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An improved method for *in vitro* morphofunctional analysis of mouse dorsal root ganglia

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Running title: DRG analysis in a whole-mount preparation

Abstract

Sensory neurons in dorsal root ganglia (DRGs) are the first-order neurons along the pathway conveying sensory information from the periphery to the central nervous system. The analysis of the morphological and physiological features of these neurons and their alterations in pathology is the necessary prerequisite to understand pain encoding mechanisms. Here, we describe an *in vitro* procedure for combined morphofunctional analysis of mouse DRGs. Freshly excised DRGs obtained from adult mice were incubated in collagenase to dissolve the ensheathing connective capsule. The degradation of the connective tissue facilitates both access to the neurons by classical recording glass pipettes and the penetration of primary antibodies for immunohistochemical procedures. The entire DRGs were then imaged using a confocal microscope obtaining a fine 3D representation of their cytoarchitecture without requiring tissue sectioning. Thus, our proposed whole-mount preparation represents a flexible *in vitro* approach for both functional and phenotypic analysis of DRG neurons by at the same time preserving their neuroanatomical relationships.

Keywords: nociceptors, DRG, whole-mount, patch clamp, immunohistochemistry

1. Introduction

Combining histological and functional analysis on intact samples of nervous system tissue is a powerful approach for understanding cell function in relation to their actual tridimensional context. Unfortunately, achieving this goal at cellular level is hampered by the intrinsic limitations of both histological and physiological techniques. Indeed, to properly investigate single cell function and phenotype, tissue slicing or dissociation is often required, thus disrupting anatomical connections or requiring complicated reconstruction procedures. In recent times, a number of powerful whole-mount approaches have been developed to investigate large areas of the nervous system. In particular, CLARITY and derivate techniques, consisting in lipid extraction procedures, result in a nearly transparent light permissive brain-hydrogel hybrid which allows visualization of fluorescence-tagged neuronal networks (Chung et al., 2013) and/or identify single neurons by *ad hoc* modified immunohistochemical or in situ hybridization techniques (Yang et al., 2014). However, clearing procedures are quite demanding in terms of time and difficult to couple with functional studies at single cell level.