Identification of the different types of spinal afferent nerve ending that underlie pain perception from the large intestine using a novel anterograde tracing technique

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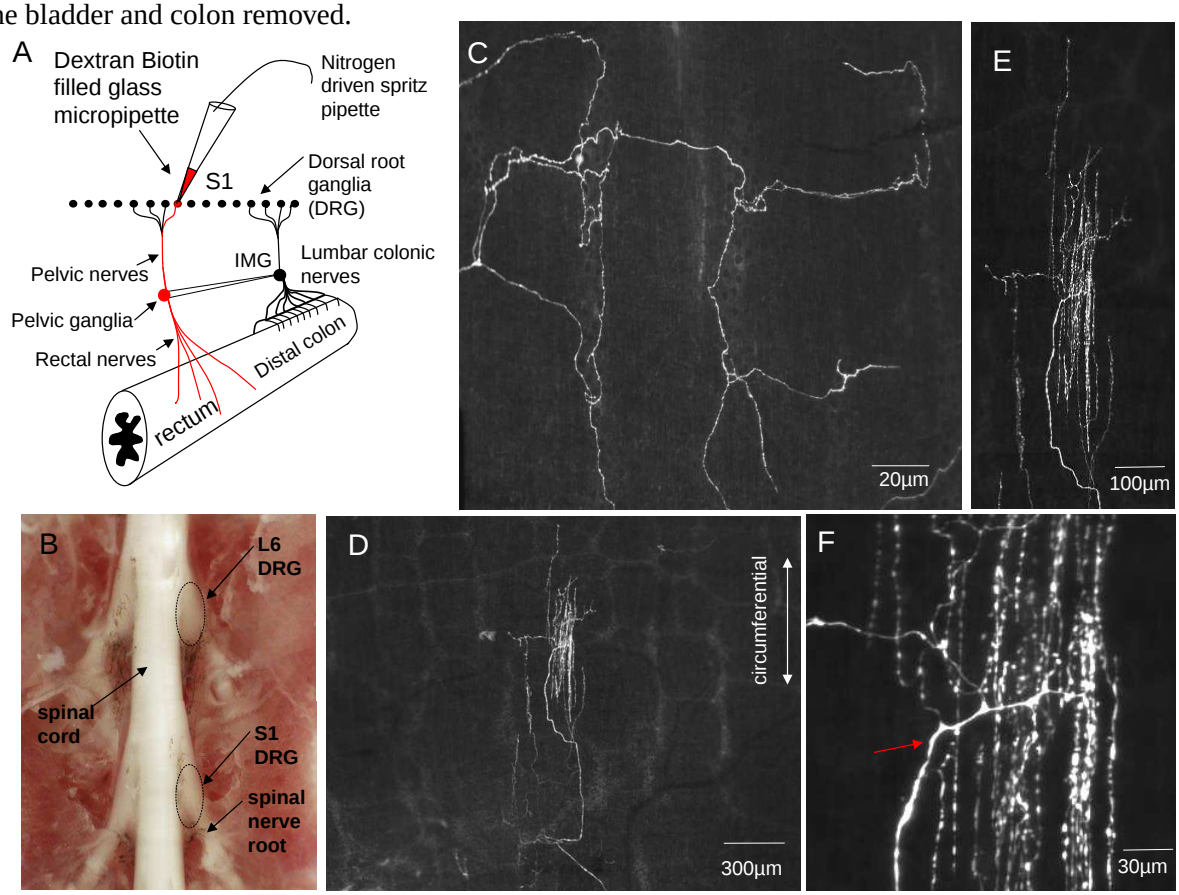
Background: One major weakness in our understanding of pain perception from visceral organs is the lack of knowledge in the location, morphology and neurochemistry of all the different types of spinal afferent nerve endings, which detect noxious and innocuous stimuli. This is because we lack techniques to selectively label only spinal afferents. Our aim was to develop an anterograde tracing technique that labels only spinal afferent nerve endings in visceral organs, without also labeling all other classes of extrinsic afferent and efferent nerves. We have in this study identified, for the first time, all the different classes of spinal afferent nerve ending that underlie pain perception from the large intestine.

Methods: Mice were anesthetized with isoflurane and dextran-biotin injected, *via* glass microelectrodes (Figure panel A), into L6 and S1 dorsal root ganglia (DRG). Mice recovered for 7 days, were then euthanized and the bladder and colon removed.



Results: In 14 mice, anterograde labeling revealed 10 unique classes of afferent endings that terminated within distinct anatomical layers of the colon and rectum, and a substantial proportion of these were immunoreactive to the sensory peptide, Calcitonin Gene Related Peptide (CGRP). One of these major classes is an intramuscular ending in the circular muscle (CM) layer of the colon that consists of multiple varicose axons that project circumferentially (Figure panels C-F). The diversity of different types and morphologies of nerve ending identified reveals a far greater complexity of sensory processing than ever expected from the large intestine.

Conclusions: We demonstrate a technique for selective anterograde labeling of spinal afferent nerve endings in visceral organs. This approach facilitates selective visualization of the precise morphology and location of the different classes of spinal afferent endings, without visual interference from indiscriminant labeling of other classes of afferent and efferent nerve axons that also innervate internal organs. For the first time, we have been able to identify all the different classes of spinal afferent nerve ending that innervate the large intestine without ambiguity. At least 10 different classes of spinal afferent ending can be identified, that innervate specific anatomical target sites, and consist of unique morphologies and neurochemistries. This new technique can be used to now identify all the different classes of spinal afferent nerve ending that underlie pain perception in visceral organs, such as the bladder, uterus and gastrointestinal tract.