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FRONT MATTER: COMMENT

Thermoregulation in mice: The road to understanding torpor hypothermia and the shortcomings of a circuit for generating fever

Comment on: Machado NLS, Saper CB. Genetic identification of preoptic neurons that regulate body temperature in mice. Temperature. 2022;9(1):14-22. DOI:10.1080/23328940.2021.1993734

In their review, "Genetic identification of preoptic neurons that regulate body temperature in mice", Machado and Saper [1] summarize and interpret the results of several recent studies in which the latest genetic and molecular approaches were employed to genetically specify populations of thermally responsive neurons in the preoptic area (POA) of mice and to observe the changes on core body temperature (Tc) evoked by stimulating or inhibiting their cell bodies or axon terminals. This review is a useful summary of many of the key findings related to POA thermoregulatory neurons that would need to be incorporated in functional models of the neural circuitry mediating mouse thermoregulatory responses, including not only cold- and warm-defense, but also fever and the hypothermia of cold-evoked torpor. In stark contrast to rats and humans, mice depend heavily on the cold-defense mechanisms of somatic activity thermogenesis and torpor, suggesting that there must be several aspects of the functional organization of their thermoregulatory circuitry, including that in the POA, that are unique to mice. Thus, it will be of particular interest to determine the wider applicability to other mammalian species of the new discoveries regarding central thermoregulatory circuits being made through genetic manipulation approaches in mice. However, despite several detailed studies on thermoregulatory neurons in mice, including those described in this review, many of the fundamental aspects of the neural circuits that function to explain even the most basic aspects of mouse thermoregulation, such as cold- or warm-defense, energy-conserving torpor hypothermia, and pathogen-combating fever, remain to be elucidated.

The authors describe some of what is known of the considerable heterogeneity with regard to genetics, projection patterns, and receptor and neurotransmitter expression within the population of VGLUT2-expressing neurons in the mouse POA. Such POA neurons would presumably include projection neurons and interneurons, as well as those potentially involved in either sympathoexcitatory or sympathoinhibitory pathways [2,3]. Against this background, it is surprising that the authors make sweeping conclusions about the thermoregulatory roles of VGLUT2- and VGAT-expressing neurons based on the effects on Tc observed after activating or inhibiting all of the POA neurons in either of these populations. A simple example illustrates the problem with deriving conclusions from this unphysiological (i.e., it is unlikely that all neurons in either of these populations are excited or inhibited simultaneously) approach to circuit analysis. Activating all VGLUT2-expressing neurons in the POA would simultaneously drive two pathways: (a) the VGLUT2-expressing neurons in the authors' graphic targeting GABAergic interneurons in RPa and inhibiting BAT sympathetic premotor neurons to reduce BAT thermogenesis and decrease Tc, and (b) the VGLUT2-expressing, glutamatergic neurons that excite thermogenesis-promoting neurons in the DMH to drive BAT during cold defense and fever [3]. However, because RPa neurons are being inhibited by pathway (a), the BAT activation that would normally be caused by potential (in mice) pathway (b) would not occur, leading to the incorrect conclusion that most VGLUT2-expressing neurons in the POA are "hypothermic". Buying into such an erroneous conclusion masks the opportunity to reveal a potential glutamatergic excitatory input to DMH from MnPO that could provide the excitation of thermogenesis-promoting neurons required for the febrile increase in Tc.

The review by Machado and Saper highlights their recent discovery of an important component within the neural circuit for fever generation in mice. A subset of the EP3R-expressing neurons in the median preoptic nucleus (MnPO) that also express VGLUT2 mRNA, which is considered as a marker of glutamatergic neurons, are required for the complete expression of PGE_2-mediated fever in mice. Such neurons could comprise a fundamental piece of the fever-generating circuit in mice since they would transduce the interstitial PGE_2 inflammatory signal in the POA into alterations in the activity of the thermoeffector-regulating neurons in the dorsomedial hypothalamus (DMH)

and rostral raphe pallidus (RPa) [2] that are responsible for the febrile increases in Tc. Machado and Saper provide a graphical illustration of how their findings might fit into the general thermoregulatory circuitry that has been elucidated primarily from experiments in rats [2]. PGE_2 is generally accepted to hyperpolarize EP3R-expressing neurons in the POA, and thus would reduce the activity of the EP3R- and VGLUT2-expressing neurons in MnPO that project to the DMH and the RPa. Beyond this graphical illustration of their basic finding, the remaining aspects of their "model" for fever generation remain conjectural, and thus, their roles in fever generation in mice remain to be determined.

