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FAST DiI Injection Protocol from Neural Tracing in Porcine SAN

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ABSTRACT

Protocol for injecting FAST DiI into the sinoatrial node of porcine test subjects for neural tracing purposes.

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MATERIALS TEXT

FAST DiI from Invitrogen, catalog D7756
CitiFluor AF1 Mounting Medium Solution (Electron Microscopy Sciences, Hatfield, PA, USA, Cat. #17970-100

Injection of FAST DiI

1 Sedated, anesthetized, and intubated pig (induction: ketamine 10 mg/kg IM/midazolam 1 mg/kg IM, maintenance: isoflurane 1-2% inhalation).

2 Performed right unilateral thoracotomy by dividing the pectoral muscle.

3 Made a small incision in the pericardium and exposed the right atrial (RA)-superior vena cava junction.

4 Used 27-guage needle to inject 5 mg of an analog of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (FAST DiI, Invitrogen, D7756) in 250 µL of 100% methanol (MeOH) into the sinoatrial nodal (SAN) region.

5 Placed chest tube and closed incision.

6 Aspirated chest tube immediately prior to its removal.

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Tissue Dissection and Preparation

7 Harvested tissue in a terminal procedure at least 3 weeks after injection procedure.

8 Immersed tissue in cold 4% PFA for 24 hours.

9 Washed tissue in PBS 4 times.

10 Transferred tissue to cold 0.01M PBS + 0.02% Na azide + 20% sucrose solution for shipment to East Tennessee State University.

11 Dissected tissue as needed to isolate specific regions.

12 Froze tissues on dry ice and stored at -80°C until ready to be sectioned.

Tissue Sectioning Using the Cryostat

13 Removed tissues from -80°C freezer and mounted onto specimen plate using Tissue-Tek® O.C.T. Compound (Sakura Finetek USA, Cat. No. 4583).

14 Cut tissues into 30µm sections at -20 to -25°C using a Leica CM3050S cryostat (Leica Microsystems Inc., Bannockburn, IL, USA).

15 Collected sections on charged slides in a sequence that yields at least 4 sets of tissue sections per region of the heart, with each set spanning the entire thickness of the specimen.

16 Stored sets of tissues in slide boxes wrapped in aluminum foil at -20°C until further processing.

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Immunohistochemistry

17 Removed every second slide from a set to perform immunolabel for PGP9.5. (Described in separate protocol, found here: dx.doi.org/10.17504/protocols.io.8rmhv46)

18 Applied cover glass to adjacent tissue slides using a drop of CitiFluor AF1 Mounting Medium Solution (Electron Microscopy Sciences, Hatfield, PA, USA, Cat. #17970-100).

Imaging

19 Imaged tissue sections using fluorescence illumination with an Olympus BX41 microscope equipped with an Olympus DP74 digital camera and cellSens software (Olympus America Inc., Center Valley, PA; RRID:SCR_016238).

20 Loaded slide onto stage and collected representative images of ganglia.

Quantification

21 Counted DiI-labeled neurons and total number of neurons (i.e., PGP9.5-positive).

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