMH (153).

Located in close proximity to the DMH and containing sympathetic premotor neurons, the paraventricular hypothalamic nucleus (PVH) has often been considered as a significant source of supraspinal drive in the regulation of BAT thermogenesis and energy expenditure. However, increasing the activity of PVH neurons with local nanoinjections of NMDA or bicuculline failed to increase BAT SNA or BAT temperature, but rather completely reversed the activations of BAT thermogenesis evoked by body cooling or by nanoinjections of PGE_2 into the MPO or of bicuculline into the DMH (140). In contrast, activation of PVH neurons was without effect on the BAT SNA increases evoked by injection of bicuculline in to the rRPa (140). Thus, activation of a population of neurons in the PVH potently inhibits BAT SNA, likely via activation of a GABAergic input to BAT sympathetic premotor neurons in the rRPa. Although the extensive connectivity between PVH and DMH (167) and the ability of bicuculline injections into the DMH to elicit Fos expression in the PVH (168) would support an interaction between DMH and PVH in the control of thermoregulatory responses, potentially through alterations of thyroid regulating hormone or thyroid stimulating hormone secretion, such mechanisms remain to be investigated.

4.3.3. Spinal sympathetic mechanisms controlling BAT thermogenesis and CVC—The discharges of BAT and CVC sympathetic preganglionic neurons (SPN) that determine the levels and the rhythmic bursting characteristics of BAT and CVC SNAs and, in turn, BAT thermogenesis and cutaneous heat loss, are governed by their supraspinal and segmental inputs, some of which are mediated through a network of spinal interneurons that influence BAT and CVC SPN excitabilities. A significant fraction of the BAT and CVC sympathetic premotor neurons in the rRPa are glutamatergic and/or serotonergic neurons. Consistent with these findings from viral retrograde tracing studies, 5-hydroxytryptamine (5-HT)-containing (169, 170) and VGLUT3-containing terminals synapse on SPNs (135, 171) or make close appositions with SPN dendrites (89, 171). SPNs contain ionotropic glutamate receptors (172, 173) and several subtypes of 5-HT receptors are located within the IML (174-178), although the specific neuronal subtypes expressing these receptors have not been determined. Viral inoculations of sympathetically-innervated tissues (179), including interscapular BAT (90), consistently label a population of spinal interneurons in the vicinity of the IML. Spinal GABAergic interneurons would appear to be among this population since they influence the discharge of SPNs (180). That such interneurons could receive inputs from the BAT premotor area in the rostral ventromedial medulla is suggested by the demonstration that VGLUT3- and GAD-67-containing terminals synapse on GABAergic neurons in the IML (135).

Nanoinjection of glutamate or NMDA into the upper thoracic IML activates BAT SNA and BAT thermogenesis (Figure 13C) (89, 181) and blockade of glutamate receptors in the upper thoracic IML suppresses the increase in BAT thermogenesis evoked by bicuculline injection into rRPa (Figure 13A) (89). Axon terminals of rRPa neurons containing VGLUT3, a marker of glutamate neurotransmission, make close appositions to sympathetic preganglionic neurons in the IML (Figure 13B) (89). These findings support an essential role for spinal glutamate receptor activation in the IML in the control of BAT thermogenesis.

Serotonin injected into the T4 IML elicits a delayed activation of BAT SNA and BAT thermogenesis (Figure 13C) (181). Significantly, IML injection of 5-HT potentiates the BAT SNA response to subsequent NMDA injections into the IML (Figure 13C, 13D) (181). This potentiation by serotonin of glutamate receptor-mediated BAT activation even allows

subthreshold doses of NMDA into the IML to increase BAT SNA (181). Interestingly, some putatively inhibitory inputs to the IML synapse on GABAergic dendrites (135) and 5-HT terminals also form close appositions with GABAergic interneurons in the central autonomic area (182). Such anatomical data in conjunction with the inhibitory signal transduction mechanisms for 5-HT_{1A} receptors are consistent with the possibility that the serotonergic potentiation of glutamate receptor-mediated increases in BAT SNA, which is blocked by antagonists of 5-HT_{1A}/5-HT_7 receptors (Figure 13D) (183), could occur via 5-HT_{1A} receptor-mediated inhibition of GABA inputs to BAT SPNs (183). An interaction of serotonin and glutamate neurotransmission in the IML is also significant in determining the CVC outflow, although the relevant serotonergic receptor is of the 5-HT_{2A} subtype (120). This is illustrated in Figure 13E, in which spinal application of a 5-HT_{2A} receptor antagonist markedly reduced the CVC sympathetic response to rRPa stimulation and subsequent blockade of spinal glutamate receptors eliminated the residual CVC activation following rRPa stimulation (120).

That the serotonergic innervation provided by raphe neurons to IML neurons, including SPNs, plays a significant role in eliciting physiologically-relevant levels of BAT thermogenesis in cold defense is suggested by the increase in spinal 5-HT turnover (an index of 5-HT release) during cold exposure (184, 185) and by the effectiveness of 5-HT receptor antagonism in the T3-T6 IML in preventing the cooling-evoked stimulation of interscapular BAT SNA (186). While the mechanisms of the interaction between glutamatergic and serotonergic neurotransmission in the IML remain to be elucidated, their interdependence in normal cold defense responses is supported by the recent finding that mice that lack almost all central serotonergic neurons exhibit intact BAT thermogenesis in the environment of 10°C, but show blunted BAT thermogenesis in 7°C (187). The current data clearly demonstrate that the spinal cord is much more than a simple relay circuit driving BAT SNA and thermogenesis, however, the details of the spinal neurocircuitry involved in the rhythmicities in BAT sympathetic outflow and in the regulation of this thermogenic effector await future studies.

4.4. Cardiac thermogenesis

The central thermoregulatory network stimulates BAT and shivering thermogenesis in response to environmental cold challenges, to a fall in core body temperature or to the presence of pyrogenic cytokines and a sympathetically-mediated increase in heart rate occurs during each of these conditions. Since cardiac thermogenesis arises primarily from the inefficiency of energy utilization in cross-bridge cycling and calcium ion sequestration, increases in heart rate will elicit increased cardiac thermogenesis. In the rat, the increases in heart rate in response to cold or during fever (Figures 11, 12) parallel the activation of sympathetic BAT thermogenesis (1, 77, 146) and of shivering. Indeed, the tachycardia elicited under these conditions could contribute to the maintenance of cardiac output during the significant dilation of BAT blood vessels and would thus serve to distribute BAT- and shivering-generated heat more efficiently and to increase the availability of energy substrates to activated BAT and skeletal muscle.

The tachycardia accompanying thermoregulatory responses to activation of cold thermal afferents and to other stimuli expected to reduce the activity of warm-sensitive POA neurons, is driven in a feedforward manner rather than by activation of cardiovascular reflexes. The neural network mediating thermoregulatory and febrile increases in heart rate appears to parallel that described above for activating BAT thermogenesis (Figure 2). Thus, briefly, cutaneous, and possibly visceral, cold afferent signals to dorsal horn neurons are relayed via neurons in the LPBel (Figure 6) (27) to the MnPO (Figure 7) (37) where they activate GABAergic interneurons that inhibit warm-sensitive MPO neurons that project to inhibit sympathoexcitatory DMH neurons (Figure 12) that project to cardiac premotor

neurons in the rRPa (80, 138) providing excitatory drive to cardiac sympathetic preganglionic neurons. Viral retrograde tracing to identify central sympathetic pathways controlling the heart (188, 189) revealed infected neurons in the rostral ventrolateral and ventromedial (including rRPa) medulla and either disinhibition (with bicuculline) or activation (with glutamate receptor agonists) of neurons in the restricted region of the rRPa increases heart rate (92, 138, 145).

4.5. Shivering thermogenesis

Thermogenesis occurs to a greater or lesser extent in all tissues that use the energy in chemical bonds such as in ATP, since heat generation is a by-product of the inefficiency of mitochondrial ATP production and of ATP utilization. During the rapid, repeated skeletal muscle contractions of shivering, thermogenesis arises primarily from the inefficiency of energy utilization in cross-bridge cycling and calcium ion sequestration, and, to a lesser degree from mitochondrial membrane proton leak in the course of ATP production from fuel substrate oxidation. Shivering thermogenesis is the last cold-defense mechanism to be activated as its thermal threshold is lower than that for either cutaneous vasoconstriction or BAT thermogenesis, presumably reflecting the existence of a distinct population of preoptic warm-sensitive neurons that regulate shivering, but also in keeping with the high metabolic energy cost of shivering and the relative vulnerability of an animal during shivering as escape behavior would be more slowly mobilized. Although heat generation through shivering has long been recognized as an essential mechanism in cold defense and in the elevated body temperature in fever in both experimental animals and in humans (190, 191), the central neural mechanisms generating shivering and the thermoregulatory circuits controlling shivering are almost entirely unknown.

