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Do catecholaminergic TrkC DRG neurons represent a class of cardiovascular enteroceptor?

Highlights

- The functional specialization of TrkC$^+$ TH$^+$ neurons remains unknown
- Deafferentation, spinal cord lesion, and sympathectomy are not usually lethal
- Vasoconstriction via TrkC neuron activation is consistent with somatosympathetic reflex
- Lethality could be explained by loss of vagal TrkC neurons

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In brief

In a recent issue of *Cell Reports*, Morelli et al. (2021) propose that catecholaminergic TrkC DRG neurons represent a baroreceptor class of homeostatic enteroceptor. McMullan et al. present an alternative interpretation of their findings and suggest priorities for future studies.

McMullan et al., 2022, Cell Reports *38*, 110082
January 18, 2022
https://doi.org/10.1016/j.celrep.2021.110082

Cell Reports

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Matters Arising

Do catecholaminergic TrkC DRG neurons represent a class of cardiovascular enteroceptor?

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https://doi.org/10.1016/j.celrep.2021.110082

SUMMARY

In a recent issue of *Cell Reports*, Morelli et al. (2021) identify a subpopulation of mechanosensitive peripheral sensory neurons that coexpress tyrosine hydroxylase (TH) and tropomyosin receptor kinase C (TrkC) and innervate cutaneous arterioles. They show that activation of TrkC sensory neurons causes cutaneous vasoconstriction and, most remarkably, that their lesion is associated with sudden death of an undetermined cause, preceded by a progressive drop in blood pressure, and conclude that $TrkC^+$ TH^+ neurons represent a baroreceptor class of homeostatic enteroceptor. This represents a radical departure from current consensus models for the central control of blood pressure. Here, we offer an alternative perspective on their findings and suggest priorities for further investigation. This Matters Arising paper is in response to Morelli et al. (2021), published in *Cell Reports*. See also the response by Heppenstall et al. (2022), published in this issue.

The sympathetic nervous system is the master controller of peripheral vascular resistance, representing the mechanism through which the body balances supply and demand of oxygen, nutrients, warmth, and waste elimination. Populations of sympathetic premotor neurons within the medulla and hypothalamus achieve this using a combination of feedforward signals, which transmit stereotyped patterns of sympathetic output according to behavioral state (e.g., diverting blood from the gut to muscles in anticipation of exercise) and feedback from peripheral receptors that convey information about the internal and external environments (e.g., peripheral chemoreceptor afferents encode arterial oxygen tension) (Dampney, 1994; Guyenet, 2006). This intricate machinery is entirely autonomous and requires no input from the "conscious" brain; the neural circuits that regulate the body's metabolic economy become active before birth and remain so throughout life, their silence defining the moment of brain death.

In a recent issue of *Cell Reports*, Morelli et al. (2021) describe a population of peripheral sensory neurons, defined by their coexpression of tropomyosin receptor kinase C (TrkC) and tyrosine hydroxylase (TH), which they propose plays an important role in cardiovascular regulation. Using intersectional genetics, immunohistochemistry, physiological recordings, and *in vivo* imaging, the authors show that $TrkC^+$ TH^+ neurons innervate small blood vessels, that optogenetic or chemogenetic activation of TrkC sensory neurons causes cutaneous vasoconstriction, and most remarkably, that lesion of TrkC sensory neurons is associated with sudden death that is preceded by a progressive drop in blood pressure.

The discovery is striking on several levels. First, sensory signals that are relevant to the ongoing control of blood pressure largely originate from vagal afferent neurons that reside within the jugular-nodose complex, project to visceral targets, and terminate directly in the brainstem. In contrast, the population described by Morelli et al. (2021) resides within the dorsal root ganglia (DRGs), innervates arteries and arterioles in the extremities, and (presumably) provides input to sympathetic neurons via spinal pathways. Although surprising, such an arrangement is not without precedent: it has long been established that noxious input from receptors in the viscera is important for the local and systemic regulation of sympathetic nerve activity, and that such input is encoded by a combination of vagal and spinal pathways (e.g., during angina pectoris; Longhurst et al., 2001). Similarly, somatic nociceptors that innervate the skin, muscle, and bone are a source of excitatory drive to sympathetic nerves via both spinal and supraspinal pathways (Beacham and Perl, 1964; Burke et al., 2011; Janig, 1975; Korim et al., 2011; McMullan et al., 2008; Sato and Schmidt, 1973), driving reductions in cutaneous and visceral blood flow, tachycardia, and pressor responses to acute noxious stimuli that reflect generalized sympathoexcitation observed at all spinal levels. Indeed, sympathoexcitation is so reliably evoked by nociceptor stimulation that pressor responses and cutaneous vasoconstriction are reliable indicators of anesthetic efficacy in clinical contexts (Ikuta et al., 1998). Importantly, under normal circumstances, such viscerosympathetic and somatosympathetic pathways are not considered a source of *ongoing* input to the central autonomic circuits: their activation is exceptional and always associated with actual or potential tissue injury.

