Prep:

* Order perfusion

Day 1:

**Perfusion**

* Keep in post-fix overnight at 4 degrees C

**Solution Prep:**

* Make up stock solution of 0.1M NaA - 4.1g/500mL H20
* Make up stock solution of 0.1% CaCl2 in 0.1M NaA - 0.1g CaCl2 into 100 ml NaA
* Make up glycerol dilutions (10%, 20%, 30%) in 0.1M NaA

Day 2:

**Vibratome**

1. Block brain to include V1
2. Mount on vibratome
3. Cut 200um thick slices into cold 0.1M PB
4. Collect sections back into chilled 0.1M PB

**Pre-Treatment – All done in chilled Styrofoam box on shaker**

1. 3 x 10 minutes in 0.1M NaA
2. 1 hour in 0.1% CaCl2/0.1M NaA
3. 3 x 5 minutes in 0.1M NaA

**Cryoprotection – All done in chilled Styrofoam box on shaker; add new ice packs before overnight step**

1. 30 min in 10% glycerol/0.1M NaA
2. 30 min in 20% glycerol/0.1M NaA
3. 30 min 30% glycerol/0.1M NaA
4. Overnight in 30% glycerol/0.1M NaA

**Prep**

* Clean metal dishes and bake overnight
* Cut, clean, and bake Aclar strips overnight

Day 3:

**Prep**

1. Fill and chill AFS to -90 deg C
2. Chill down metal dishes and mesh-bottomed capsules in acetone

**Dissection**

1. In 30% glycerol/0.1M NaA, dissect out desired number of V1sections
2. Place back in 30% glycerol/0.1M NaA after dissection