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Labeling of neuronal morphology using custom diolistic techniques

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HIGHLIGHTS

- An advancement in the study of neuronal morphology is demonstrated.
- Customization allows for laboratory specific modification and increases availability.
- A low-cost option for diolistic labeling of fixed or living neurons.

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ABSTRACT

Background: Diolistic labeling is increasingly utilized in neuroscience as an efficient, reproducible method for visualization of neuronal morphology. The use of lipophilic carbocyanine dyes, combined with particle-mediated biolistic delivery allows for non-toxic fluorescent labeling of multiple neurons in both living and fixed tissue. Since first described, this labeling method has been modified to fit a variety of research goals and laboratory settings.
New method: Diolistic labeling has traditionally relied on commercially available devices for the propulsion of coated micro-particles into tissue sections. Recently, laboratory built biolistic devices have been developed which allow for increased availability and customization. Here, we discuss a custom biolistic device and provide a detailed protocol for its use.
Results: Using custom diolistic labeling we have characterized alterations in neuronal morphology of the lateral/dentate nucleus of the rat cerebellum. Comparisons were made in developing rat pups exposed to abnormally high levels of 5-methyloxytryptamine (5-MT) pre-and postnatally. Using quantitative software; dendritic morphology, architecture, and synaptic connections, were analyzed.
Comparison with existing method(s): The rapid nature of custom diolistics coupled with passive diffusion of dyes and compatibility with confocal microscopy, provides an unparalleled opportunity to examine features of neuronal cells at high spatial resolution in a three-dimensional tissue environment.
Conclusions: While decreasing the associated costs, the laboratory-built device also overcomes many of the obstacles associated with traditional morphological labeling, to allow for reliable and reproducible neuronal labeling. The versatility of this method allows for its adaptation to a variety of laboratory settings and neuroscience related research goals.

1. Introduction

Three-dimensional morphological reconstructions of individual neurons and their dendritic arbors have served as the bases for analyzing the structure-function relationships within the nervous system (Staffend and Meisel, 2011a,b; Wu et al., 2004). For over a century, the main method for these studies consisted of various modifications of the famed Golgi staining procedure (Fairen, 2005), which has proved invaluable in developing our modern understanding of the nervous system (Staffend and Meisel, 2011a,b). However, this method and the various modifications of the silver impregnation of a percentage of the neuronal population are not without drawbacks. In particular, the small population of stained cells, while an advantage in morphological studies, elicits the lingering concern of selection bias (Staffend and Meisel, 2011b; Spergel et al., 2001). The Golgi method also suffers from a limited

Abbreviations: DiI, dialkylcarbocyanine; IACUC, Institutional Animal Care and Use Committee; PVP, polyvinylpyrrolidone; S, seconds; Ms, milliseconds; Min, minutes; H, hours; PBS, phosphate buffered saline; 5-MT, 5-methoxytriptamine.

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compatibility with modern immunolabeling techniques and confocal microscopy. Additionally, data analysis following the Golgi method of staining may be negatively influenced by inconsistent neuronal impregnation, sectioning artifacts, and the overlapping of fine dendritic processes that leads to an indistinguishable morphology.

More recently, the use of neuronal transfection and electroporation methods have allowed for neuronal morphological labeling that circumvent the limitations experienced with Golgi staining (Buhl and Lubke, 1989; Spacek, 1989). While the administration of dye into individual cells through microinjection using intracellular or patch pipettes generates excellent single-cell labeling, the technique is technically demanding and may be vulnerable to sampling bias (Buhl and Lubke, 1989; de Lima et al., 1990; Schmidt et al., 1996; Surkis et al., 1996). Neuronal transfection relies on the introduction of DNA constructs into target cells and tissues through a variety of methods. One such method of DNA transfer is known as "Biolistic" delivery, in which a gene gun utilizes a pressurized release of gas to propel DNA-coated micro-particles into tissue, crossing the plasma membrane to target the cells (Wu et al., 2004). This method does not require the same level of technical expertise needed for conventional intracellular injections (Bridgman et al., 2003). Furthermore, the random sampling used in biolistic delivery is ideal for full-scale quantitative analysis. However, the reliance on DNA transfection for fluorescence expression limits the method to use on living tissues in prepared cultures.

A more recent advancement from the biolistic approach to the morphological labeling of live or fixed neurons was first reported by Gan et al. (2000) and is known as diolistic labeling. Diolistic labeling utilizes the lipophilic fluorescent dye dialkylcarbocyanine (DiI). While DiI has traditionally been used in anterograde and retrograde neuronal tracing, it has also proven to be effective in the fluorescent labeling of the neuronal cell membrane. Utilizing a diolistic approach (Gan et al., 2009), the ballistic delivery of DiI-coated micro-carriers to fixed or cultured tissue slices allows the DiI to be incorporated into the cellular membrane. This occurs through lateral diffusion in living or fixed tissue, illuminating the cellular morphology. When ballistically delivered to tissue sections through a single pulse of high-purity gas (*i.e.,* helium or nitrogen), individual DiI-coated tungsten particles are embedded in various neurons of the tissue. The micro-carriers pass into the soma of the neuron, while the DiI is captured in the neuronal membrane. Thus, the DiI is allowed to diffuse along the cellular membrane of a single neuron, fluorescently labeling the fine neuronal architecture of dendritic branches and spines (Gan et al., 2009). DiI-labeled neurons can be observed through high-resolution imaging, such as confocal or two-photon microscopy, and can be digitally reconstructed in precise detail. The quantification and classification of dendritic branching and dendritic spines can be accomplished with appropriate software packages (O'Brien et al., 2001; Noterdaeme et al., 2002).