The muscle contractions of shivering result from rhythmic bursts of activity in the alpha-motoneurons innervating skeletal muscle fibers. The increased IA-afferent input in response to the simultaneous activation of gamma-motoneurons could play a role in determining alpha-motoneuron responsiveness and, in turn, in setting the shivering threshold and intensity (192) and frequency. The central neural network generating the cold-evoked bursts of alpha-motoneuron activity (Figure 2) is not well understood, but includes transmission of cutaneous cold afferent signals through the lateral parabrachial nucleus (27), integration of thermoregulatory signals in the preoptic area (121) and activation of fusimotor neurons that is dependent on neurons in the rostral ventromedial medulla (193, 194). A potential role for neurons in the rostral ventromedial medulla, including the rRPa, in shivering thermogenesis is suggested by the intense viral retrograde labeling in this region following inoculation into skeletal muscle (195). Increases in muscle EMG activity and shivering are also elicited by disinhibition of rostral ventromedial medullary neurons (196).

4.6. Evaporative cooling

Evaporation increases heat loss from the body surface and is a critical thermoregulatory strategy in ambient temperatures exceeding core temperature. With their large surface area of hairless skin, sweating is an efficient evaporative cooling mechanism in humans. In contrast, rodents rely on increased salivary secretion and saliva spreading for evaporative cooling, while in other hairy animals, evaporation occurs from the respiratory tract during panting. The pathway mediating the increased sympathetic cholinergic outflow to sweat glands in a warm environment is unknown, but presumably includes the cutaneous warm thermal receptor afferent pathway involving the dorsal horn and the LPBd (Figure 2) and control of sweat gland-specific, spinal sympathetic preganglionic neurons by their supraspinal sympathetic premotor neurons. Saliva spreading is a more complex evaporative cooling behavior, involving both an autonomically-mediated increase in the secretion of protein-poor saliva and the somatically-mediated behavior of spreading the saliva on the

body surface. Little is known about the pathways for the thermoregulatory increase in salivary secretion, although it is likely to include the cutaneous warm thermal receptor afferent pathway involving the dorsal horn and the LPBd (Figure 2) and salivatory premotor neurons in the PVH (197, 198). The loss of body water during evaporative cooling, which usually occurs in parallel with a large increase in cutaneous blood flow, has a significant effect on blood volume and plasma osmolarity and emphasizes the strong interaction between thermoregulatory and cardiovascular and osmotic control circuits during prolonged heat exposure, dehydration and exercise (199). Increased osmolarity has central effects on thermoregulatory responses to heating, eliciting an increase the internal temperature threshold for cutaneous vasodilation (200) and evaporative heat loss (201) and a reduction in sweating (202) and respiratory stimulation (203). As with saliva spreading, evaporative heat loss through panting has an autonomic component to increase fluid secretion in the respiratory tract and a somatic component to increase respiratory air flow across the upper respiratory tract. The pathways mediating thermoregulatory influences on respiratory activity remain to be investigated.

5. Summary and Perspective

The principal thermoregulatory effectors are the cutaneous blood vessels for control of heat loss, the brown adipose tissue and skeletal muscle for thermogenesis and various, species-dependent mechanisms such as sweating, panting and saliva spreading for evaporative heat loss. The activation of these effectors is regulated by parallel but distinct, effector-specific, core efferent pathways within the central nervous system (Figure 2) that are strongly influenced by shared cutaneous thermal afferent signals. Cutaneous (and likely visceral) thermal sensory information is integrated, in an as yet unknown manner, at synapses in the spinal and trigeminal dorsal horns and subsequently within the lateral parabrachial nucleus, where cool and warm afferent signals are processed within anatomically distinct regions with projections to the POA. Within the POA, different, effector-specific populations of temperature-sensitive neurons, principally warm-sensitive neurons, provide the substrate not only for local (sometimes called “core”) temperature to influence the activation of each type of thermoregulatory effector, but also for thermal sensory inputs, arriving via the LPB projection neurons, to be integrated with these local temperature influences to control the activation of each type of thermoregulatory effector. Different thermal sensitivities among populations of temperature-sensitive POA neurons may allow differential responsiveness of different effectors to changes in cutaneous vs. brain temperatures. It remains unknown whether and how these differing thermal sensitivities may be maintained under a variety of perturbing alterations in the external or internal thermal and neurochemical environments.

The core efferent pathway for thermoregulatory activation of BAT thermogenesis and heart rate involves a tonically-active inhibitory input from the POA to sympathoexcitatory neurons in the DMH, which project to sympathetic premotor neurons in the rRPa, which, in turn, provide the excitatory drive to sympathetic preganglionic neurons in the thoracic spinal cord that is transmitted via sympathetic ganglion cells to brown adipocytes and to cardiac pacemaker cells. From preliminary results, a similarly-organized core effector pathway is expected for the control of shivering thermogenesis, but considerable additional experimentation is necessary to define this circuit. The core efferent pathway for CVC also involves a tonically-active inhibition emanating from the POA. However, these POA projection neurons send axons to the rRPa where they influence the discharge of CVC sympathetic premotor neurons and, consequently, the level of excitation to CVC sympathetic preganglionic neurons in the thoracic and lumbar spinal cord that drive the release of transmitter from CVC sympathetic ganglion cells to elicit cutaneous vasoconstriction. Although they are expected to involve warm-sensitive neurons in the POA, the further details of the core efferent pathways for the thermoregulatory effectors mediating

evaporative heat loss remain to be elucidated. Identifying key structures within each of these core thermoregulatory pathways provides a framework for increased understanding of the sites and mechanisms through which body temperature regulation is influenced by a wide variety of neurotransmitters, peptides, cytokines, and genetic, nutritional and perinatal (204) manipulations.

Within these core efferent pathways controlling thermoregulatory effectors, the sources and mechanisms responsible for the tonic discharge of key neuronal populations and for the synchronous bursting that characterizes the sympathetic outflows are unknown. Similarly puzzling is the neural basis for the tremor-like discharge in somatic motor neurons during shivering. The sites and mechanisms underlying the critical integration of thermogenesis with other homeostatic systems regulating oxygen and fuel substrate availability, body water, salt appetite and energy balance remain to be investigated. The projection neurons connecting important regions in the central thermogenic pathways have yet to be characterized, including the inhibitory output neurons of the POA, the excitatory output neurons in the DMH and the sympathetic premotor neurons in the rRPa and PPy regions of the medulla. The marked differences in temperature response thresholds among thermoregulatory effectors suggests that significant differences should be expected among the populations of warm-sensitive POA projection neurons that control these effectors. We expect that the model circuit for central thermoregulatory control that we have proposed (Figure 2) will be embellished and corrected as we gain a further understanding of the functional organization of central thermoregulation.

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Abbreviations

5-HT	serotonin
BAT	brown adipose tissue
CVC	cutaneous vasoconstriction
DMH	dorsomedial hypothalamus
IML	intermediolateral nucleus
LPB	lateral parabrachial nucleus

LPBd	dorsal subnucleus of the LPB
LPBel	external lateral subnucleus of the LPB
LPO	lateral preoptic area
LPS	lipopolysaccharide
MnPO	median preoptic nucleus
MPO	medial preoptic area
NMDA	N-methyl D-aspartate
PAG	periaqueductal gray
PG	prostaglandin
POA	preoptic area
PPy	parapyramidal area
rRPa	rostral raphe pallidus
RVLM	rostral ventrolateral medulla
SNA	sympathetic nerve activity
SPN	sympathetic preganglionic neurons
TRP	transient receptor potential
VGLUT3	vesicular glutamate transporter 3

