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INVITED REVIEW

Cellular populations and thermosensing mechanisms of the hypothalamic thermoregulatory center

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Abstract

Temperature affects all aspects of life down to the diffusion rates of biologically active molecules and reaction rates of enzymes. The reciprocal argument holds true as well and every biological process down to enzymatic reactions influences temperature. In order to assure biological stability, mammalian organisms possess the remarkable ability to maintain internal body temperature within a narrow range, which in humans and mice is close to 37 °C, despite wide environmental temperature variations and different rates of internal heat production. Nevertheless, body temperature is not a static property but adaptively regulated upon physiological demands and in the context of pathological conditions. The brain region that has been primarily associated with internal temperature regulation is the preoptic area and the anterior portion of the hypothalamus. Similar to a thermostat, this brain area detects deep brain temperature, integrates temperature information from peripheral body sensors, and—based on these inputs—controls body temperature homeostasis. Discovered more than a century ago, we still know comparatively little about the molecular and cellular make-up of the hypothalamic thermoregulatory center. After a brief historic outline that led to the discovery of the thermoregulatory center, we here review recent studies that have considerably advanced our understanding of hypothalamic thermoregulation. We touch upon proposed mechanisms of intrinsic deep brain temperature detection and focus on newly identified hypothalamic cell populations that mediate thermoregulatory responses and that provide novel entry points not only to shed light on the mechanistic underpinnings of the thermoregulatory center but also to probe its therapeutic value.

Keywords Thermoregulation · Thermosensation · Preoptic area · Hypothalamus · TRP ion channels · Temperature homeostasis · Temperature detection

Introduction—hypothalamic temperature detection and thermoregulation

Pioneering studies conducted by, among others, the Nobel Prize awardee Charles Richet [74] and Aronsohn and Sachs [6] established the existence of a "warming center"—an anatomical region within the brain that regulates body temperature. Specifically, these researches observed that lesions afflicted to the base of the brain (very probably injuring the hypothalamus and surrounding areas) caused a rapid increase in body temperature in rabbits, dogs, guinea pigs, and other animals. Further evidence of the role of the brain in body temperature regulation came from Barbour's work with rabbits [7]. He observed that heating the brain by introducing thin water-conducting metal tubes reaching down to the hypothalamus effectively stimulated a decrease of body temperature in rabbits. Using radio-frequency-induced heating of specific brain areas in the cat, Magoun and colleagues refined the location of a heat-responsive region to the anterior part of the hypothalamus [61]. Subsequent experiments done by numerous research groups confirmed these early observations and showed that temperature changes in a brain region just above the optic chiasm, known as preoptic area (POA), are followed by counterbalancing alterations in body temperature in essentially all mammalian species studied [12]. Additionally, it has been reported that thermal stimulation of the POA in conscious mammals triggers autonomic thermoregulatory mechanisms such as shivering, activation of brown adipose tissue (BAT) and peripheral vasoconstriction when the POA is cooled or, oppositely, vasodilation, sweating, and panting when it is warmed [39]. Interestingly, the magnitude

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of the thermoregulatory responses elicited by hypothalamic temperature stimulation was found to depend on the ambient temperature. It was concluded that both anatomical locations, the POA and sensory-neuron innervated skin, detect temperature changes relevant for body temperature regulation and homeostasis. It was further elaborated that both sites act in unison and warming the POA and the skin simultaneously had a stronger thermoregulatory effect than warming one of the two areas alone. These results suggest that the POA receives thermal information from peripheral thermoreceptors and this information can modify the way in which the POA responds to changes of internal temperature [30, 46]. Collectively, these results demonstrate that the POA is a preeminent neuroanatomical location concerned with thermoregulatory functions, and—although not the only thermoresponsive site within mammals—it has the capability to intrinsically detect temperature changes with remarkable sensitivity.

However, despite the identification of the POA as an important region for intracranial temperature detection more than 80 years ago, the cellular and molecular nature of its thermosensitivity has largely remained elusive and is only starting to emerge now. A ground-breaking discovery in this respect was the identification of neurons in the POA that respond to local changes in brain temperature [37, 68, 69]. Specifically, in vivo electrophysiological studies showed that approximately 20 to 40% of single units recorded from the POA display a spontaneous and regular firing and this ongoing activity can be modified by changes in local brain temperature. Further studies performed in acute brain slices support these observations [23, 34, 43, 50] and determined that approximately 30% of all POA neurons increase their discharge frequency in response to warming and, hence, have been classified as warm-sensitive neurons (WSNs), while 5% of intermingled POA neurons respond to cooling, and thus are regarded as cold-sensitive neurons (CSNs) [10]. However, the percentages of WSNs and CSNs vary across a number of different studies depending on (i) the exact hypothalamic location studied, (ii) the species analyzed, and (iii) the parameter used to determine neuronal thermosensitivity [12].

The majority of preoptic WSNs have been shown to retain their ability to respond to temperature changes even in the absence of synaptic inputs [50], although the precise number of truly intrinsically temperature-sensitive WSNs remains uncertain [44]. Independently of the exact proportions, these results strongly suggest that there are POA WSNs that harbor the molecular machinery necessary to detect and transduce temperature changes into electrical signals [56]. The evidence for intrinsic cold-sensitivity of POA neurons is less clear and may depend on synaptic inputs from intermingled WSNs [23, 50], although exceptions have been reported [3, 97]. In addition to this and in contrast to POA CSNs, a great proportion of WSNs respond to changes in extracranial temperature by altering their firing activity. This observation implies that WSNs, in addition to detect local brain temperature, receive thermal inputs from extra-hypothalamic thermoreceptors such as those located in the spinal cord and the skin [13].

On the basis of abundant evidence collected over more than 100 years (here only introduced in a summarized version), it has been proposed that POA WSNs are a crucial part of the central thermoregulatory circuit. Specifically, the current model of central body temperature regulation proposes that WSNs receive peripheral thermal inputs and integrate this information with intracranial temperature as well as information on metabolic fuel availability and other homeostatic parameters to produce appropriate autonomic thermodefensive responses [67]. Four key findings support this model: (1) POA WSNs are to a large extent intrinsically thermosensitive and respond to temperatures within the physiological range. (2) POA WSNs are located in a brain region that controls most—if not all—of the autonomic thermoregulatory mechanisms in mammals. (3) POA WSNs receive somatosensory afferent input from thermoreceptors located in the skin, spinal cord, and likely also from internal organs. (4) POA WSNs respond to inflammatory and metabolic signaling molecules (such as pyrogens and diverse neuropeptides) [9]. For a more comprehensive overview about the extra-hypothalamic pathways that control core body temperature, we highly recommend consulting the following authoritative reviews [10, 66, 67].

