**Supplement A: Fabrication of Indifferent (Ground) Electrode**

1.6.1. Make a 100 mL solution of 4% agarose in 1 M KCl in a small, glass beaker. Set the beaker on a hot plate with magnetic stir bar to heat and mix the agarose until it is fully dissolved.

1.6.2. Place 50 mL of 1 M KCl solution into a beaker and keep the beaker on ice to chill.

1.6.3. Coil a long chloridized silver wire (see step 1.3) into a helix. The wire must be long enough to extend half-way into a Pasteur pipette and protrude out of the pipette.

1.6.4. If needed, heat the thin part of the Pasteur pipette in order to bend it at an angle appropriate for the electrophysiology setup. The indifferent electrode will have its tip in the solution of the recording chamber during experimentation.

1.6.5. Draw the warm agarose solution into the barrel of the Pasteur pipette using a syringe and suitable rubber tube until agarose reaches the top of Pasteur pipette.

1.6.6. Place the tip of the Pasteur pipette into chilled 1 M KCl for a few seconds to solidify the agarose in the tip and prevent leakage of the solution.

1.6.7. Embed the coiled silver wire into the warm agarose filing the Pasteur pipette such that the wire is protruding out the back. Place the electrode at room temperature to allow the rest of the agarose to solidify.

1.6.8. Seal completely the back of the indifferent electrode with silicone sealant (as used for an aquarium or domestic bath). Ensure the wire is still sticking out of the sealant.

1.6.9. Place the electrode tip in room temperature 1M KCl and allow sealant to dry. The sealant prevents drying out of the agarose and serves to stabilize the wire.

1.6.10. Wrap the indifferent electrode in plumber’s tape or similar to ensure electrical isolation when placed in a pipette holder; leave the tip and wire exposed.

1.6.11. Place the indifferent electrode in a jar of 1 M KCl such that the tip of the electrode is submerged. Store at 4 °C. Reuse these electrodes as long as the agarose remains homogenous and the signal produced by them provides a stable ground.

**Supplement B: Calibration of ISM in Experimental Setup**

Note: Calibration of an ISM in a dedicated calibration station is also performed using the procedure below except the slice chamber (step 3.29.1) is replaced by the dedicated calibration chamber.

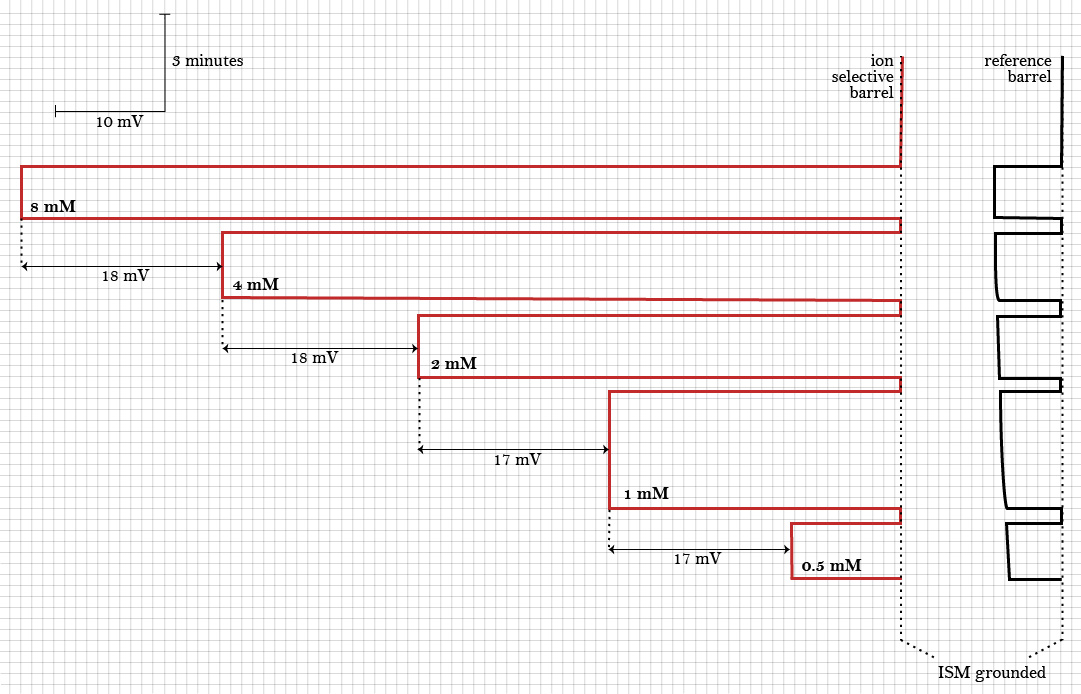
3.29.1. Place a small cup containing a standardized solution (step 1.8) in slice chamber. Insert an indifferent electrode in the cup and connect the electrode to the ground of the recording setup.

3.29.2. Secure an ISM to the pipette holder of one micromanipulator and connect the ISM to its head stage (Fig 2b).

3.29.3. Lower the tip of the ISM into the standardized solution and record ISM voltage in the solution. This voltage will be used for calibration by software Wanda in a later step.

3.29.4. Repeat steps 3.29.1 – 3.29.3 with each of the five standardized solutions.

3.29.5. Confirm that ISM voltage responds rapidly and predictably to changes in stock solution concentration.



**Supplemental Figure: Representative Calibration Data**

Voltage measured by ion selective barrel (red) and reference barrel (black) over time when an ISM is submerged in five TMA calibration solutions of increasing [TMA]. When the ISM is moved from 0.5 mM TMA calibration solution to 1 mM TMA solution, the voltage recorded by ion selective barrel increases rapidly by 16 mV. When ISM is submerged in subsequent calibration solutions, the voltage recorded by the ion selective barrel responds predictably to changing [TMA] concentration. The reference barrel demonstrates a negligible response to small changes in [TMA]. This is an ideal ISM for RTI experiments.