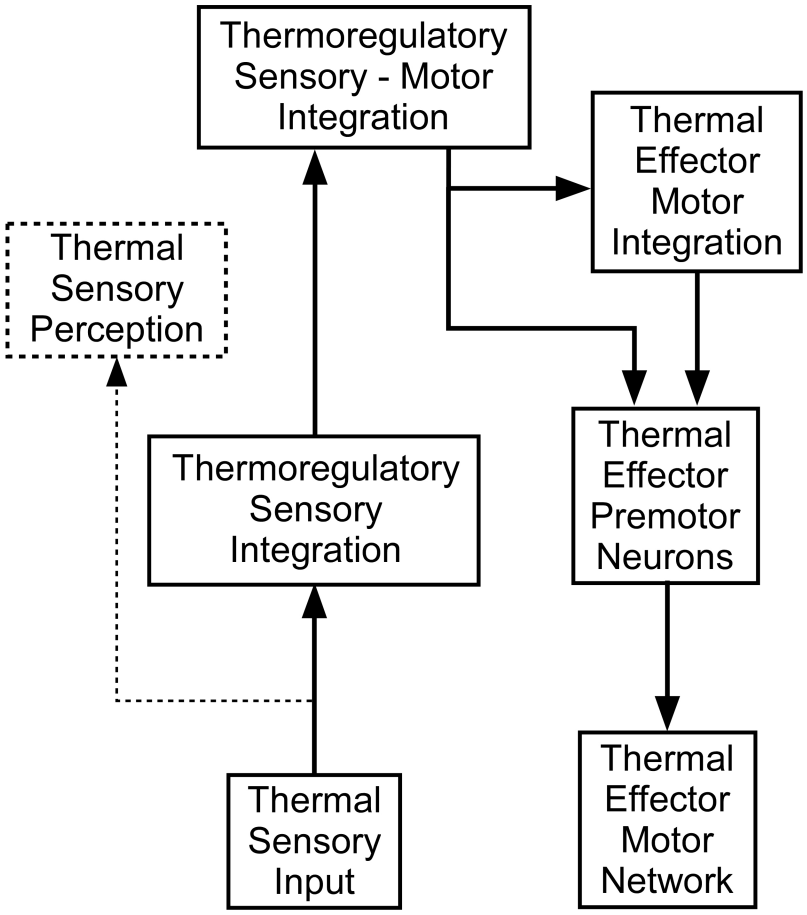

Figure 1.
Block diagram of the functional components of a model for the central neural circuit providing cutaneous thermal afferent and thermally-sensitive neuronal control of thermoregulatory effectors. Thermal sensory signals are conveyed via separate pathways to sites involved in conscious perception and localization of thermal sensation and in the integration of thermal sensory information with other sensory inputs that influence thermoregulatory responses. Thermoregulatory sensory-motor integration: integrated cutaneous and visceral thermal sensory signals influence the discharge of effector-specific populations of hypothalamic, thermally-sensitive neurons with projections to motor regions controlling thermal effectors. Thermal effector motor integration: thermally-modulated inputs are integrated with non-thermal signals that contribute to the regulation of the activity of neurons that provide an excitatory drive to the premotor neurons controlling thermal effectors. Thermal effector premotor neurons: supraspinal neurons with descending inputs to spinal motor networks controlling thermal effectors.

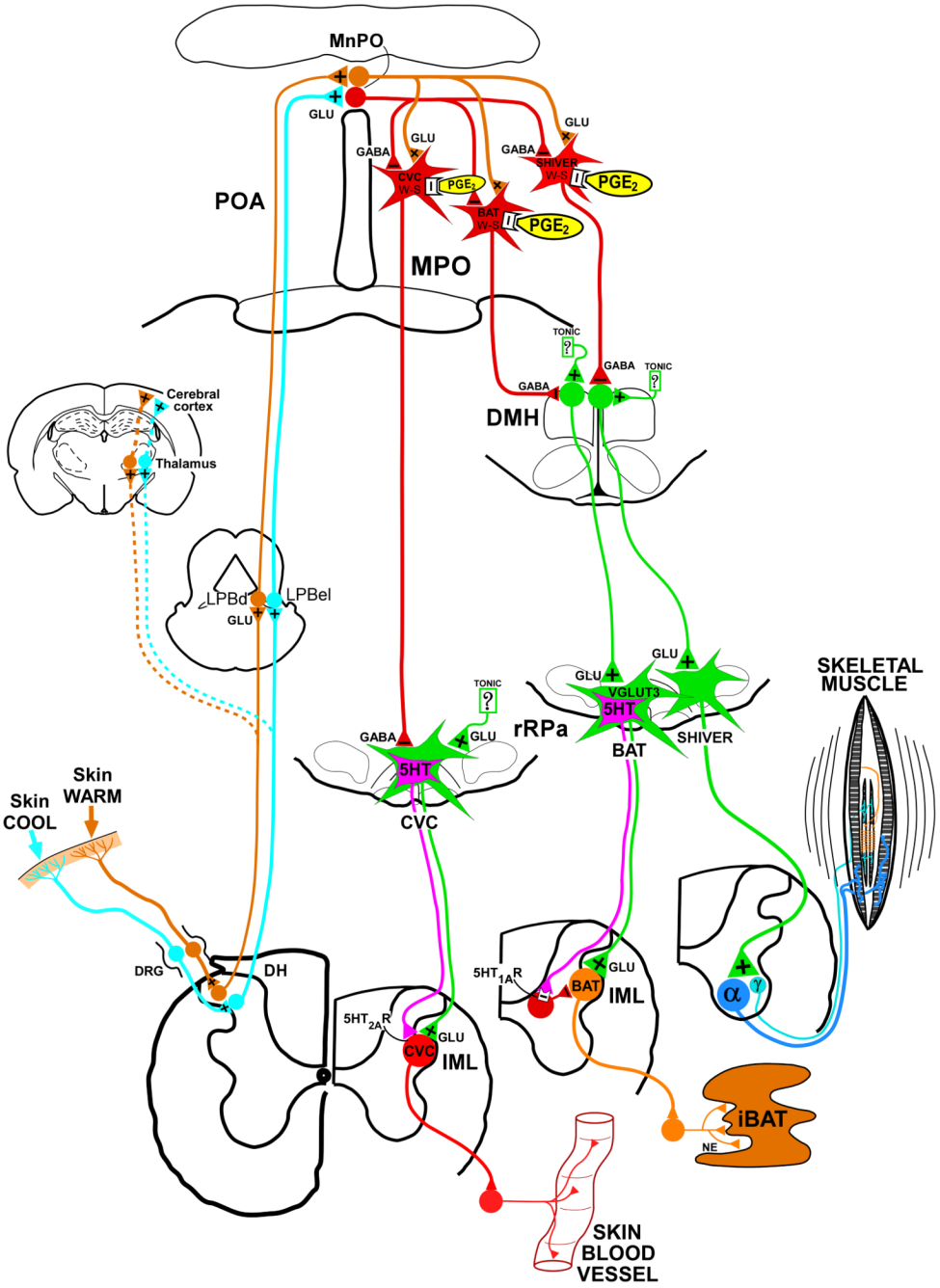

Figure 2.

Functional neuroanatomical and neurotransmitter model for the fundamental pathways providing the thermoregulatory control and pyrogenic activation of CVC, BAT and shivering thermogenesis. Cool and warm cutaneous thermal sensory receptors transmit signals to respective primary sensory neurons in the dorsal root ganglia (DRG) which relay this information to second-order thermal sensory neurons in the dorsal horn (DH). Cool sensory DH neurons glutamatergically activate third-order sensory neurons in the external lateral subnucleus of the lateral parabrachial nucleus (LPBel), while warm sensory DH neurons project to third-order sensory neurons in the dorsal subnucleus of the lateral parabrachial nucleus (LPBd). Thermosensory signals from DH neurons are also relayed to

the thalamus and then to the cortex for conscious thermal perception and localization. Thermosensory signals for thermoregulatory responses are transmitted from the LPB to the preoptic area (POA) where GABAergic interneurons in the median preoptic (MnPO) subnucleus are activated by glutamatergic inputs from cool-activated neurons in LPBel and inhibit the distinct populations of warm-sensitive (W-S) neurons in the medial preoptic (MPO) subnucleus that control cutaneous vasoconstriction (CVC), brown adipose tissue (BAT) and shivering. In contrast, glutamatergic interneurons in the MnPO, postulated to be excited by glutamatergic inputs from warm-activated neurons in LPBd, excite W-S neurons in MPO. Prostaglandin (PG) E_2 binds to EP3 receptors on W-S neurons in the POA to inhibit their activity. Preoptic W-S neurons provide thermoregulatory control of CVC by inhibiting CVC sympathetic premotor neurons in the rostral ventromedial medulla, including the rostral raphe pallidus (rRPa), that project to CVC sympathetic preganglionic neurons in the intermediolateral nucleus (IML). CVC premotor neurons can increase CVC sympathetic tone by release of glutamate and/or serotonin (5-HT) within the IML. Preoptic W-S neurons providing thermoregulatory control of BAT thermogenesis inhibit BAT sympathoexcitatory neurons in the dorsomedial hypothalamus (DMH) which, when disinhibited during skin cooling, excite BAT sympathetic premotor neurons in the rRPa that project to BAT sympathetic preganglionic neurons in the IML. Some BAT premotor neurons, containing vesicular glutamate transporter 3 (VGLUT3), can release glutamate to excite BAT sympathetic preganglionic neurons and increase BAT sympathetic nerve activity by release of glutamate, while others can release 5-HT to interact with 5-HT1A receptors, potentially on inhibitory interneurons in the IML, to increase the BAT sympathetic outflow and thermogenesis. Preoptic W-S neurons provide thermoregulatory control of shivering responses by inhibiting shivering-promoting neurons in the DMH which are postulated to provide excitation to medial medullary shivering premotoneurons in the rRPa, that project to the ventral horn to excite alpha and gamma motoneurons during shivering in skeletal muscles.