Temperature detection in the POA—is it physiologically relevant?

Despite the general acceptance of the aforementioned model of thermoregulation in mammals, the physiological role of deep brain temperature detection has remained controversial [12, 14, 40, 86]. One argument raised is based on the assumption that intracranial temperatures are rather static and devoid of any fluctuations under normal physiological conditions. According to this argument, central thermoreceptors would be functionally irrelevant because there are no temperature changes to be detected deep in the brain. However, this view is clearly oversimplified and brain temperature measurements have shown that the deep brain temperature fluctuates considerably: just taking into account the basal circadian temperature rhythm of a rat, high-speed temperature recordings reveal thermal brain fluctuations of ~ 3 °C. These fluctuations become even more pronounced if the animals are exposed to arousing sensory stimuli or are engaged in physical activity [51]. Additionally, a number of reports have observed differences between deep brain temperatures and temperatures at other internal body parts including the rectum, skeletal muscles, and arterial blood vessels in rodents, monkeys, and humans, among others species [14, 38, 41, 53–55]. The general picture that emerges from these studies is that brain

temperatures are constitutively higher than temperatures at other internal locations. For example, in humans, an average difference between brain (measured at a distance of 2 cm from the tip of a ventroculostomy catheter) and rectal temperatures between 0.1 to 2.0 °C have been observed [41]. In rats, brain temperatures have also been found to be significantly higher than arterial blood temperatures in steady state conditions (with mean values of 37.6 °C at the ventral striatum, 37.3 °C at the cerebellum and 36.6 °C at the abdominal aorta) [54]. Interestingly, the arterial blood entering the brain is persistently cooler than the brain tissue itself, suggesting that heat production within the brain may be the origin of elevated intracranial temperatures [52]. This hypothesis is consistent with the observation that the brain is a highly metabolically active tissue, requiring up to 20% of all oxygen consumed at resting conditions [52, 77, 80]. An additional observation is that diverse stimuli comprising tail pinching, cage transfer, and social interaction produce a faster and more prominent change in brain temperature than in arterial blood temperature in rats, possibly reflecting a sudden increase in metabolic expenditure due to neuronal activation [54]. Localized changes in brain temperature have been observed in discrete neuroanatomical areas including the hypothalamus [51], suggesting that deep brain temperature changes constitute a physiologically relevant parameter. In this regard, it is also interesting to note that a dorsal-ventral brain temperature gradient has been observed in several species including humans [26, 38, 45, 51, 63, 78] rendering the most ventral brain regions to be among the hottest body parts of the mammalian organism and making the most ventrally located structures—such as the hypothalamus—prime candidates to detect internal body heat.

Overall, these data challenge the notion that brain temperature is merely a static parameter but rather suggest that it is changing dynamically, perhaps in a highly regulated manner.

A second recurrent argument challenging a role of central thermoreceptors in thermoregulation is that many thermodefensive reflexes in animals, such as BAT activation in response to skin cooling or vasodilation after skin warming, can be initiated almost immediately after exposure to an environmental temperature challenge and before any changes in internal thermal conditions occur [14, 29, 86, 100]. In these cases, hypothalamic temperature may seem to be physiologically irrelevant to effectively orchestrate thermodefensive responses and to preserve a normal internal thermal state. Although nobody would dispute that the activity of peripheral thermoreceptors endows the thermoregulatory system with predictive capabilities, particularly under high environmentally challenging conditions [39], a correct regulation of body temperature may still require multiple sources of thermal information, such as those provided by thermoreceptors located in the brain, spinal cord, viscera, and skin [67]. These coexisting groups of thermoreceptors may confer robustness to the thermoregulatory system and generate a more reliable picture of the thermal state of the organism [39]. Indeed, there is evidence to suggest that peripheral and central thermoreceptors provide parallel and, to some degree, redundant temperature information: it has been found that the gene encoding the capsaicin receptor Trpv1 serves as a lineage marker for all peripheral somatosensory thermoreceptive (cool to hot) neurons [17, 64, 98] and thus utilization of Trpv1-Cre mice allows genetic ablation of all known peripheral thermoreceptive neurons. Mice that lack peripheral (skin and putatively also visceral) thermoreceptor cells (and thus are unable to detect environmental temperatures) maintain a core body temperature indistinguishable from non-ablated littermates. Importantly, these ablated mice are able to preserve a normal body temperature at 25 °C, a temperature that constitutes a mild cold challenge and thus requires active thermoregulatory mechanisms to be engaged (such as BAT activation) to defend body temperature [32]. These findings suggest that, in the absence of peripheral thermoreceptors, additional temperature-sensitive cells, likely located in the hypothalamus (and potentially in other CNS areas such as the spinal cord [15]), can provide sufficient thermal information to allow the thermoregulatory system to preserve body temperature [64]. These results strongly argue that peripheral (skin) temperature sensors are dispensable for body temperature homeostasis, at least in the absence of environmental temperature challenges.

By a similar argument, it is intuitive and reasonable to assume that for the detection of *internal* body temperature changes, such as those associated with strenuous exercise, the circadian- and menstrual cycle or fever, *internal* body temperature sensors in the POA are much better located to monitor these types of interior temperature "challenges" compared to temperature sensors residing in the skin [39], again arguing for additional—internal—temperature sensors next to cutaneous ones. In this regard, it is noteworthy that POA neurons, in particular those in the ventromedial part of the POA, are in close vicinity to the third ventricle and vessels that supply the brain with blood and thus are ideally positioned to sample temperatures in those circulating fluids.

Additional evidence of the role of central thermoreceptors in body temperature homeostasis was provided by Conti and colleagues [21]. Using a mouse model that intrinsically generates heat within the hypothalamus, this group studied the effects of a chronic increase in hypothalamic temperature on thermoregulation. Specifically, mice were engineered to overexpress the uncoupling protein 2 (UCP2) exclusively in orexin neurons, a group of approximately 3000 neurons located in the lateral hypothalamus, a region located in close proximity (about 800 μm) to the POA [24]. They observed that the additional energy dissipated by UCP2-expression generated a raise in POA temperature of approximately 0.32 °C. This modest increase in hypothalamic temperature was sufficient to permanently reduce the body temperature, a feature that correlated with an increased longevity of these mice [21]. These

results suggest that thermal information from the periphery (skin, viscera, and spinal cord) cannot completely offset the sensory inputs from central thermoreceptors located in the hypothalamus. By means still unknown, the thermoregulatory system computes the elevated hypothalamic temperature (and integrates it with peripheral thermosensory information) to correspondingly adjust thermal and metabolic states.