With this in mind, some of the details of the study by Morelli et al. (2021) warrant careful attention: is it possible that the vasoconstriction observed in their gain-of-function studies is

attributable to nociceptor stimulation and activation of the classic somatosympathetic reflex? We think so. While the authors demonstrate sparse co-expression of TrkC with conventional markers of unmyelinated nociceptors (e.g., IB4, CGRP; see figure 1F), recent transcriptomic analyses (Zeisel et al., 2018) indicate expression of TrkC in other DRG populations that likely include myelinated mechanoreceptive and unmyelinated heat/itch nociceptors, corresponding to the PEP2 and NP2.1 DRG cell types defined by Emery and Ernfors (2018), for which there are no reliable immunohistochemical markers. Functional evidence of nociceptor activation is also clear from the authors' behavioral experiments, in which acute chemogenetic stimulation of $TrkC^{CreERT2}$::$Avil^{hM3Dq}$ neurons resulted in an enormous increase in sensitivity to punctate mechanical stimuli. The authors concede that such responses are consistent with nociceptor activation but suggest that this effect may be secondary to acute cutaneous ischemia. This seems unlikely: whereas chronic ischemia is certainly associated with a number of painful conditions, we are unfamiliar with any evidence that acute ischemia can evoke cutaneous hyperalgesia of this sort (indeed, common experience is that acute ischemia, as happens when a limb "falls asleep," is accompanied by anesthesia until reperfusion, an effect attributable to the high dependence of the sensory neuronal membrane potential on Na-K-ATPase) (Hofmeijer et al., 2013).

What then of the study's most striking finding: that ablation of TrkC sensory neurons is rapidly lethal and associated with a cardiovascular phenotype? Three questions are immediately raised.

First, what life-sustaining sensory modality might $TrkC^+$ DRG neurons convey? Using whole-cell patch recordings of isolated $TrkC^+$ DRG neurons, Morelli et al. (2021) confirm the mechanosensitive properties of these neurons; an expected finding given the widespread expression of the mechanotransducer Piezo2 in TrkC neurons in general and their association with cutaneous lanceolate endings (Fünfschilling et al., 2004; Kupari et al., 2019; Zeisel et al., 2018). However, the authors draw short of suggesting what sensory modality the subpopulation of $TrkC^+$ TH^+ neurons at the heart of this study might encode, without which a functional context for the other components of the study remains opaque.

Second, do $TrkC^{CreERT2}$::$Avil^{iDTR}$ mice die because of autonomic dysfunction, or might the cardiovascular abnormalities reported simply be a portend of impending doom because of something else? In an attempt to elucidate the functional relevance of TrkC afferents, Morelli et al. (2021) genetically ablated all peripheral sensory TrkC neurons. Over the 48-h period following diphtheria toxin-induced cell death, the authors observed transient cutaneous hypoperfusion, a gradual reduction in blood pressure, and an increase in heart rate variability, which they believe underlies the rapid deterioration of the animals.

However, no evidence of having considered other potentially deleterious mechanisms is presented in the paper (e.g., Was there respiratory distress? Metabolic or thermogenic dysfunction? Disturbed food or water intake or electrolyte balance?), and there appear to be some contradictions that hamper acceptance of this simple narrative. For one, the reported cardiovascular effects do not seem enough, on their own, to account for the demise of the animals: neither spinal cord trauma (lesion, transection, etc.), which disrupts both ascending sensory and descending sympathoexcitatory signals, nor chemical sympathectomy are lethal, and in fact the short-term loss of vascular tone associated with sympathetic lesion is rapidly buffered by vasoactive hormones (renin-angiotensin, vasopressin) that restore blood pressure within a few days. Second, the reported reduction in cutaneous blood flow seen at 16 h (indicating sympathetic *activation*) seem at odds with the hypotension (indicating sympathetic *inhibition*) seen at the same time point, whereas increased heart rate variability, interpreted by the authors as a *negative* prognostic indicator, is in fact, at least for short-term beat-to-beat measurements, normally considered to be a *positive* prognostic indicator, as outlined by the statement of the Task Force of the European Society of Cardiology referenced by Morelli et al. (2021). The value of these measurements is undermined by the notoriously inaccurate indirect technique used to measure blood pressure, which was performed under isoflurane anesthesia, and apparent errors in the labeling or analysis of these data. We note the Poincaré plots submitted in supplemental figures 4 and 6 have axes labeled as R-R interval in milliseconds, but electrocardiogram (ECG) R wave was not measured in these experiments, and the data suggest R-R intervals around 500 ms (i.e., a heart rate of 120 beats per minute [bpm], compared with the expected heart rate of ~500 bpm), most probably indicating that the measurement plotted was heart rate in bpm. The ellipses are normally plotted to include 95% of data points, and the lengths of the long and short axes used to determine long- and short-term variability, respectively. Figure 6D also appears to be mislabeled: experiments plotted in red (TrkC-hM3Dq+C21+Prop) seem to be the same experimental group as the red (averaged) plot in figure 6C (TrkC-hM3D+C21).