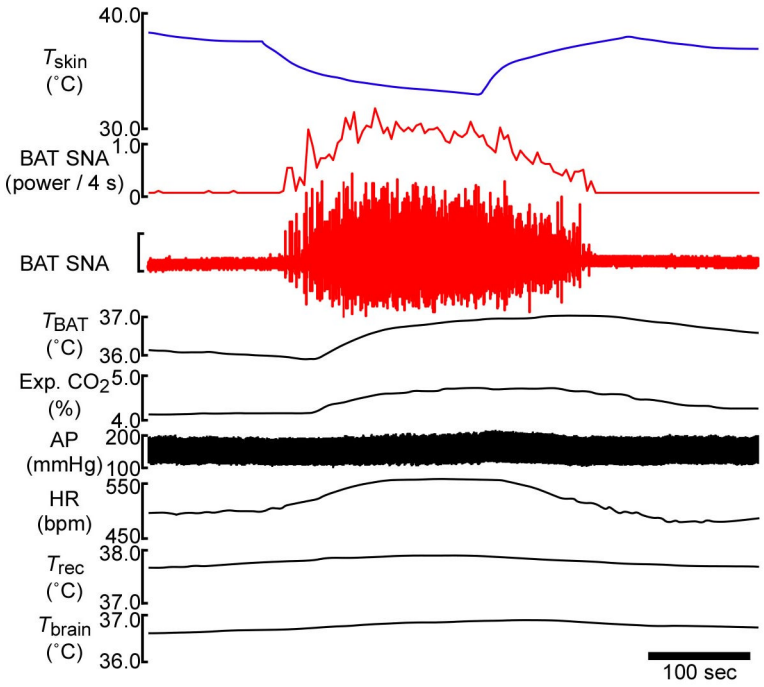

Figure 3.

Changes in BAT sympathetic nerve activity (BAT SNA, red traces), BAT temperature (T_{BAT}), expired (Exp.) CO_2, heart rate (HR), arterial pressure (AP), rectal temperature (T_{rec}) and brain temperature (T_{brain}) in response to cooling the rat trunk skin (T_{skin}, blue trace). The vertical scale bar for the BAT SNA trace represents 100 microvolts. Note that T_{rec} and T_{brain} do not change substantially during the skin cooling and rewarming, indicating that the observed changes in BAT SNA, T_{BAT}, Exp. CO_2, HR and AP were evoked by changes in skin temperature rather than by changes in body core or in brain temperatures. Reproduced with permission from (1).

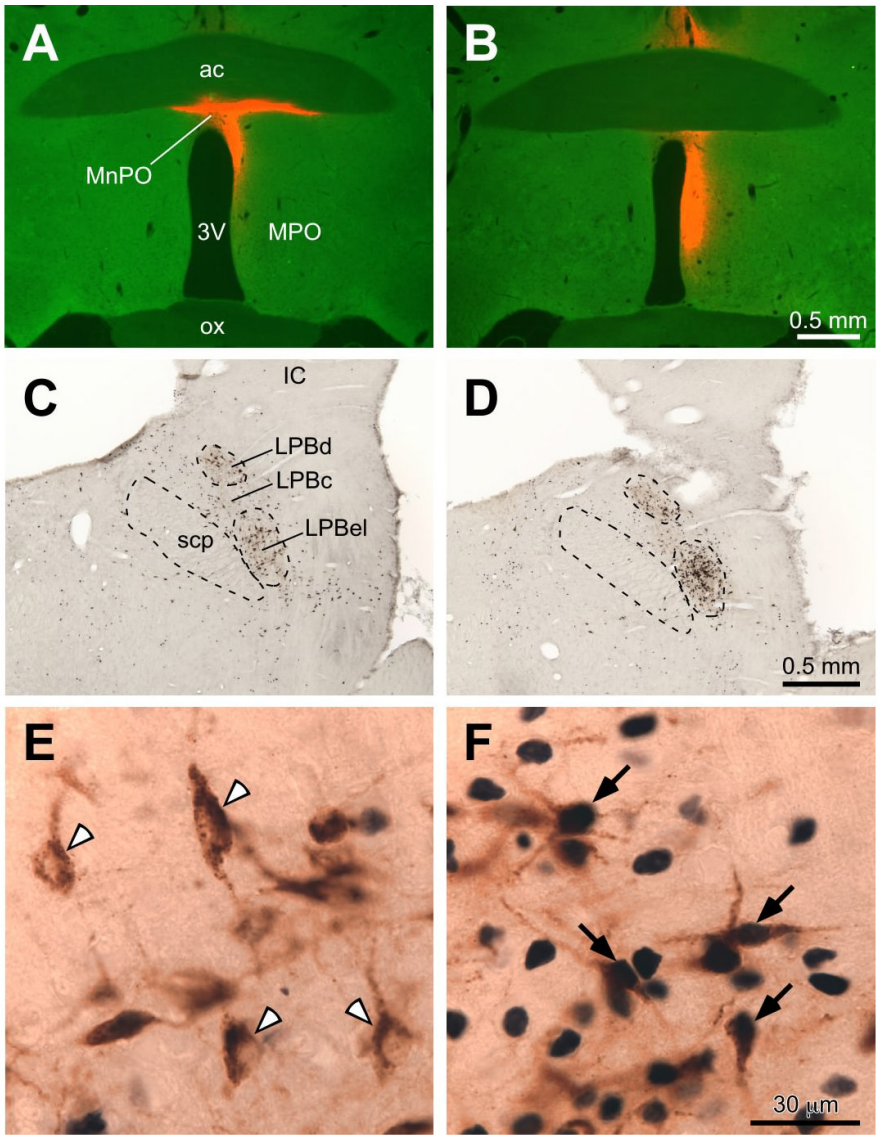

Figure 4.

POA-projecting LPB neurons are activated in a cold environment. (A–F) Fos expression in LPB neurons retrogradely labeled with the retrograde tracer, cholera toxin b-subunit (CTb), injected into the POA in rats exposed to 24°C (A, C, E) and 4°C (B, D, F). A and B show injection sites of CTb (red). In C–F, CTb (brown) and Fos (blue-black) immunoreactivities in the LPBel of the animals shown in A and B are visualized. In E and F, arrowheads indicate Fos-negative, CTb-labeled neurons and arrows indicate Fos-positive, CTb-labeled neurons. 3V, third ventricle; ac, anterior commissure; IC, inferior colliculus; LPBc, central part of the lateral parabrachial nucleus; MPO, medial preoptic area; ox, optic chiasm; scp, superior cerebellar peduncle. Reproduced with permission from (27).

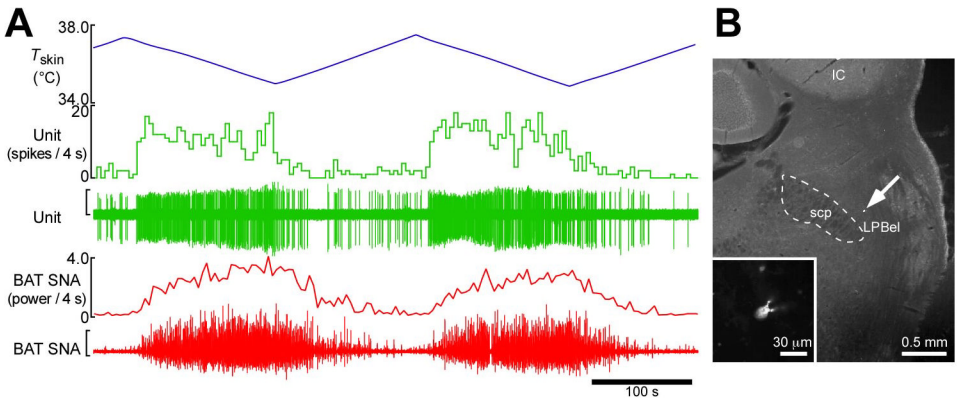

Figure 5.

Skin cooling-evoked response of a single LPB neuron antidromically activated from the POA. A, *in vivo* extracellular unit recording of the action potentials of an LPB neuron (unit, green traces) and changes in BAT SNA (red traces) in response to trunk skin cooling (T_{skin}, blue trace). The vertical scale bars for the unit and BAT SNA traces represent 300 microvolts and 100 microvolts, respectively. The unit firing rate increased in response to skin cooling and returned to the baseline level in response to rewarming the skin. The changes in firing rate of this neuron paralleled the changes in BAT SNA. A collision test confirmed that this neuron projected to the POA. B, juxtacellular labeling located this neuron in the LPBel (arrow and magnified in inset). Reproduced with permission from (27).