Strong evidence for the physiological relevance of brain thermoreceptors comes from comparative studies done in a great diversity of species comprising the major vertebrate lineages. In all classes of vertebrates, thermoregulatory functions are coordinated by the POA and an adjacent brain region, the anterior hypothalamus (POA/AH). Additionally, manipulations of POA/AH temperature trigger counterbalancing thermodefensive responses in most vertebrates, perhaps with the exception of birds, where brain thermosensitivity seems to be somehow reduced—birds have a higher body temperature compared to mammals, a feature that is possibly linked to altered thermoreceptive functionality of the bird POA. While most non-mammalian vertebrates respond to changes in POA/AH temperature mainly by modifying their thermal preference (the most basic and widely used thermodefensive mechanism in vertebrate species), mammals can additionally complement this behavioral response by activating their well-developed autonomic mechanisms of thermoregulation. Furthermore, electrophysiological studies showed that the proposed central thermoreceptors, the WSNs, populate POA/AH in virtually all vertebrate groups [12, 22, 39]. Overall, this data suggests that the role of the POA/AH in thermoregulation is highly conserved and predates the separation of vertebrates into independent lineages, an event that took place more than 500 million years ago [57, 79]. Given that the vertebrate hypothalamus and preoptic area are ancient brain regions that contain highly conserved neural circuits involved in multiple basic life functions [76], the most parsimonious explanation for such a deep phylogenetic conservation is that brain thermoreceptors fulfill an important function in vertebrate biology related to temperature homeostasis. However, it is also possible that brain thermosensitivity has persisted as a vestigial property as part of a "trade-off": a consequence of natural selection acting upon another functional feature [33]. In this scenario, another property more relevant than central thermoreception but collaterally connected to it, is the driving force for conservation. Along the lines of these considerations, many temperature-sensitive channels that participate in environmental temperature detection, such as TRP ion channels, have been found to work as multimodal transducers that are able to respond to more than one type of stimulus (e.g., temperature, pH, steroids, inflammatory mediators, osmotic stimuli, and others) [94]. Therefore, POA thermosensitivity (mediated by TRP ion channels and potentially other molecular entities) might potentially have emerged as a "collateral" feature of natural selection that principally is acting upon a non-thermal function of such channels. In other words, these receptors may serve in the hypothalamus predominantly as sensors of other modalities (such as stress, inflammatory status, hormone levels, metabolic cues, and others) and not primarily as temperature sensors, and the evolutionary preservation of temperature sensitivity is merely a by-product.

Interestingly, recent molecular evolutionary studies suggest that the neurosecretory brain centers in bilateral animals, which in vertebrates comprise the POA and hypothalamus, are—in evolutionary terms—older than proposed previously. These studies suggest that an early hypothalamus-like structure may have emerged before the divergence of protostomes (annelids and insects, among others) and deuterostomes (the group that includes the vertebrates) [25, 87, 88]. Specifically, it has been proposed that neurons in this ancestral multimodal sensory-neurosecretory brain region would have been in direct contact with the animal's body surface, where they would detect changes in environmental conditions (such as presence of chemicals, mechanical forces, and temperature) and would transduce those stimuli internally via neuropeptide release [87, 88, 90]. In this scenario, it is reasonable to speculate that vertebrate POA/AH neurons would have retained some of those ancestral sensory-neurosecretory properties—including the capacity to detect temperature—from their ancient predecessors that were in direct contact with stimuli emanating from the environment [88, 90]. In agreement with this hypothesis, the proposed thermoreceptor cells in the vertebrate POA/AH [10, 56], the WSNs, were found to express a rich repertoire of neuropeptides, which include, among others, tachykinin 1, somatostatin, galanin, and prodynorphin [27]. Furthermore, there is some additional data that is consistent with a prevertebrate origin of brain thermoreceptors in animals. Specifically, it has been shown that in the fruit fly, the brain contains a small group of neurons, the anterior cells (ACs) that can directly detect warmth [35]. While peripheral thermoreceptors in the fly are important for detecting steep (environmental) changes in thermal conditions, warmth-detecting ACs are involved in a functionally different thermoregulatory response: They participate in a slowly-developing thermal preference when flies are exposed to very shallow thermal gradients [35, 70]. Although this functional division between peripheral and central thermoreceptors in the fly very much resembles the proposed model of body temperature homeostasis in vertebrates [70], and thermosensitive ACs may share functional similarity with vertebrate WSNs, it is important to notice that the homology between fruit fly central cells and vertebrate brain thermoreceptors has thus far remained unexplored. Clearly, more detailed comparative genetic and molecular analysis is needed to track down the origin of hypothalamic warm-sensitive neurons in the vertebrate brain.

In summary, from an evolutionary perspective, the existence of thermoreceptors deep in the brain can be now

considered less surprising as previously thought. These central sensory neurons may originate from a multimodal sensory-neurosecretory organ that was present in the last common bilaterian ancestor [88, 90]. In vertebrates, some of the cellular components of this early homeostatic organ could have been repurposed and fine-tuned to preferentially detect internal brain temperature and potentially integrate it with other internal signals and parameters. This interoceptive function is likely critical to regulate homeostasis in vertebrates, especially in large-bodied and big-brained species, where brain temperature can significantly differ from temperatures at other anatomic locations [52]. In these larger species, more complex representations of the internal thermal and metabolic states are required to preserve stability of the interior milieu and adapt to changes and challenges in a concerted and balanced way. Furthermore, the high thermal dependence of the POA/AH circuits controlling body temperature may add an additional level of control to the system and may operate as a feedback signal. However, a deeper understanding of the physiological role of central thermoreceptors in vertebrates, including mammals, requires the identification of the molecular basis that sustains POA/AH thermosensation, namely the ion channels and/or other proteins involved in intrinsic temperature detection. Molecular and mechanistic insight into WSN physiology would not only deepen our understanding of the thermoregulatory system but will also be instrumental to generate controlled alterations of deep brain thermosensitivity and to study the impact of these manipulations on temperature- and metabolic homeostasis. Given the intricate connection of thermoregulation to many (arguably all) aspects of our physiology, it stands to reason that the understanding of our central thermostat poses untapped therapeutic potential.