As previously proposed in rats, although with a slightly different circuit [2], the PGE_2-mediated inhibition of EP3R-containing neurons that project to the DMH and the RPa leads to a "disinhibitory" model in which PGE_2 binding to EP3R on POA neurons reduces an inhibitory input to neurons in the DMH and the RPa, thereby increasing their activity and resulting in the febrile elevation in Tc. Since the EP3R-expressing neurons discovered by Machado and Saper express VGLUT2 and are thus presumed to be glutamatergic, they have hypothesized that these neurons synapse on GABAergic interneurons in the DMH and RPa. In this scenario, PGE_2-mediated inhibition of the MnPO excitatory inputs to DMH and RPa would reduce the drive to these GABAergic interneurons and disinhibit the sympathoexcitatory neurons in DMH and RPa, leading to an increase in Tc. However, the actual DMH and RPa target neurons of the VGLUT2- and EP3R-expressing MnPO neurons remain a critical, but unknown aspect of the proposed mouse fever circuit. In this regard, there are neurons in the DMH that project to other brain regions besides the RPa that are involved in regulating thermoeffector activity. Thus, since the postsynaptic targets of the MnPO, VGLUT2-expressing input to DMH are unknown, it is not unreasonable to entertain a potential location outside of DMH (e.g., the ventrolateral preoptic area [3]) for the disinhibition-relevant GABAergic input to DMH and RPa. Along these lines, since EP3R deletion from VGLUT2-Cre neurons would affect both POA interneurons and projection neurons, Machado and Saper's data do not exclude a potential role for EP3R- and VGLUT2-expressing, warm-responsive interneurons in the POA that could activate POA GABAergic neurons projecting to the DMH (or RPa) [2]. PGE_2 signaling to these EP3R-expressing interneurons would inhibit POA GABAergic projection neurons to disinhibit the sympathoexcitatory neurons in the DMH and RPa – in essence a parallel disinhibitory pathway to that described in the review.

In addition, simply removing an inhibition from DMH or RPa neurons will not increase their activity – they must have active excitatory inputs, and the identification of these inputs is critical to elucidating the mouse fever circuit. Machado and Saper seem to recognize this aspect, since they illustrate a "stress" excitation of DMH neurons, but in this scenario, unstressed mice could not generate fever. Regarding stress-related inputs to the DMH, a recent study in rats led to the discovery of an important glutamatergic stress pathway from the prefrontal cortex to the DMH that drives a PGE_2-independent, psychogenic fever through DMH-mediated thermogenesis and cardiovascular stress responses, including cutaneous vasoconstriction [4], the latter in contrast to stress responses in mice [5]. The actual source of the glutamatergic excitatory inputs to the mouse DMH and RPa required for PGE_2-mediated fever, perhaps arising from neurons in the MnPO as has been demonstrated in rats [3], remains unknown.

In rats, the thermoregulatory circuits controlling cutaneous vasoconstriction are quite distinct from those regulating BAT thermogenesis [2], and this would appear to be the case in mice as well [5]. Thus, a simple and generalized graphic in which the control of all of the fever-relevant thermoeffectors is lumped together does not accurately represent the mouse thermoregulatory circuit for fever generation. For instance, the simplified graphic in this review could easily be misinterpreted to suggest that neurons in the DMH drive the febrile increase in cutaneous vasoconstriction, which the authors' recent publication [5] as well as data in rats [2] suggests is not the case. Along these same lines of the differential regulation of thermoeffectors, shivering thermogenesis is the most significant source of facultative heat production in humans, but the significance of shivering (or of somatic activity) thermogenesis in mouse fever appears to be unknown and is not even represented in the authors' graphic. Along these lines, it will also be of considerable clinical significance to understand the relevance of our increasing insight into the neural mechanisms underlying COX/PGE_2-dependent fevers to the symptomatic (e.g., shivering) treatment of COX-independent hyperthermias such as psychogenic and viral fevers, and those arising from ischemic and traumatic brain injuries.