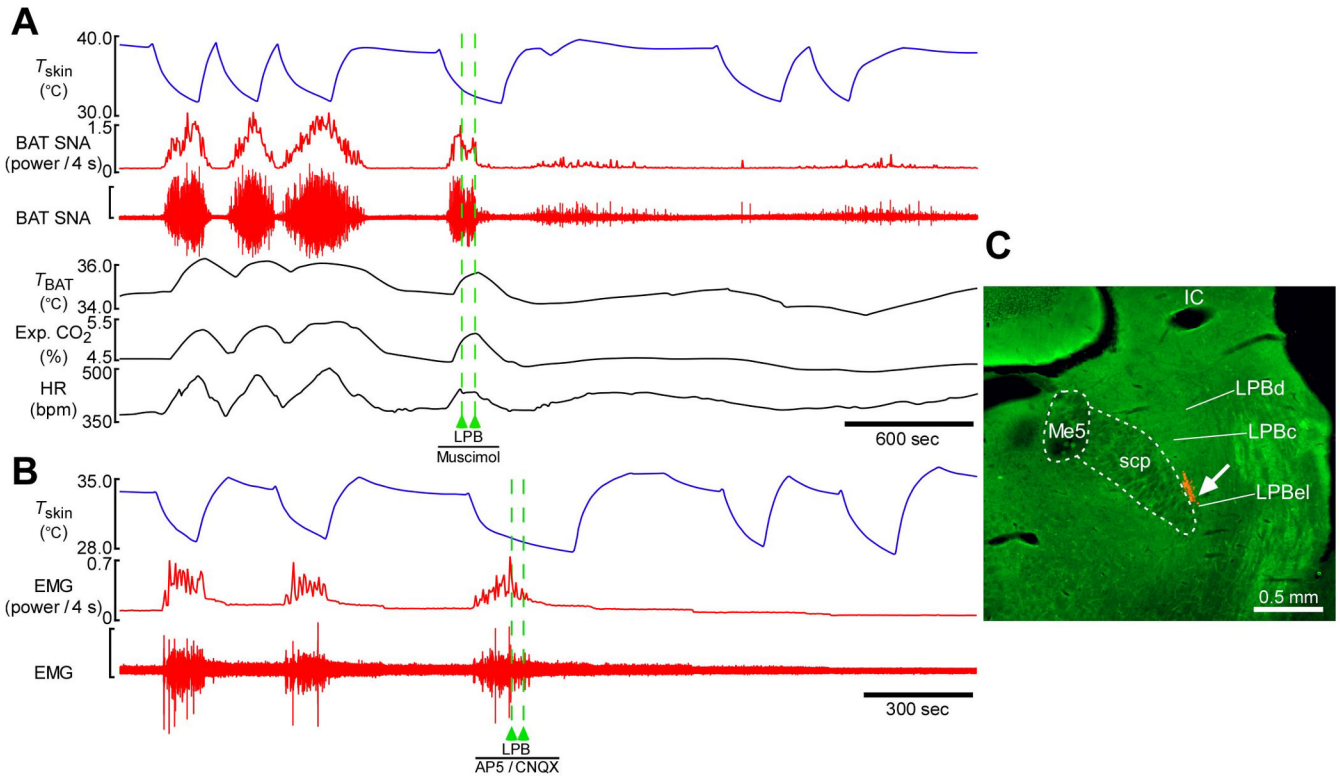

Figure 6.

Inhibition of neuronal activity or blockade of ionotropic glutamate receptors in the LPBel reverses shivering and non-shivering thermogenesis and metabolic and cardiac responses that are evoked by skin cooling. A, skin cooling-evoked changes in BAT SNA, T_{BAT}, Exp. CO_2 and HR before and after bilateral nanoinjections (green dashed lines) of the $GABA_A$ receptor agonist, muscimol, into the LPBel. The vertical scale bar for the BAT SNA trace represents 100 microvolts. B, skin cooling-evoked changes in nuchal EMG before and after bilateral nanoinjections (green dashed lines) of a mixture of the glutamate receptor antagonists, AP5 and CNQX, into the LPBel. The vertical scale bar for the EMG trace represents 400 microvolts. C, representative view of a nanoinjection site in the LPBel as identified with a cluster of fluorescent beads (arrow). Reproduced with permission from (27).

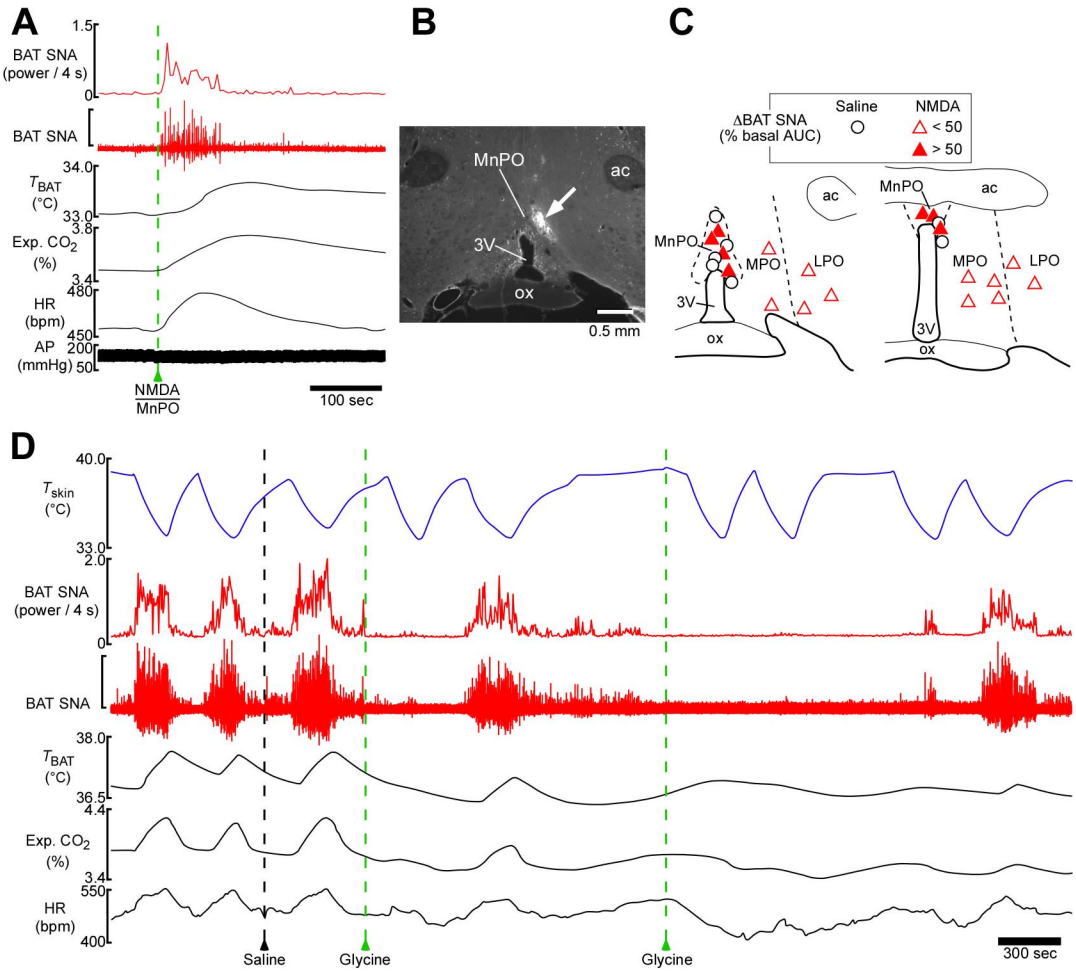

Figure 7.

Glutamatergic stimulation of MnPO neurons, but not MPO or LPO neurons, evokes thermogenic, metabolic and cardiac responses and inhibition of MnPO neurons blocks thermogenic, metabolic and cardiac responses to skin cooling. A, changes in physiological variables evoked by a nanoinjection (green dashed line) of NMDA into the MnPO. The vertical scale bars for the BAT SNA trace represent 50 microvolts. B, representative injection site in the MnPO, identified by a cluster of fluorescent beads (arrow). C, composite drawing of saline or NMDA injections sites in the subregions of the POA, with their stimulatory effects on BAT SNA. No saline injections increased BAT SNA by more than 50 percent of the basal area under the curve (AUC) of the power trace during the 3 minute period after the injection. D, changes in BAT SNA, T_{BAT}, Exp. CO_2 and HR evoked by repeated skin cooling (T_{skin}, blue trace). Saline or glycine, an inhibitory neurotransmitter, was nanoinjected into the MnPO at the broken lines. The vertical scale bar for the BAT SNA trace represents 400 microvolts. Reproduced with permission from (37).