Temperature-sensing mechanisms in the POA and the proposed role of TRPM2 in brain temperature detection

Surprisingly, although POA thermosensitivity was discovered 80 years ago [61] and WSNs have been postulated as interoceptive POA thermoreceptors as early as the 1960s [37, 68, 69], molecular mechanisms of temperature detection within the POA have remained highly speculative [10, 28, 43, 56, 81, 95]. Based on electrophysiological studies using acute brain slices, two different ion channel-based models explaining POA thermosensitivity have been proposed. The first model is based on work by Kobayashi and colleagues. They found that POA warming leads to depolarization of the resting membrane potential in intrinsically temperature-sensitive WSNs. In turn, this warming-induced depolarization (or receptor potential) increased the firing frequency of WSNs. Isolating the underlying current, Hori and colleagues were able to record a heat-activated nonselective cationic inward current in POA WSNs [43, 56].

The second model has been derived by Boulant and colleagues, who suggested that the basis of WSN thermosensitivity relies—at least in part—on a transient temperature-sensitive outward potassium current (I_A). This I_A generates a brief hyperpolarizing current that slows down the rate of depolarization triggered by a pacemaker current that is present in WSNs. Contrary to Kobayashi's hypothesis, temperature would not significantly alter the resting membrane potential of WSNs but rather control the rate of inactivation of I_A. Since heat presumably increases the inactivation rate of I_A, depolarization in between two action potentials would occur faster. The result would be an increase in firing frequency in WSNs upon warming [11, 34]. Along these theoretical considerations, it has been proposed that WSNs and temperature-insensitive intermingled POA neurons may essentially express the same set of temperature-sensitive and insensitive ion channels, comprising, among others, pacemaker current channels, background potassium leak channels, and transient receptor potential channels. Differences in the relative expression levels of these channels would determine whether POA neurons are warm-sensitive, temperature insensitive, or silent [95].

It is important to highlight that different criteria have been used in these studies to identify POA WSNs. In the first case, Kobayashi and colleagues considered a POA neuron to be warm-sensitive if it displayed a thermal sensitivity steeper than 0.5 action potentials per second per °C in conditions where synaptic transmission is completely abolished, thus restricting the study only to intrinsically thermosensitive WSNs. Notably, using this approach, the authors described a type of warm-sensitive neuron that rarely fires at temperatures lower than ~ 36 °C but displays a striking temperature-sensitive firing activity at temperatures > 36 °C. This behavior is consistent with the proposed role of a temperature-sensitive nonselective cationic inward currents with an activation temperature around the average body temperature of 37 °C and, consequently, a high sensitivity of the firing rate across the physiological temperature range [43]. Conversely, Boulant's group classified WSNs as those POA neurons that display an ongoing activity and thermal sensitivities > 0.8 action potentials per second per °C, independently of whether the spontaneous activity is synaptically driven or not. Their criterion is based on the observation that in vivo most POA neurons with thermosensitivities higher than 0.8 action potentials per second per °C receive thermal inputs from the skin and spinal cord and therefore are regarded as the POA temperature integrators within the hypothalamic thermoregulatory center [11]. Using the latter definition, identified WSNs show a very regular firing activity within the entire range of testing temperatures (32 to 39 °C) in vitro, without any obvious "thresholds of activation" [34].

On the other hand, in vivo [37] and in vitro [23, 49] electrophysiological studies have identified an additional group of

temperature-sensitive neurons in the POA, the cold-sensitive neurons (CSNs). Contrary to WSNs, preoptic CSNs are characterized by having an inversely related firing rate-temperature relation such that their ongoing activity decreases with increasing temperature. Their spontaneous activity in vivo was found to be greatly insensitive to changes in peripheral temperatures [37]. Additionally, it has been found that the synaptic inputs reaching CSNs are greatly influenced by temperature. Two mechanisms of temperature-dependent synaptic modulation of spontaneous activity in CSNs have been described: (i) excitatory postsynaptic potentials (EPSP) that increase with cooling and inhibitory postsynaptic potentials (IPSP) that decrease by lowering the temperature [23]. These observations led to the suggestion that CSNs are not intrinsically temperature sensitive and, additionally, are not connected to inputs from peripheral thermoreceptors. Considering these findings, it has been proposed that POA CSNs are probably not a principal neuronal population controlling core body temperature [67].

However, the existence of intrinsic CSNs in the POA has not been ruled out completely and apparently intrinsically cold-sensitive neurons have been identified in cultured rat preoptic preparations [3]. In this study, the identified intrinsic CSNs showed a low threshold of activation of around 27 °C [3]. It is unclear whether this (non-physiological) low activation temperature is a result of the in vitro culture condition and whether these CSNs display a shifted response profile in the intact POA. In this regard, it is noteworthy that several recent studies have shown that temperature sensation in peripheral locations is more complex as envisioned before. In fact, it is well established that temperature responses of peripheral temperature-sensitive neurons are strongly modulated by factors released upon tissue injury. For example, after injury trigeminal warmth responses are enhanced while cold responses are greatly reduced [98]. Thus, it is conceivable that temperature responsiveness of preoptic WSNs and CSNs is likewise plastic (state-dependent) and modulated by internal conditions, such as inflammation (fever), metabolic state, circadian cycle, and others. Indeed, previous work suggests that temperature sensitivity of hypothalamic WSNs is in fact plastic and subject to modulation [31, 72].

Despite some efforts to uncover the molecular mechanism of POA thermo-responsiveness, until recently, the proposed models of central thermosensation were regarded as largely hypothetical, principally due to the lack of comprehensive experimental data exploring the role of specific ion channels in POA thermosensitivity. Perhaps the single most important factor that critically hampered progress was the lack of molecular marker(s) of WSNs and CSNs, a "molecular signature" that would allow their (visual) identification for (i) directed electrophysiological experimentation, (ii) their selective isolation and molecular analysis and, eventually, (iii) their manipulation in vivo.

In contrast to the elusive identity and mechanism(s) of hypothalamic WSNs and CSNs, marker genes and thermosensory ion channels of the peripheral somatosensory temperature-sensitive neurons have been identified and a wealth of data has accumulated over the recent years. Particularly, these studies have emphasized that members of the family of "thermoTRP" channels, serve as "molecular thermometers" and act as bona fide temperature transducers in neurons of the somatosensory system. Moreover, using in vivo approaches, many TRP channels have been confirmed to participate in environmental temperature detection [8, 16, 89, 92–94].

The identification of TRP channels as part of the environmental temperature detection machinery, together with the observation that some POA WSNs show a temperature-sensitive nonselective cationic inward current (15)—a hallmark feature of TRP channels—raised the possibility that peripheral and central temperature detection mechanisms may share a similar molecular toolkit comprising, among others, TRP channels [56, 94]. Several TRP ion channels, in particular TRPV1 and TRPV4, have previously been implicated in hypothalamic interoceptive sensory processes, albeit in the context of water/osmotic homeostasis and not in the context of POA thermosensitivity [20, 47, 59, 65, 73, 83].

