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Supplementary information

A neuronal architecture underlying autonomic dysreflexia

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Supplementary Note 1. Autonomic dysreflexia engages Vglut2^{ON} neurons

We asked whether the neurons embedded in the lumbosacral and lower thoracic spinal cords, and activated during autonomic dysreflexia, exhibited an excitatory or inhibitory phenotype. To answer this question, we exposed Vglut2^{Cre}::Ai9$^{(RCL\text{-}tdT)}$ and Vgat^{Cre}::Ai9$^{(RCL\text{-}tdT)}$ mice to repetitive autonomic dysreflexia. We quantified the proportion of activated neurons that colocalized with each neurotransmitter phenotype in the lumbosacral and lower thoracic spinal cords (**Extended Data Fig. 2a-b**). We found that autonomic dysreflexia triggered a disproportionate activation of Vglut2^{ON} neurons in the lumbosacral and lower thoracic spinal cords compared to Vgat^{ON} (**Extended Data Fig. 2b**) [1,2].

We next sought to determine the causal role of these neuronal subpopulations in triggering autonomic dysreflexia. To manipulate each neuronal subpopulation in each location, we expressed inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)[3] in Vglut2^{ON}, Vgat^{ON} or Chat^{ON} neurons located either in the lumbosacral or lower thoracic spinal cord using targeted infusions of AAV5-hSyn-DIO-hM4Di-mCherry in Cre-driver mouse lines (**Extended Data Fig. 2c**) [4].

Chemogenetic inactivation restricted to Vglut2^{ON} neurons located in the lumbosacral or lower thoracic spinal cord blunted autonomic dysreflexia (**Extended Data Fig. 2c**). In contrast, chemogenetic silencing of Vgat^{ON} neurons had no effect (**Extended Data Fig. 2c**).

These experiments suggested that Vglut2^{ON} neurons located in the lumbosacral spinal cord must establish axonal projections within the lower thoracic spinal cord.

To expose these putative projections, we labeled the axons and synapses of Vglut2^{ON} neurons with infusions of AAV-DJ-hSyn-flex-mGFP-2A-synaptophysin-mRuby[5] into the lumbosacral spinal cord of Vglut2^{Cre} mice (**Extended Data Fig. 2d**). We detected long-distance projecting axons that established synapses throughout the thoracolumbar spinal cord. SCI altered this projection pattern, whereby the density of synaptic terminals increased significantly within the lower thoracic spinal cord segments (**Extended Data Fig. 2d**).

Supplementary Note 2. Absence of contribution of thoracic-projecting bowel afferents in the neuronal response to autonomic dysreflexia

The presence of discrete yet distant enrichments in neuronal activity demonstrates that autonomic dysreflexia due to bowel distension activates neuronal subpopulations in the lower lumbosacral spinal cord, and suggests that these neurons may establish axonal projections onto neurons located within the lower thoracic spinal cord, coinciding with the *hemodynamic hotspot* that regulates blood pressure. However, the bowel is innervated by sensory afferents that project to both the lumbosacral and lower thoracic spinal cord [6,7]. Consequently, we could not rule out the possibility that autonomic dysreflexia emerged from the sensory volleys travelling to the lower thoracic spinal cord when eliciting colorectal distension. To address this possibility, we tested the blood pressure responses to colorectal distension in mice with SCI that also underwent bilateral dorsal rhizotomies at T11-T13, thus eliminating all afferent projections to these segments. In every tested mouse, quantifications revealed that the severity of autonomic dysreflexia was comparable to mice without rhizotomies (**Extended Data Fig. 1h-i**). Moreover, the pattern of transcriptional activation to colorectal distension was indistinguishable to that of mice without rhizotomies (**Extended Data Fig. 1j)**. These experiments indicate that the emergence of autonomic dysreflexia following colorectal distension in mice with SCI is not dependent on afferent input from the bowel to the lower thoracic spinal cord. Instead, we interpreted these results as evidence that neuronal subpopulations in the lumbosacral spinal cord must access neurons in the

lower thoracic spinal cord via projections travelling within the spinal cord in order to trigger autonomic dysreflexia.

Supplementary Note 3. Longitudinal monitoring of autonomic neurorehabilitation

We surgically inserted an electronic dura mater (e-dura [8]) implant with an optimized configuration of electrodes to target the dorsal root entry zones projecting to the last three thoracic segments of the spinal cord, which coincide with the *hemodynamic hotspot*. Closed-loop adjustment of EES amplitudes with a proportional-integral controller enabled the maintenance of blood pressure to a predefined target [9] (**Extended Data Fig. 9a**). We previously established the efficacy of this technology for the treatment of orthostatic hypotension, which we named the *neuroprosthetic baroreflex*[9].

We exposed rats with SCI to daily sessions of autonomic neurorehabilitation. During these sessions, we configured the closed-loop controller to augment blood pressure to a predefined target. Careful monitoring of hemodynamics during autonomic neurorehabilitation revealed that blood pressure was maintained within and did not exceed the defined target range, indicating that EES targeting the *hemodynamic hotspot* promoted controlled elevations of blood pressure that were not reminiscent of autonomic dysreflexia (**Extended Data Fig. 9a**). Moreover, blood pressure returned to baseline levels as soon as EES was switched off.

We conducted formal weekly hemodynamic assessments to quantify the severity of autonomic dysreflexia (**Extended Data Fig. 9b-c**). These assessments revealed that autonomic dysreflexia vanished in all tested rats. Rats with chronic SCI exhibited an increase in the number of axonal projections emanating from neurons located in lumbosacral segments that formed synaptic appositions with $Vsx2^{ON}$ neurons in the lower thoracic spinal cord, comparable to observations in mice (**Extended Data Fig. 9d-g)**. Autonomic neurorehabilitation reversed this aberrant connectome, and in turn augmented the density of $vGlut1^{ON}$ synaptic-appositions from PV^{ON} neurons onto $Vsx2^{ON}$ neurons located in the lower thoracic spinal cord (**Extended Data Fig. 9g-j)**.

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