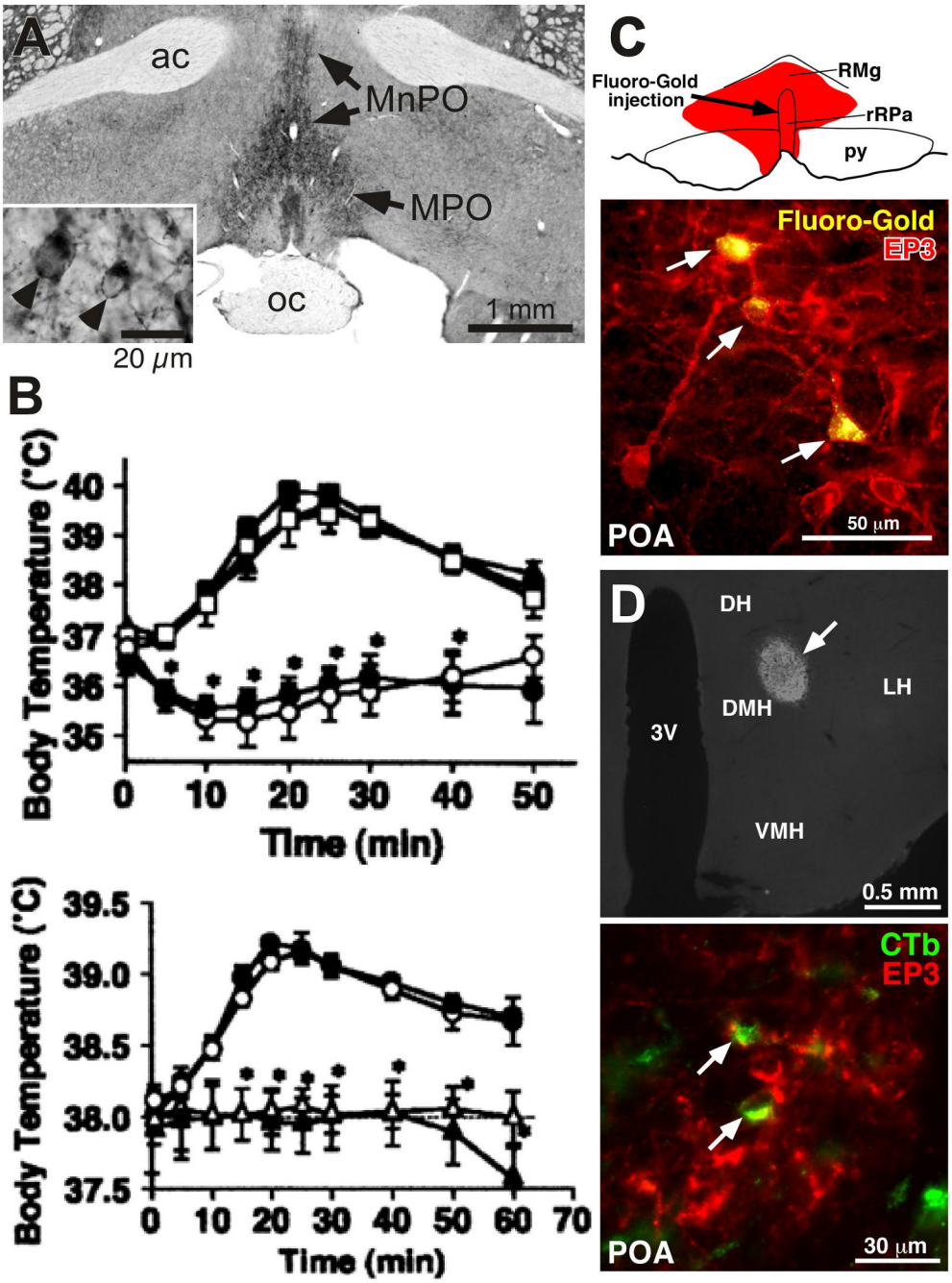

Figure 8.

Pyrogenic role of prostaglandin EP3 receptors in the preoptic area. A, within the rat POA, prostaglandin EP3 receptor immunoreactivity is distributed in the somatodendritic part of MnPO and MPO neurons (inset, arrowheads) (modified from (71), with permission). B, loss of PGE_2-induced (top) or lipopolysaccharide (LPS)-induced (bottom) febrile response selectively in *EP3*$^{-/-}$ mice. Top graph shows changes in body temperatures of *EP1*$^{-/-}$ (filled squares), *EP2*$^{-/-}$ (open squares), *EP3*$^{-/-}$ (filled circles) and *EP4*$^{-/-}$ (filled triangles) mice following icv PGE_2 injection. Vehicle was injected icv into *EP3*$^{-/-}$ mice (open circles). Asterisk indicates *P* less than 0.01 for *EP3*$^{-/-}$ mice versus wild-type mice injected with PGE_2. Bottom graph shows changes in body temperatures of wild-type (open circles), *EP1*$^{-/-}$

(filled circles) and *EP3*$^{-/-}$ (filled triangles) mice following iv LPS injection. Vehicle was injected iv into wild-type mice (open triangles). Asterisk indicates *P* less than 0.01 for *EP3*$^{-/-}$ mice versus wild-type mice injected with LPS. Reproduced with permission from (74). C, EP3 receptor-expressing POA neurons directly project to the rRPa. Top panel shows a site of injection of Fluoro-Gold, a retrograde neural tracer, that was centered at the caudal one-third of the rRPa (arrow) and spread into the surrounding raphe magnus nucleus (RMg) (red area); bottom, fluorescence photomicrograph shows POA neuronal cell bodies (arrows) double-labeled with Fluoro-Gold fluorescence (yellow) and EP3 receptor immunoreactivity (red). Reproduced with permission from (63). D, EP3 receptor-expressing POA neurons directly project to the DMH. Top, a CTb injection site (arrow) in the DMH; bottom, fluorescence photomicrograph of POA neuronal cell bodies (arrows) double-labeled with EP3 receptor (red) and CTb (green) immunoreactivities. DH, dorsal hypothalamic area; LH, lateral hypothalamic area; VMH, ventromedial hypothalamic nucleus. Reproduced with permission from (77).

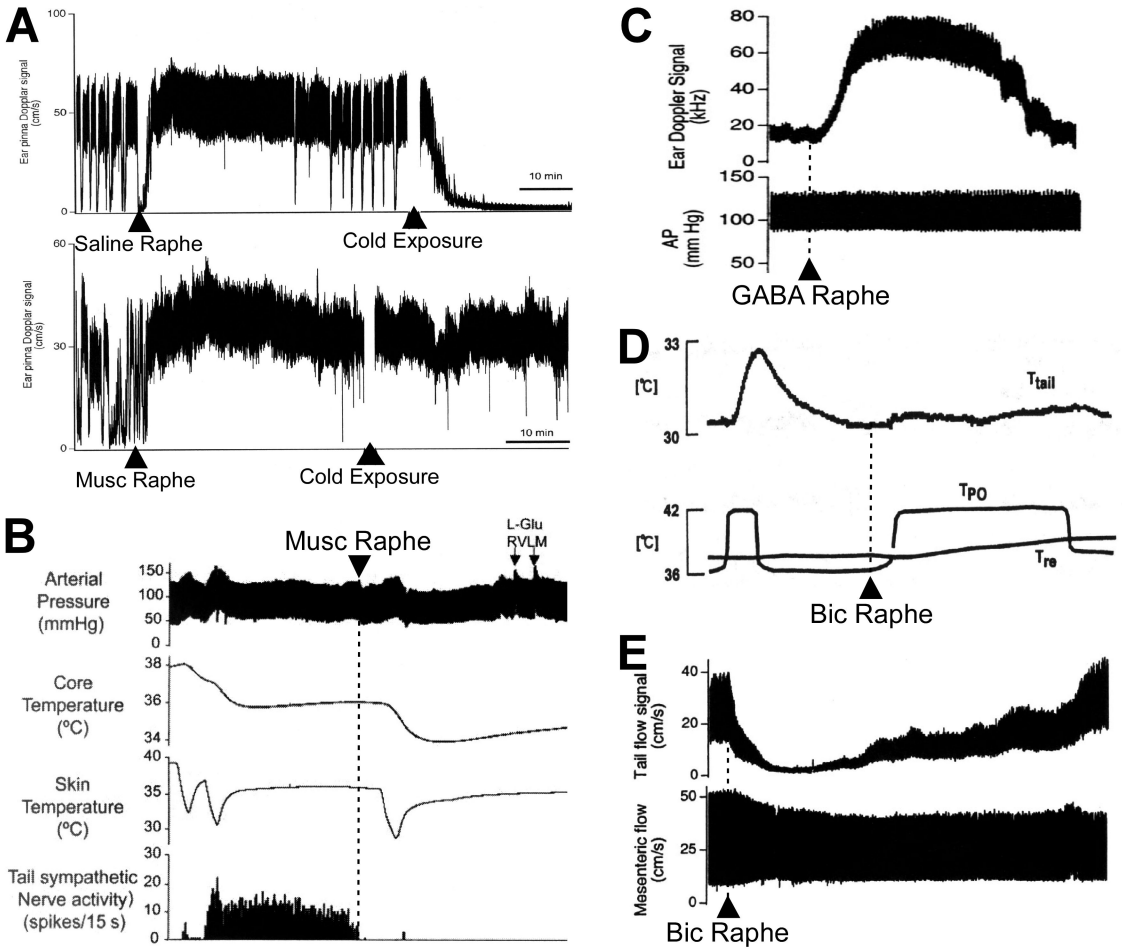

Figure 9.

Rostral medullary raphe neurons play a major excitatory role in determining cutaneous vasoconstrictor (CVC) sympathetic outflow and cutaneous blood flow. Inhibition of raphe pallidus neurons with microinjection of muscimol (Musc) or GABA prevents the cold-evoked decrease in rabbit ear pinna blood flow (A, reproduced with permission from (119)) and the cold-evoked increase in rat tail CVC activity (B, reproduced with permission from (117)) and increases rabbit ear pinna blood flow from spontaneous levels at room temperature (C, modified from (118), with permission). Increases in raphe pallidus neuronal activity following microinjection of bicuculline (Bic) prevents the increase in rat tail temperature evoked by hypothalamic warming (D, reproduced with permission from (115)) and decreases rat tail blood flow from spontaneous levels at room temperature (E, Reproduced with permission from (116)).