Thus, far and until very recently, the only report demonstrating a role for a TRP channel in brain temperature detection came from studies carried out in *Drosophila melanogaster*, where it was found that dTRPA1 mediates warmth detection in a small group of neurons, the aforementioned anterior cells in the fly's brain [35].

Very recently, we have implicated a TRP channel in deep brain temperature detection in the mouse POA. Using calcium-imaging and electrophysiological studies in cultured POA neurons as well as acute brain slices, we found that the ion channel transient receptor potential melastatin type 2 (TRPM2) is involved in temperature detection in a subset of mouse POA neurons. Additionally, we have reported that activation and inhibition of Trpm2-expressing POA neuron activity in vivo affects body temperature, triggering severe hypothermia and hyperthermia, respectively. These results suggest that preoptic TRPM2 may detect high temperatures to trigger "venting" and body cooling. Although it has not yet been proven whether the TRPM2 channel serves as a physiologically relevant temperature sensor in the POA in vivo, aforementioned manipulations of the activity of Trpm2-expressing neurons in the intact animal have demonstrated that they are an integral part of the neuronal circuitry that controls body temperature [82]. Together, these data offer the first direct evidence for a role of intrinsically warm-sensitive preoptic neurons in body temperature regulation and constitute a long-awaited experimental support to the current model of temperature homeostasis in mammals.

Using calcium-imaging in acute mouse brain slices, we showed that Trpm2-positive POA WSNs begin to show robust responses at ~38 °C, a thermal stimulus slightly above the physiological set point (also referred to as balance point) around 37 °C. Moreover, these warmth responses are significantly reduced in TRPM2-deficient (TRPM2-KO) mice, pointing to a possible role of preoptic TRPM2 during conditions of elevated body temperature (> 37 °C) [82]. Interestingly, in parallel with the identification of TRPM2 as a warmth sensor in the POA, it was reported that TRPM2 is expressed in dorsal root ganglia (DRG) neurons where it participates in environmental warmth detection. In this parallel study, it was shown that TRPM2-KO mice are impaired in their ability to discriminate environmental temperatures between 33 and 38 °C [85]. Together, the two reports strongly suggest that TRPM2 is implicated in temperature detection within a narrow range across the physiological warmth➔hot range in both central and peripheral locations [91].

Detailed examination of the thermal responses in cultured POA neurons led us to the identification of warmth-activated currents with a linear current/voltage relationship that reverts near 0 mV. Congruently with our hypothesis, the TRPM2 agonist adenosine 5′-diphosphoribose (ADPR) induces a TRPM2-characteristic current indistinguishable from the one observed during warming [82]. Interestingly, this current bears striking similarity to the linear current Kobayashi and colleagues had recorded in warmth-sensitive rat POA neurons [43]. Overall, these new results support the hypothesis that nonselective cationic currents promote temperature responses in POA neurons [56] and advocate a role of TRP ion channels in warmth sensitivity of WSNs.

However, we observed that in the absence of TRPM2 temperature responsiveness in POA neurons derived from adult mice was not completely abolished [82]. Thus, TRPM2 alone cannot explain all warmth responses and additional molecules (perhaps in combination with TRPM2) may participate in POA thermosensitivity. Importantly, the likely existence of additional molecular POA temperature sensors—and/or compensatory mechanisms in the absence or TRPM2—may explain the lack of a central thermoregulatory phenotype in TRPM2-KO mice [82]. Among possible candidates that may contribute to central thermosensitivity, we and others have identified a number of additional thermoTRP channels expressed in the POA [27, 82, 86, 95]. However, so far, the role of these channels in POA temperature detection has not been experimentally explored. Moreover, the finely tuned, temperature-triggered firing rate changes of WSNs in the POA very likely are shaped by other (non-TRP) ion channels [95]. One likely scenario is that the interplay of different temperature-sensitive—and temperature-insensitive—ion channels generates WSN responses to subtle temperature fluctuations deep in the brain. Future work will shed light on the contribution and potential cooperation of these ion channels in central thermosensation.

In addition, we showed that pan-null TRPM2-deficient (TRPM2-KO) mice have an exaggerated fever response when compared to littermate controls. This result is consistent with the observation that TRPM2 seems to be activated by temperatures slightly above 37 °C and suggests that this channel may promote heat dissipation and protects against body overheating [82]. However, we cannot completely rule out that the observed fever phenotype in our global TRPM2-KO mice results from a combination of TRPM2 acting at central and peripheral locations. In agreement with the second possibility, it has been found that mice lacking all types of peripheral thermoreceptors (both cold- and warm/heat sensors) also have overshooting fever responses, probably due to the lack of peripheral feedback to the thermoregulatory center in the brain [64]. Disentangling the contribution of central vs. peripheral (TRPM2-dependent and independent-) temperature detection to body temperature regulation is an exciting research avenue that is worth exploring. This endeavor has now become feasible given that both, marker genes for peripheral and central thermosensitive neurons as well as genetic tools for neuronal inhibition and ablation have become available.

TRPM2 has been described as a multimodal channel that is gated by a number of stimuli including temperature, reactive oxygen species (ROS) [36, 48, 96] and adenosine diphosphate ribose (ADPR) [71, 75]. Thus, additional intracellular stimuli may, perhaps together with changes in local POA temperature, modulate TRPM2 activity. Furthermore, TRPM2 itself may function as a multimodal sensory receptor, detecting different clues from internal and external cellular environments.

