Cell Reports

Matters Arising Response

TrkC-CreERT2-mediated recombination supports evidence that TrkC^{+}/TH^{+} DRG neurons contribute to cardiovascular homeostasis

Highlights

- The TrkC^{CreERT2} BAC transgene does not fully reproduce the expression pattern of TrkC
- Cre-mediated recombination is absent in nociceptors and sympathetic or vagal ganglia
- Vasoconstriction is likely mediated by TrkC^{+}/TH^{+} DRG neurons and not nociceptors
- Strategies targeting only TrkC^{+}/TH^{+} DRG neurons are required to understand lethality

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In brief

Heppenstall et al. demonstrate that the absence of TrkC-CreERT2-mediated recombination in nociceptors or sympathetic or vagal ganglia suggest that it is TrkC^{+}/TH^{+} DRG neurons that regulate cardiovascular parameters.

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TrkC-CreERT2-mediated recombination supports evidence that TrkC^{+}/TH^{+} DRG neurons contribute to cardiovascular homeostasis

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SUMMARY

In their Matters Arising article, McMullan et al. (2022) offer alternative explanations for the phenotypes we observed upon stimulation and ablation of TrkC^{CreERT2}-positive neurons in mice. Their interpretations are focused on two aspects: first, whether the vasoconstriction we observed upon activation of TrkC^{CreERT2} neurons is really mediated by TrkC/TH-positive neurons, or whether it might stem from stimulation of somatic nociceptors that also express TrkC; and second, whether the lethality observed after ablation of TrkC^{CreERT2} neurons might be a result of ablation of vagal afferents and not TrkC/TH neurons located in the spinal ganglia. Central to both of these concerns is the expression and recombination efficiency of the TrkC^{CreERT2} transgene in these other cell types. This Matters Arising Response paper addresses the McMullan et al. (2022) Matters Arising paper, published concurrently in *Cell Reports*.

Vasoconstriction upon activation of TrkC^{CreERT2} neurons

McMullan et al. (2022) suggest that vasoconstriction driven by activation of TrkC^{CreERT2} neurons is a result of nociceptor stimulation. In support of this interpretation, they cite well-documented evidence that noxious input from the periphery is a strong driver of sympathoexcitation. They further point out that transcriptomic analysis indicates that in addition to its expression in myelinated mechanoreceptors (termed PSNF2 by Zeisel et al., 2018) and TH (tyrosine hydroxylase) neurons (PSNP1), TrkC is also expressed by a subpopulation of unmyelinated nociceptors corresponding to the PEP2 (PSPEP1) and NP2.1 (PSNP4) cell types defined by Usoskin et al. (2015). In our study, it is important to remember that we are using Cre recombinase to target and manipulate cells *in vivo*, and that Cre-mediated recombination often does not fully reproduce the expression pattern of the driver gene. The TrkC^{CreERT2} mouse we generated is also based upon BAC (bacterial artificial chromosome) transgenic technology, which is known to be less reliable than knockin strategies for replicating endogenous gene expression patterns. Thus, the most reliable indicator of recombination in a cell type or tissue is through the use of a highly sensitive reporter. Indeed, when we used the Rosa26$^{ChR2\text{-}YFP}$ reporter (the most sensitive reporter in our hands), we observed that recombination in the DRG was largely limited to PVALB^{+}, TH^{+}, and Ret/NF200 neurons, with minimal expression of the YFP transgene in other cell types, including peptidergic and non-peptidergic nociceptors (figures 1A–1F in Morelli et al. [2021]). Moreover, upon DRG-specific recombination using intrathecal injection of 4-hydroxytamoxifen, we detected ChR2-YFP reporter in TH^{+} neurons innervating blood vessels and subsequent vasoconstriction upon optical stimulation (figures 2H, 2I, and S2 in Morelli et al. [2021]). Collectively, these data suggest that vasoconstriction is indeed mediated by TrkC/TH DRG neurons rather than by nociceptors.

McMullan et al. (2022) also refer to our behavioral experiments in which chemogenetic stimulation of TrkC^{CreERT2} neurons provoked increases in sensitivity to punctate mechanical stimuli as a further indication that we are in fact directly activating nociceptors in TrkC^{CreERT2}::Avil^{hM3Dq} mice. However, we never detected reporter expression in any neuron morphologically resembling a nociceptor in the skin (figure 2A in Morelli et al. [2021]). Instead, we suggest that locally, the mechanical pain hypersensitivity may be explained by the reduced blood flow leading to tissue hypoxia. Lack of oxygen initiates anaerobic glycolysis, resulting in decreased ATP levels and increased concentration of lactate (Birklein et al., 2000b). Low tissue pH could thus trigger the hypersensitivity with the same mechanism described in complex regional pain syndromes (CRPS) patients where pain has been linked to low tissue pH (Birklein et al., 2000a; Koban et al., 2003). Also, in animal studies, it has been shown that low pH in the skin acts on nociceptors, exciting them and thus contributing to pain sensitivity (Steen et al., 1995), and in the case of the TrkC^{CreERT2} mice, this could be the result of the hypoxia generated by vasoconstriction.

Lethality after ablation of TrkC^{CreERT2} neurons

McMullan et al. (2022) propose that the lethal effects of ablation of TrkC^{CreERT2} neurons may arise from loss of vagal rather than DRG neurons. They point out that both of the drivers we used for our intersectional genetic approach, Advillin and TrkC, are

expressed in vagal ganglia, and that we would therefore expect to see ablation of vagal and DRG neurons upon injection of diphtheria toxin. Using the sensitive Rosa26$^{ChR2\text{-}YFP}$ reporter, we were not able to detect any Cre-mediated recombination in the vagal nodose-petrosal-jugular ganglion complex (figure 2I in Morelli et al. [2021]), or indeed in the sympathetic superior cervical ganglion (figure 2J in Morelli et al. [2021]), despite TrkC being expressed there. Again, we put this down to a lack of fidelity of the TrkC^{CreERT2} BAC transgene in reproducing the endogenous expression pattern of TrkC. Why the TrkC^{CreERT2} driver would recombine better in DRG than in other ganglia is unknown to us. It is also possible that the Cre-dependent chemogenetic or diphtheria receptor transgenes might be more sensitive to recombination than the Rosa26$^{ChR2\text{-}YFP}$ reporter. However, in the absence of detectable Cre-mediated recombination in vagal (or sympathetic) ganglia, we thus favor a role for DRG neurons in mediating the lethal effects of ablation. As pointed out by McMullan et al. (2022), further detailed experiments monitoring respiratory, metabolic, homeothermic, arterial, and other essential body functions are required to understand the cause of death. Developing an improved genetic strategy to selectively target only TrkC^{+}/TH^{+} DRG neurons in the DRG would also be useful in this regard.

McMullan et al. (2022) point out some errors in the labeling of the figures. The Poincaré plots (figures S4A, S4B, and S6 in Morelli et al. [2021]) should indeed be labeled as BPM (beats per minute). The ellipses indicate the standard deviations of the scatter plot (SD1 and SD2) and not 95% of data point as they suggest. Finally, figure 6D should also be color coded in the same way as the other panels in figure 6: CTRL + C21, black; TrkC-hM3Dq + C21, red; CTRL + C21 + Prop, green; TrkC-hM3Dq + C21 + Prop, yellow.

AUTHOR CONTRIBUTIONS

All authors wrote and edited the manuscript

DECLARATION OF INTERESTS

The authors declare no competing interests.

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