Physiological recordings made during chemogenetic gain-of-function add to the confusion. Acute chemogenetic activation of TrkC neurons evoked considerable pressor and tachycardic responses in $TrkC^{CreERT2}$::$Avil^{hM3Dq}$ mice that, surprisingly, were attenuated by the non-selective β-adrenoceptor blocker, propranolol. The finding was interpreted by Morelli et al. (2021) as evidence that pressor and heart rate responses are sympathetically mediated, but they neglected to consider that sympathetic vasoconstriction is mediated by α-adrenoceptors, with minimal interaction by propranolol. Although it is conceivable that the pressor response to activation of TrkC neurons was driven by the observed tachycardia, this possibility seems unlikely given the highly variable heart rate responses that suggest that, at least in some animals, no tachycardia was observed.

Third, are there any mechanisms beyond the DRG/sympathetic nervous system that could conceivably underlie the lethal effects of advillin-TrkC ablation? The answer to this question may reside within the vagal sensory ganglia. Although the authors' double-transgenic strategy limited expression of diphtheria receptor to cells that express the advillin gene, it is important to recognize that advillin is also widely expressed in sympathetic, enteric, and vagal ganglia, as well as DRG, suggesting other potential contributors to the observed effects (Hasegawa et al., 2007; Hunter et al., 2018; Nonomura et al., 2017). Like the DRG, TrkC is expressed in functionally diverse vagal afferent neurons (Lieu et al., 2011), with recent transcriptome mining expeditions

reporting significant expression of Ntrk3, the genetic precursor to TrkC, in jugular and nodose ganglion neurons likely corresponding to low-threshold and nociceptive mechanoreceptors, lung volume/stretch receptors, itch/heat nociceptors, and baroreceptors (Kupari et al., 2019). Could ablation of any of these account for the lethal phenotype of the TrkC^{CreERT2}::Avil^{iDTR} mouse? The available data suggest this is highly plausible: vagal sensory neurons that express Piezo2 encode lung stretch and airway distension, and Piezo2 deletion is associated with an embryo-lethal respiratory phenotype (Nonomura et al., 2017; Zhang et al., 2019), while inducible advillin-Piezo2 knockdown attenuates airway distension feedback in the adult mouse.

The authors report no recombination of YFP in sympathetic or vagal ganglia of TrkC^{CreERT2}::Rosa26$^{ChR2\text{-}YFP}$ mice, and therefore attribute the effects of their gain- and loss-of-function experiments to selective stimulation or ablation of DRG neurons. This is itself unexpected: TrkC is expressed in a significant proportion of adult sympathetic efferent (Ernsberger, 2009; Wetmore and Olson, 1995; Zhou and Rush, 1996) and visceral afferent neurons (Kupari et al., 2019; Lieu et al., 2011), and indeed nodose neurons are developmentally and transcriptionally indistinct from DRG neurons (Kupari et al., 2019; Zeisel et al., 2018), so one might expect to see significant YFP expression in these tissues.

In our view it seems premature to credit these neurons with a role in the ongoing regulation of the cardiovascular system until a more thorough investigation of their functional properties, elimination of physiological dysfunction in other vital systems, and proposal of a plausible mechanism of action. Direct measures of arterial blood pressure in conscious animals following TrkC ablation and additional measures of respiratory and homeothermic function, metabolic status, arterial blood chemistry, and pulmonary function would be helpful in establishing a cause of death.

The idea that DRG afferents encode the mechanical properties of the peripheral vasculature, and that such input is crucial for the neural control of the circulatory system, is radical. The findings of Morelli et al. (2021) are provocative and original, but they require careful validation and exploration of other potential explanations.

AUTHOR CONTRIBUTIONS

All authors reviewed Morelli et al. (2021) and developed the discussion points. S.M. drafted the manuscript with support from C.M.H. and P.G.R.B. All authors approved the final version of this Matters Arising.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: July 2, 2021
Revised: August 22, 2021
Accepted: November 10, 2021
Published: January 18, 2022

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