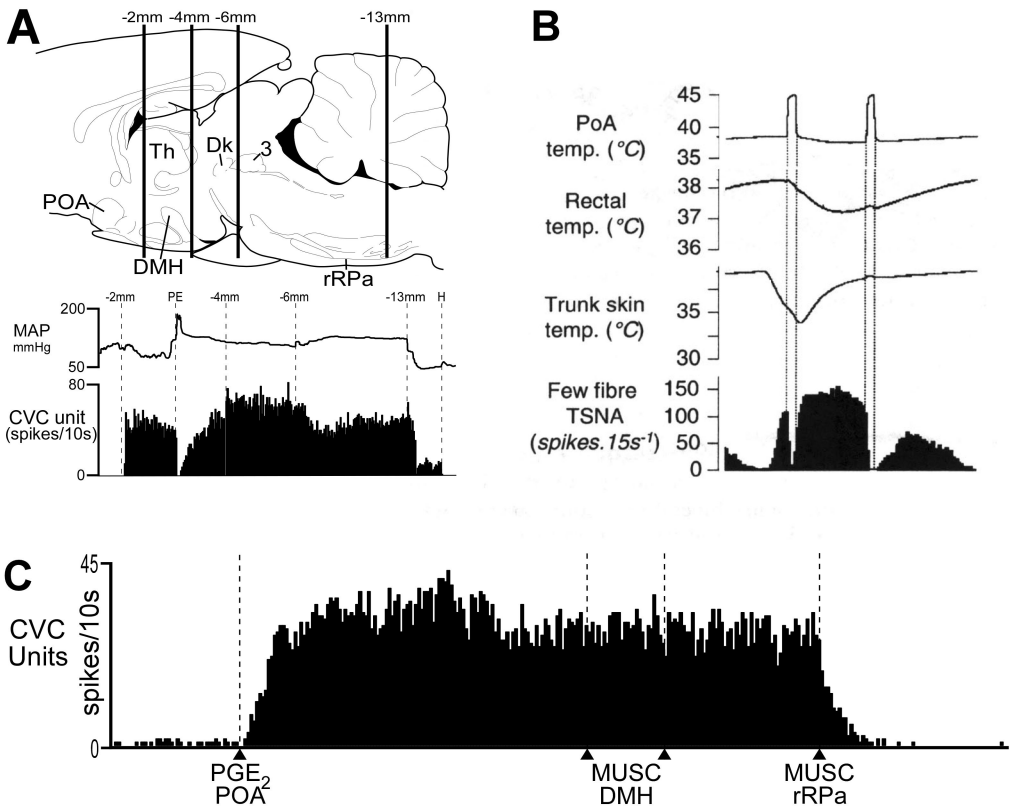

Figure 10.

Warm-sensitive preoptic area (POA) projection neurons provide an inhibitory influence on cutaneous vasoconstrictor (CVC) outflow and neurons in dorsomedial hypothalamus (DMH) are not required for PGE_2-evoked CVC activation. A, transection of the neuraxis caudal to the POA produces an increase in rat tail CVC activity. Reproduced with permission from (93). B, POA warming inhibits rat tail sympathetic nerve activity (TSNA) controlling tail CVC. Reproduced with permission from (205). C, inhibition of neurons in the DMH with microinjections of muscimol (Musc) does not affect the activation of rat tail CVC neurons evoked by microinjection of PGE_2 into the POA, but inhibition of putative CVC sympathetic premotor neurons in the raphe pallidus (rRPa) reverses the febrile-like activation of CVC activity. Reproduced with permission from (93).

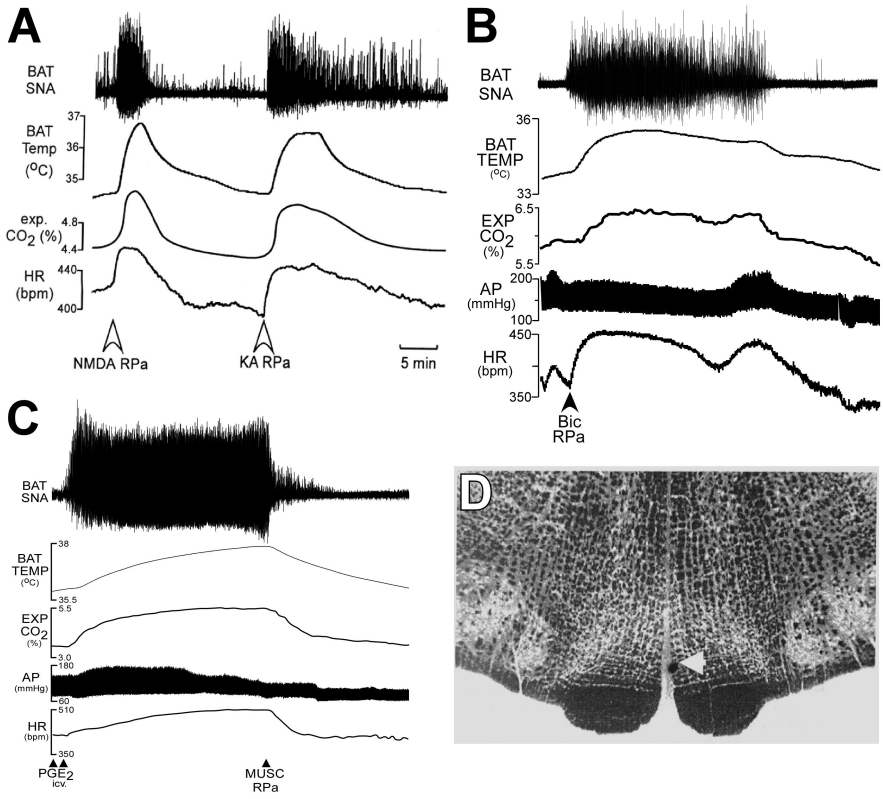

Figure 11.

A, both NMDA and kainic acid (KA) are highly effective at increasing BAT SNA, BAT temperature, expired (exp.) CO_2 and heart rate (HR) when nanoinjected into the raphe pallidus (RPa). Reproduced with permission from (92). B, disinhibition of local neurons in the RPa elicits large and sustained increases in BAT SNA, BAT temperature, expired CO_2 and HR. Reproduced with permission from (92). C, inhibition of the activity of neurons in the RPa with a local injection of muscimol (MUSC) reverses the increases in BAT SNA, BAT temperature, expired CO_2 and HR evoked by PGE_2 icv. Reproduced with permission from (146). D, histological coronal section through the rat brainstem approximately 12.3 mm caudal to bregma, illustrating a typical nanoinjection site (arrowhead) in the RPa. Reproduced with permission from (146).

