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Protocols for "Characterizing the diversity of enteric neurons using Dopamine Transporter (DAT)-Cre reporter mice".

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Abstract

Dopaminergic neurons (DA) are the predominant cell type in the midbrain that synthesize dopamine, a neurotransmitter implicated in a number of behavioural processes, including motor function, the reward pathway, and satiety. In diseases affecting these neurons, such as in Parkinson's disease (PD), there is growing evidence that the gut-brain axis and selective vulnerability of DA neurons plays a crucial role in disease. Most investigations relating to DA neurons in the gut rely on immunoreactivity to tyrosine hydroxylase (TH) - a rate-limiting enzyme in the production of dopamine. However, the reliability of TH staining as a marker of DA neurons has been questioned in recent years. Our aim is to perform a comprehensive characterization of DA neurons in the gut using a well-accepted reporter mouse line, expressing a fluorescent protein under the dopamine transporter promoter (DAT). Our findings confirm a unique localisation of DA neurons in the gut, and also unveil that there are discrete subtypes of DA neurons in the gut, which we characterised using both immunofluorescence and single cell transcriptomics. We observed distinct subtypes of DAT neurons expressing co-transmitters and modulators; some of them likely co-releasing acetylcholine, and a smaller population likely releasing nitric oxide; while others were positive for a slew of canonical DA markers (Vmat2, Girk2, Foxa2). Given the clear heterogeneity of DA gut neurons, further investigation is warranted to define their functional signatures and discover their inherent biological differences that put these cells at risk for neurodegeneration.

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