Genetically defined POA thermoregulatory neurons

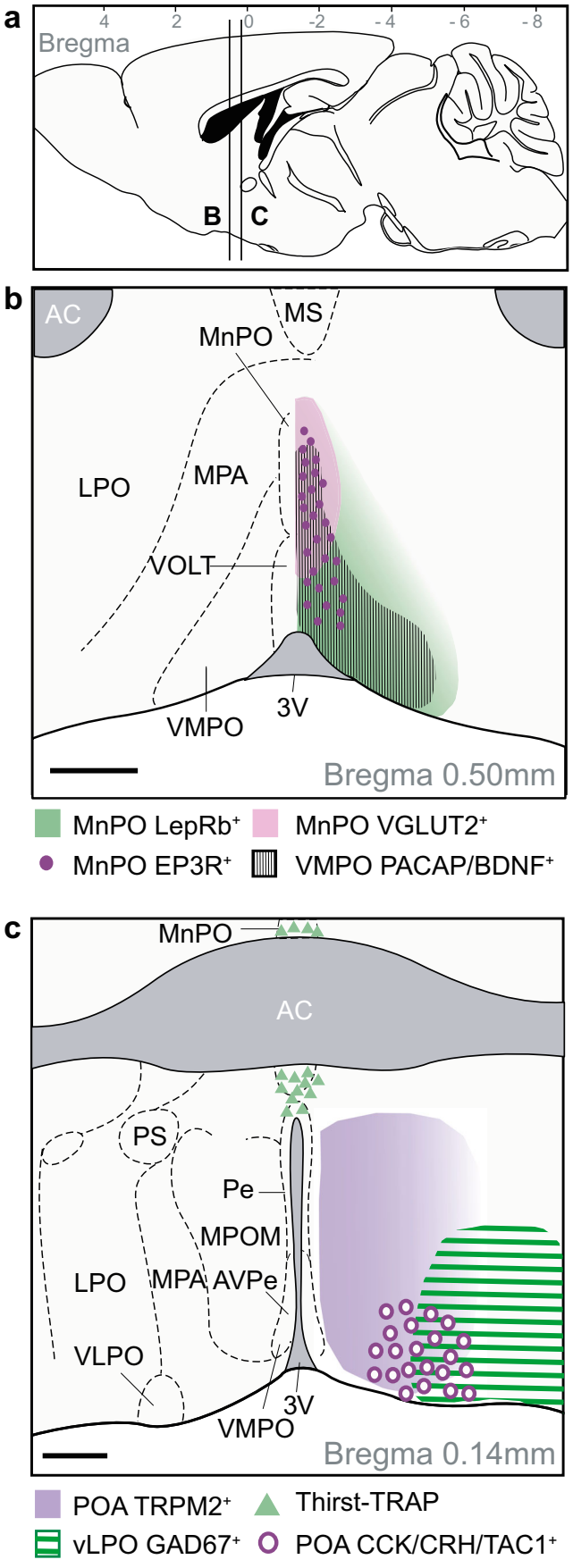
Prevailing models of thermoregulation propose that peripheral thermoreceptors located primarily in the skin and spinal cord send local temperature information to temperature-insensitive neurons of the median preoptic nucleus (MnPO). Consecutively, GABAergic and glutamateric MnPO neurons project to the medial and lateral preoptic area (MPO and LPO) where they modulate the activity of WSNs. In turn, WSNs integrate these peripheral thermal inputs with deep brain temperature information. WSNs are also believed to further integrate inflammatory and metabolic signals to control the level of activation of different downstream brain regions involved in thermoregulation [67]. Based on the findings that knife transections done directly caudal to the POA activate BAT thermogenesis [18] and lesions in the POA significantly increases body temperature [84], it has been suggested that WSNs are mainly GABAergic and inhibit heat-generating peripheral processes. According to this model, distinct groups of WSNs control parallel but discrete thermoregulatory pathways that exert tonic inhibition over specific downstream relay stations

involved in the control of thermoeffector organs including BAT activation, cutaneous blood flow, cardiac output, and metabolic heat production [67].

Although formulated in its simplest version decades ago, this model of body temperature regulation, and in particularly the role of WSNs in temperature homeostasis, was based mainly on circumstantial evidence and direct experimental support for this hypothesis was lacking. In particular, the extensive functional heterogeneity of POA neurons and the lack of cell markers to identify WSNs and to discriminate them from intermingled but functionally unrelated POA neurons hindered further progress toward the identification of the molecular mechanisms and neuronal circuits involved in thermoregulation.

Very recently, this situation has changed thanks to a number of studies published within the last 2 years [2, 82, 86, 99, 100]. In light of these publications, our knowledge about the mechanisms of central thermosensation and the cell populations involved in mammalian thermoregulation has grown considerably (Fig. 1). The key of this ground-breaking progress was (i) the use of cellular and biochemical assays that allowed the identification of molecules specifically expressed in thermoregulatory neurons, and the combination of (ii) "classic physiology" with (iii) state of the art genetic tools to distinguish and manipulate the activity of cell-type specific groups of neurons (namely optogenetics, pharmacogenetics, fiber photometry, among others). The principal research scheme followed in these studies can be summarized as follows: (1) use of a stimulation paradigm that allowed the activation of neurons likely involved in thermoregulation, (2) identification of cell markers expressed in these activated POA neurons, and (3) evaluation of the effect controlled activity changes in these discrete neuron populations have on body temperature homeostasis.

Using this strategy, two independent groups observed that environmental warmth (35 °C) activated a population of neurons expressing the neuropeptides pituitary adenylate cyclase-activating polypeptide (referred to as PACAP) and brain-derived neurotrophic factor (BDNF). These neurons were found to reside largely—albeit not exclusively—in the anterior ventromedial preoptic nucleus (VMPO) and the ventral portion of the lateral preoptic area (vLPO) of mice (Fig. 1, Table 1) [86, 100], brain regions previously associated with processing of peripheral thermal information in the POA [67]. Different to the aforementioned Trpm2-expressing POA neurons that are intrinsically thermosensitive and that are found in an overlapping POA region [82], the population of PACAP/BDNF-positive (PACAP/BDNF^{+}) neurons appear to be largely temperature insensitive [86]. Therefore, we prefer to use the term warmth-activated neurons (WANs) to differentiate them from intrinsically warm-sensitive neuronal (WSN) populations in the POA, such as Trpm2-expressing cells.



◀ **Fig. 1** Newly genetically-defined populations of preoptic area (POA) neurons. **a** Mid-sagittal scheme of an adult mouse brain showing the approximated locations of coronal sections displayed in inset B and C. Bregma scale is expressed in mm. **b**, **c** Schematic spatial distribution of molecularly defined neuronal populations in the anterior (B) and middle (C) part of the POA. References: LepRb$^+$, leptin receptor-expressing neurons; EP3R$^+$, prostaglandin E receptor 3 expressing neurons; VGLUT2$^+$, vesicular glutamate transporter 2 expressing neurons; PACAP/BDNF$^+$, pituitary adenylate cyclase-activating polypeptide and brain-derived neurotrophic factor expressing neurons; TRPM2$^+$, transient receptor potential melastatin 2 expressing neurons; GAD67$^+$, glutamate decarboxylase isoform 67 expressing neurons; CCK/CRH/TAC1$^+$, cholecystokinin, corticotropin-releasing hormone and tachykinin1 expressing neurons; Thirst-TRAP, thirst-associated preoptic neurons; MS, medial septal nucleus; MnPO, median preoptic nucleus; MPA, medial preoptic area; LPO, lateral preoptic area; VOLT, vascular organ of the lamina terminalis; VLPO, ventrolateral preoptic nucleus; VMPO, ventromedial preoptic nucleus; 3 V, third ventricle, PS, parastrial nucleus; MPOM, medial preoptic nucleus medial part; AVPe, anteroventral periventricular nucleus; Pe, periventricular hypothalamic nucleus; vLPO, ventral part of the lateral preoptic nucleus. Scale bars: 250 μm (schemes modified from Paxinos and Franklin, 2001)