The most exciting and potentially clinically relevant aspect of the recent genetic analyses and targeting of mouse POA neurons involved in thermoregulation is the characterization of neurons that are active during torpor and whose experimental activation induces a long-duration hypothermia. The underlying circuit mechanisms for this prolonged and deep hypothermia, which could be highly relevant for inducing therapeutic hypothermia, remain to be elaborated. Since mouse torpor is generated by cold exposure, it may be confusing, particularly with regard to

VGLUT2-expressing, torpor-active neurons, that throughout the review, the authors conflate warm-responsive neurons and torpor-active neurons. In this regard, what the authors appear to be suggesting is that the mouse torpor-generating circuit may employ some of the same sympathoinhibitory neurons in the POA that mediate the warm-evoked inhibition of the sympathetic outflows to BAT and to the cutaneous blood vessels. Indeed, as pictured in the authors' graphic, a MnPO neuron that glutamatergically excites a GABAergic inhibition of thermogenesis-promoting neurons in the DMH, could function to inhibit BAT thermogenesis both during warm-exposure and during torpor. What the authors fail to make clear is that in such a scenario, this neuron would have to be excited both by the skin warm afferent pathway through the dorsal subnucleus of the lateral parabrachial nucleus [2] during warm exposure, and by the cutaneous cold afferent pathway through the external lateral subnucleus of the lateral parabrachial nucleus [2] during the induction and maintenance ofevoked torpor. Whether this occurs, or whether there are two, distinct, but likely PACAP-expressing, subpopulations of POA sympathoinhibitory neurons employed during warm exposure and during torpor, perhaps excited by different thermal afferent pathways from the lateral parabrachial nucleus, remains to be determined. Additionally, rather than eliciting a hypothermia as the authors suggest, the integrated response to warm exposure defends normothermia, at least in rats, via warmth-augmented, tonic inhibitory signaling from the POA to the DMH and RPa [2]. Our "thermoregulatory inversion" model [6] in rats for torpor induction and maintenance provides a novel framework for the integration of normal thermoregulatory responses and the torpor-generated inhibition of thermogenesis.

In contrast to rats and humans, mice mount a two-pronged thermoregulatory response to cold-exposure, depending on the availability of food. In a cool environment typical of laboratory vivaria in which food is constantly available, mice consume significant quantities of the metabolic energy they obtain from food to generate heat through BAT and somatic activity thermogenesis. However, if a cold ambient temperature is coupled with the absence of food, mice switch to the cold-defensive, energy-conserving response of torpor. This response is characterized by a paradoxical "thermoregulatory inversion" [6] in which cutaneous cold-sensory signals now elicit an inhibition of BAT and activity thermogenesis leading to the hypothermia and inactivity characteristic of mouse torpor. This novel state of thermoregulatory inversion is self-sustaining and could account for the maintenance of torpor hypothermia: in a feedforward manner, cold-driven inhibition of thermogenesis lowers Tcore in a cold environment, which continues to drive the inhibition of thermogenesis and thus sustain a low Tcore. The neural circuitry underlying such a state of thermoregulatory inversion that may mediate mouse torpor remains a mystery. Unlike shivering, the use of activity thermogenesis for cold-defense is unique to mice (vs rats or humans). The neural circuitry underlying this phenomenon and its inhibition during torpor have not been investigated. Clearly, the various torpor-active neurons described in this review, whose activation leads to a deep and prolonged hypothermia similar to that during mouse torpor, would be possible candidates to mediate the inhibition of BAT and somatic activity thermogenesis via a torpor induction mechanism consistent with our thermoregulatory inversion model. Having thus made the switch to thermoregulatory inversion, the torpor state would be self-sustaining in a subthermoneutral environment, accounting for the evoked hypothermia that is observed to long outlast the exogenous stimulation of the torpor-active neurons in mice. Since the thermoregulatory inversion model for induction and maintenance of the hypometabolic hypothermia of torpor has yet to be tested in mice, it remains to be determined whether some populations of torpor-active POA neurons switch their thermal responsiveness during mouse torpor, and how the several groups of torpor-active POA neurons control the many physiological mechanisms besides thermoregulation that are altered to produce the new hypometabolic state of torpor homeostasis.

In conclusion, the identification and manipulation of genetically specified populations of neurons with potential roles in mouse thermoregulation provides a useful basis for the development, testing and modification of hypotheses on the neural circuitries underlying the several strategies that mice employ for the control of their metabolism and body temperature. In turn, this information might provide important clues to molecules that could be promising candidates for therapeutic manipulation of body temperature and metabolism in humans. Nevertheless, the unique aspects of thermoregulatory control in mice demands that new insights into the central neural circuits controlling mouse thermoregulation be tested for their relevance to our understanding of thermoregulatory circuits in other mammals.

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