Since its development, diolistic labeling has relied on the use of a commercially available gene gun, designed for biolistic transfection, for the propulsion of dye-coated micro-particles into tissue. However, these handheld devices have several drawbacks in the proposed staining method. First, the handheld nature of the device can alter the exact angle of delivery of the particles, causing inconsistent dye patterning in the tissue. Next, the standard tubular barrel of the gun causes increased particle density in the middle of the field and produces a burst of gas strong enough to damage the superficial layer of fixed tissue. Finally, the cost of commercially available devices and the associated materials may preclude some laboratories from using this type of methodology. In an effort to circumvent the aforementioned obstacles, Bridgman et al. (2003) designed and constructed a custom-built device for use in biolistic applications (Fig. 1). With protocol modifications, the device has been optimized for the diolistic labeling of fixed tissues. The device consists of a solenoid valve triggered by a relay switch to fire for 50 ms and is a modification of an original model constructed by Dr. David Kirk at Washington University in Saint Louis. The main features of the device that contributes to its reliability and reproducibility include a precisely timed trigger for the solenoid valve, a precise height adjustment system, a narrow baffled barrel, and a small pore size filter (Bridgman et al., 2003). Since the gun is mounted on a fixed base, the angle of delivery remains the same throughout all procedures, while the baffled barrel (constructed based on the design described by O'Brien) (O'Brien et al., 2001) limits the amount of pressurized gas that contacts the tissue. Particle carriers (cartridges) are supported by a plastic ring that fits inside a modified filter holder (cartridge holder) (Bridgman et al., 2003). The particle carriers are cut-off yellow (200 μL) pipette tips. The device components described here, and included in the parts list, allow for a similar device to be constructed and operated with commercially available materials for a lower cost than traditional gene gun systems. Laboratory devices may be constructed with various available materials and designed to address individual research goals.

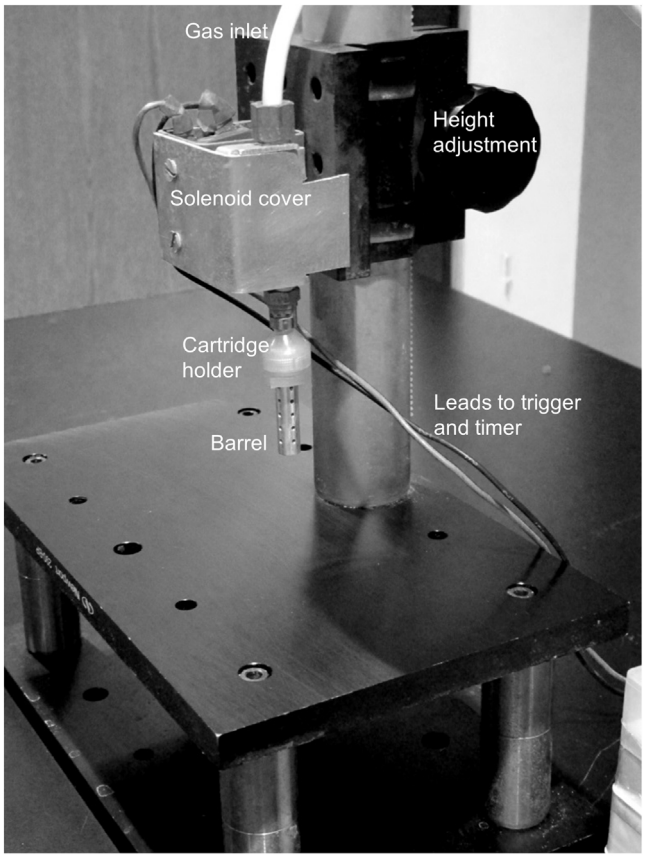

Fig. 1. Custom biolistic device.
Key features include a baffled barrel to improve particle delivery, knob for height adjustment and a fixed base. The firing mechanism consists of a solenoid valve activated by a relay switch allowing a burst of nitrogen gas to pass through the cartridge, filter assembly, and into tissue specimens.

2. Protocol

All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) and must follow officially approved procedures for the care and use of laboratory animals. This protocol has been approved by the IACUC at Missouri State University.

Note: The following protocol will detail the methods used in the diolistic labeling of fixed brain tissue obtained from rats pre-