Although slightly more ventrally located, the group of PACAP/BDNF$^+$ VMPO neurons share a number of interesting characteristics with MnPO neurons that have been previously described as the first point of entry of peripheral temperature information into the hypothalamic thermoregulatory center [66, 67]. First, both populations are believed to be primarily intrinsically temperature insensitive. Second, PACAP/BDNF$^+$ POA neurons respond to changes in ambient temperature within seconds, strongly suggesting that these neurons receive inputs from peripheral thermoreceptors located in the skin. Third, stimulation of PACAP/BDNF$^+$ VMPO neurons in vivo activates autonomic as well as behavioral heat loss mechanisms including tail vasodilation, decrease of BAT thermogenesis, and changes in preferred temperature to cooler regions in behavioral temperature-choice paradigms.

Whether the inhibitory neurons identified in the vLPO by Zhao et al. are intrinsically temperature sensitive is at present unclear [100]. These vLPO neurons bidirectionally trigger hypothermia and hyperthermia when activated and inhibited, respectively. Additionally, these neurons mediate their effect on body temperature homeostasis, at least partially, by projecting to the dorsal part of the dorsomedial hypothalamus (DMD), where they inhibit neurons involved in energy expenditure and physical activity [100]. Thus, many of the properties of these GABAergic vLPO neurons bear striking similarities to those attributed to POA WSNs, including their neuroanatomic location within the POA.

It has been proposed that the neuronal population receiving thermal information from warmth-activated skin thermoreceptors in the median preoptic area (MnPO) are principally glutamatergic [66]. Although both studies found that PACAP/BDNF$^+$ VMPO/vLPO neurons also contain glutamatergic neurons, by and large they are mostly GABAergic and exert their effect on thermoregulation, at least in part, by sending direct inhibitory projections to the DMD to inhibit BAT activity. Thus, the predominant picture of the role of MnPO neurons in thermoregulation is probably incomplete and additional groups of VMPO and vLPO neurons that receive peripheral temperature information are also part of the thermoregulatory circuit. It is of course possible that these VMPO and vLPO neurons receive input from MnPO neurons, an aspect future tracing studies will have to address.

Additionally, given the somewhat diffuse "salt-and-pepper" distribution of PACAP/BDNF$^+$ and Trpm2$^+$ neurons in only loosely anatomically defined locations across the POA, it is entirely possible that closer scrutiny may find these two populations to partially overlap. In that case, the use of intersectional genetics [5, 60] may help in future studies to further refine and identify more discrete thermoregulatory pathways and their interaction with metabolic and other homeostatic circuits.

Next to PACAP/BDNF$^+$ neurons, two recent reports identified two additional neuronal populations in the MnPO that promote heat loss in mice (Fig. 1, Table 1) [2, 99]. First, Yu et al. observed that activation of leptin receptor-expressing neurons (LepRb$^+$) in the MnPO decreases body temperature and energy expenditure and promotes body extension, a behavioral thermoregulatory response observed in animals exposed to high ambient temperatures. Moreover, the authors found that LepRb$^+$ MnPO neurons are preferentially activated by environmental warmth (30 °C) but not by cold exposure (4 °C), suggesting that LepRb$^+$ MnPO neurons also receive thermal information from skin thermoreceptors. In contrast to PACAP/BDNF$^+$ neurons, the effects on body temperature and energy homeostasis of LepRb$^+$-neuronal activation seem to be mediated principally by glutamatergic neurons [99]. In agreement with these findings, Abbott and colleagues found that specific and restricted optogenetic activation of glutamatergic MnPO neurons recapitulated the phenotype of LepR neurons and caused severe hypothermia [2]. In addition to this hypothermic effect, they observed that stimulation of glutamatergic MnPO neurons promotes water consumption and wakefulness. However, at present, it is unclear whether the cell populations mediating these two types of responses (hypothermia and water consumption) are partially overlapping or mutually exclusive [2, 4].

Taken together, these studies demonstrate that both GABAergic PACAP/BDNF$^+$ neurons and glutamatergic LepRb$^+$ MnPO neurons relay thermal information from warmth-activated skin thermoreceptors to downstream POA neurons that subsequently promote autonomic and behavioral peripheral heat loss responses. Interestingly, thermoregulatory MnPO neurons send projections to both medial and lateral POA- (MPA- and LPO-) neurons [1, 100]. It would be interesting to know whether the aforementioned MnPO neuronal populations mediate their thermoregulatory effect mainly by direct innervation of downstream nuclei outside the POA (as observed in case of some PACAP/BDNF$^+$ neurons) or whether these neurons primarily innervate WSNs located in the

Table 1 Functional properties observed in preoptic area neuronal populations involved in thermoregulation

Neuronal population	Identified cell markers	Reported properties associated with thermoregulatory functions	Ref.
MnPO LepRb$^+$	Leptin receptor	• Pharmacogenetic activation suppresses energy expenditure, promotes heat dissipation, and triggers profound hypothermia. • Exposure to ambient warmth (30 °C) activates preferentially glutamatergic LepRb$^+$ neurons in the POA.	[99]
MnPO VGLUT2$^+$	Vesicular glutamate transporter 2	• Optogenetic stimulation produces bradycardia and severe hypothermia due to activation of cutaneous vasodilatation. • In some cases, neuronal activation also promotes drinking behavior.	[2]
VMPO PACAP/BDNF$^+$	Pituitary adenylate cyclase-activating polypeptide Brain-derived neurotrophic factor	• Neurons are activated within seconds after exposure to environmental heat (35 °C). • Neurons appear predominantly intrinsically temperature insensitive. • Optogenetic stimulation reduces body temperature via autonomic (tail vasodilation and BAT inactivation) as well as behavioral thermoregulatory mechanisms. • This population projects to several brain regions including the dorsomedial hypothalamus (DMH), where they inhibit BAT thermogenesis.	[86]
POA TRPM2$^+$	Transient receptor potential melastatin 2	• Warm-sensitive neurons. • Pharmacogenetic activation and inhibition cause opposite effects on body temperature triggering severe hypothermia and hyperthermia, respectively. • Similar to results in [99], hypothermia is driven by the glutamatergic fraction of POA neurons.	[82]
vLPO GAD67$^+$	Glutamate decarboxylase isoform 67	• Neurons are activated by exposure to environmental heat (38 °C). • Optogenetic activation and inhibition produce opposing effects on body temperature inducing hypothermia and very high hyperthermia, respectively. • This population projects to the dorsal part of the dorsomedial hypothalamus where they promote heat loss responses.	[100]

lateral and medial POA. In the latter case, it can be assumed that they modulate the activity of these deep brain thermoreceptors and POA WSNs may—after all and as predicted by classic literature—integrate and process both peripheral and deep brain temperature signals.