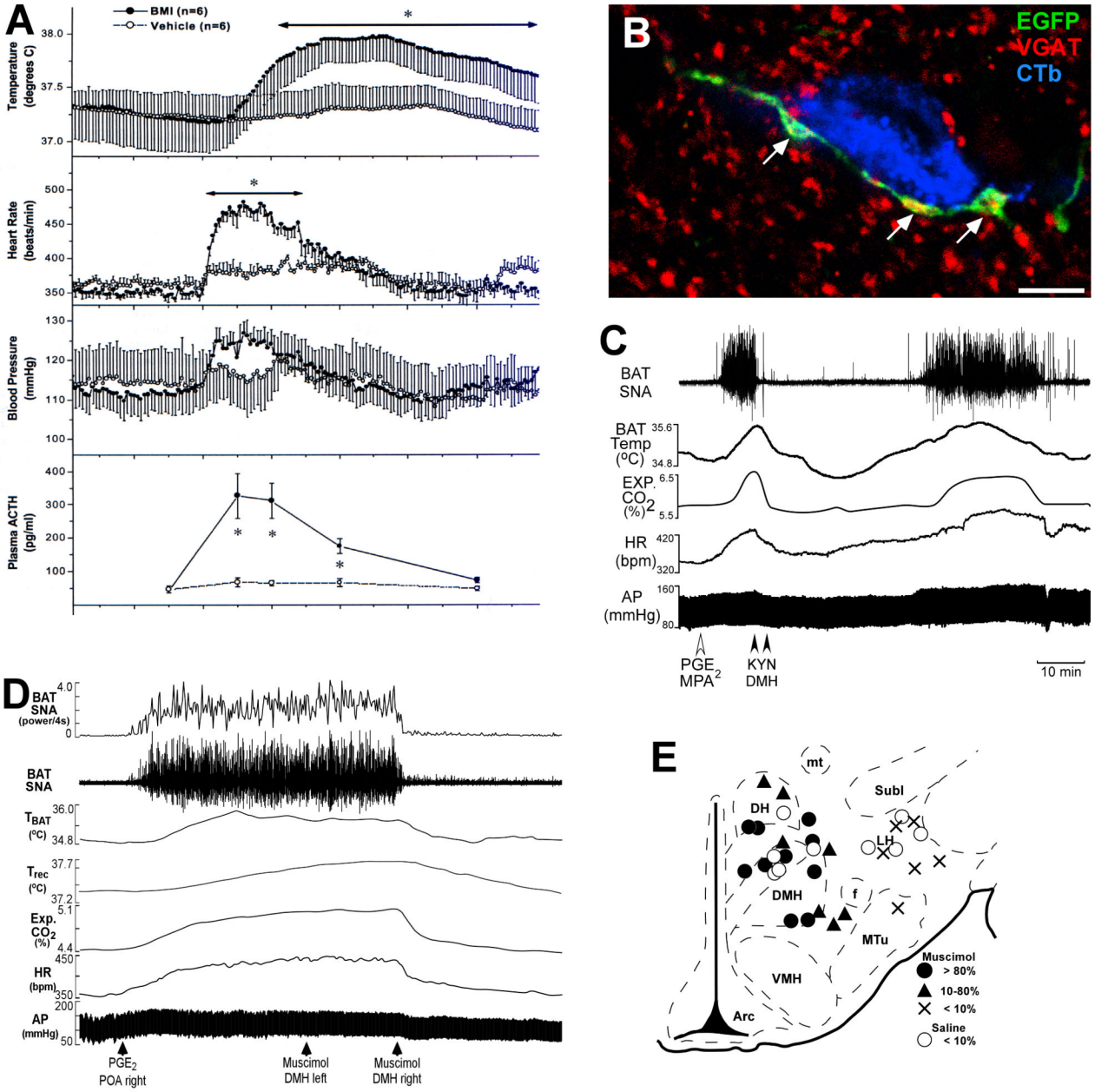

Figure 12.

Role of dorsomedial hypothalamic neurons in BAT and HR thermoregulatory responses. A, in the conscious rat, disinhibition of neurons in the dorsomedial hypothalamus (DMH) with local injection of bicuculline (BMI) elicits increases in body temperature, heart rate and ACTH secretion. Reproduced with permission from (83). B, confocal image demonstrates immunohistochemically that axon terminals of POA GABAergic neurons double-labeled (yellow) with VGAT-immunoreactivity (red) and EGFP (green, following anterograde transport of Sindbis virus) make close appositions with DMH neurons that project directly to the rRPa (labeled with CTb immunoreactivity (blue)). Reproduced with permission from (77). C, blockade of glutamate receptors in the DMH with a nanoinjection of the glutamate receptor antagonist, kynurenic acid (KYN), reverses the increases in BAT SNA, in BAT temperature (thermogenesis), in expired CO_2 and in heart rate elicited by nanoinjection of

the febrile mediator, PGE_2, into the medial preoptic area (MPA). Reproduced with permission from (94). D, inhibition of neuronal activity in the DMH with nanoinjections of muscimol eliminates the increases in BAT SNA, in BAT temperature, in expired CO_2 and in heart rate elicited by nanoinjection of PGE_2 into the POA. Reproduced with permission from (77). E, schematic map of the medial hypothalamus illustrating the concentrated localization within the DMH of sites at which injections of muscimol were efficacious in inhibiting PGE_2-evoked increases in BAT SNA. Reproduced with permission from (77).

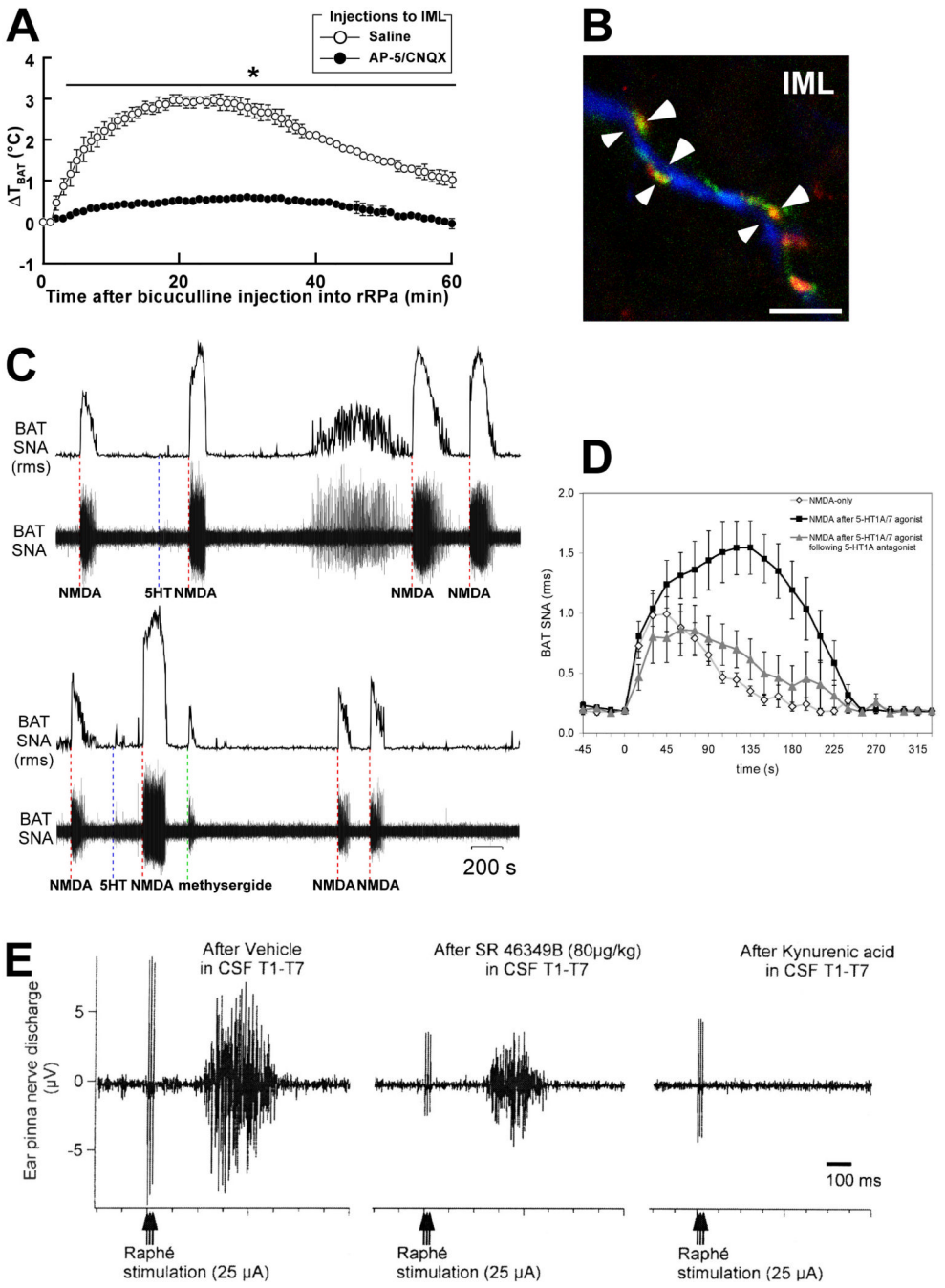

Figure 13.

Spinal glutamatergic and serotonergic regulation of thermoregulatory effectors. A, blockade of thoracic spinal glutamate receptors significantly reduced the increase in BAT temperature elicited by disinhibition of neurons in the rRPa. Reproduced with permission from (89). B, confocal image demonstrates immunohistochemically that axon terminals of rRPa neurons double-labeled (yellow) with VGLUT3-immunoreactivity (red) and EGFP (green, following anterograde transport of Sindbis virus) make close appositions with sympathetic preganglionic neurons (labeled with ChAT immunoreactivity (blue)). Reproduced with permission from (89). C, upper panel, the increase in brown adipose tissue (BAT) sympathetic nerve activity (SNA) following nanoinjection of NMDA into the IML of the T4

spinal segment is potentiated by similar nanoinjection of serotonin (5-HT) into the T4 IML, which also causes a delayed increase in BAT SNA. C, lower panel, 5-HT-induced potentiation of spinal NMDA-evoked increase in BAT SNA is reversed by nanoinjection of the 5-HT receptor antagonist, methysergide, into the T4 IML. Reproduced with permission from (181). D, nanoinjection of the 5-HT $_{1A/7}$ receptor agonist, 8-OH-DPAT, into the IML of the T4 spinal segment potentiates the increase in BAT SNA elicited by NMDA injection into the T4 IML and this potentiation is markedly reduced, but not completely reversed by T4 IML nanoinjection of the selective 5-HT_{1A} receptor antagonist, WAY 100635. Reproduced with permission from (183). E, increases in rabbit ear pinna cutaneous sympathetic nerve discharge elicited by electrical stimulation of the rRPa were markedly reduced by superfusion of the upper thoracic spinal cord with the 5-HT_{2A} receptor antagonist, SR 46349B, and completely reversed by further superfusion of the thoracic spinal cord with the non-selective glutamate antagonist, kynurenic acid. Reproduced with permission from (120).