Refining the current model of thermoregulation

We found an additional group of POA neurons that is involved in thermoregulation. These neurons express the ion channel TRPM2 (TRPM2$^+$) and are, so far, the only discrete population of POA neurons that has been shown to be intrinsically thermosensitive. Based on the observations that TRPM2$^+$ POA neurons can detect thermal changes within the physiological temperature range, their location in the MPO and LPO (Fig. 1), and the finding that their stimulation and inhibition can produce severe hypothermia and hyperthermia respectively, we propose that preoptic TRPM2$^+$ neurons belong to the pool of WSNs [82]. While TRPM2$^+$ neurons come in two flavors and—similar to PACAP/BDNF+ neurons—can be either GABAergic or glutamatergic, we observed that glutamatergic- (but not GABAergic-) neurons are primarily responsible for thermoregulation [82]. This is in agreement with another study that also showed that POA glutamatergic neurons can drive robust hypothermia [99]. Our results provided compelling evidence supporting the long-proposed role of intrinsically thermosensitive POA neurons in thermoregulation. However, the current view that WSNs are exclusively GABAergic needs to be expanded to include also excitatory WSNs.

Overall, the general picture that emerges from these studies is widely congruent with the classic model describing the central circuits controlling thermoregulation in mammals [66]. Nevertheless, there is one aspect of the prevailing view of WSNs that appears inconsistent with the latest findings: None of the new studies discussed in this review found evidence for a discrete population of POA neurons controlling a single and selective thermoregulatory effector organ as has been predicted by previous models [62, 66]. Instead, the newly described groups of POA neurons share the ability to engage a number of different thermodefensive responses, including both behavioral and autonomic mechanisms. One possible explanation for this perceived discrepancy to the current model is that the coarse molecular blueprint that we have obtained of the hypothalamic thermoregulatory center thus far has not yet yielded genetic markers for more refined subsets of POA neurons that engage, e.g., only BAT or only cutaneous blood

flow etc. It is, however, also feasible that POA neurons, and in particular WSNs, preferentially work as an integrated unit to produce coordinated responses in multiple/all thermoregulatory effector organs via the activation of downstream, more effector-specific hypothalamic and extra-hypothalamic relay stations. Future efforts are needed to refine the current model by uncovering further thermoregulatory cell populations and their connectivity to ultimately describe how body temperature is encoded and modulated.

The POA, including the MnPO, is involved in the regulation of several other homeostatic parameters besides the control of body temperature. These include (but are not limited to) sleep, sexual maturation fertility and social interaction, the control of inflammatory reactions, and the regulation of thirst and water balance [76]. Reflecting these diverse functionalities are highly diverse neuronal populations that reside in this evolutionary ancient brain region, many of which can be identified by the expression of discrete neuropeptides and receptors, such as cholecystokinin, corticotropin-releasing hormone, tachykinin 1 (regulating sleep [19]), gonadotropin-releasing hormone (GnRH), kisspeptin (regulating fertility—[42]), prostagalandin EP3 recepter (controlling fever—[58]), and PACAP & BDNF (identified in thirst-associated MnPO neurons—[4]) (Fig. 1, Table 1). Interestingly, all of these diverse homeostatic systems modulate body temperature: the sleep-wake cycle modulates body temperature in a circadian fashion; female fertility—via the estrous cycle—modulates body temperature with higher T_{core} during the luteal phase and lower T_{core} during the follicular phase; homeostatic immunological defense responses can modulate T_{core} by mounting the febrile reaction (fever) that is orchestrated by the POA.

These functional interactions suggest that the responsible neuronal populations—and their underlying circuits—are likely to (reciprocally) interact with POA thermoregulatory neurons. Given the medical importance of these physiological systems, it will thus be highly valuable to understand the crosstalk between WSNs and WANs and hypothalamic neurons involved in regulating the sleep-wake cycle, fertility, energy metabolism, and the febrile response.

Unsolved questions and perspective

The research interest in the field of thermoregulation has been freshly infused by several the recently published studies. Based on these exciting findings new questions have sprung up. Among those questions, we list here those we find most eminent in order to understand the mechanisms orchestrating body temperature homeostasis.

1. The identification of TRPM2 as a component of the temperature detection machinery in the POA constitutes a much-needed step forward to the elucidation of thermosensory mechanisms in the brain. However, current evidence indicates that additional molecules are involved in this process. Elucidating the role of further functional components participating in POA temperature detection will undoubtedly be critical to comprehend how our brain controls body temperature homeostasis. Additionally, this knowledge will be instrumental to envision novel therapies to control body temperature, especially under pathological conditions.
2. In addition, unveiling how the thermoregulatory system is modulated by factors such as circadian rhythm, estrous cycle, and metabolic state constitutes another exciting research area, where information at the anatomical, molecular, and cellular level currently is still very limited.
3. Temperature affects many (if not all) aspects of life. The reverse is true as well and many biological processes affect temperature (e.g., by generating heat). Thus, the thermoregulatory center not only receives diverse modulatory inputs but likely also generates diverse outputs. Neuronal tracing studies combined with functional interrogations will be instrumental to probe output pathways and to assess their physiological roles.
4. Plasticity, a concept that is widely studied in many areas of neurobiology, has thus far only been very superficially touched upon in the context of circuits regulating body temperature. For example, long-term changes in the external and internal conditions known to affect thermoregulatory properties, including acclimatization to novel thermal environments or development of metabolic disorders, may cause plastic changes in the brain circuits that control body temperature. If and how these persisting stimuli reshape the activity in the preoptic thermoregulatory center and associated areas remain to be elucidated. Along the same lines, how plasticity in the thermoregulatory center drives physiological (and pathological) changes is of medical importance and data provided by corresponding studies may allow the design of therapeutic paradigms geared to promote (or correct) those adaptations.
5. Finally, the aforementioned new studies have expanded our view on how the thermoregulatory information flows within the POA and downstream brain areas. A next challenge will be to further dissect the specific contribution of particular POA areas (such as the MnPO, VLPO, and MPO) to the processing of thermoregulatory inputs. In particular, the role of POA WSN versus temperature-insensitive neuronal populations in these areas remains to be elucidated.

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