

Methodology

Cockroach Preparation Procedure (Local and Global):

1. Chill the cockroach on ice to anesthetize/numb it
2. Secure cockroach with pins and fix the ventral side up
3. Surround the cockroach with cockroach Ringer's solution to keep tissue moist
4. Remove cuticle around the coxa using scalpel to access the nerve/ganglion (local) and brain tissue (global)
5. Keep tracheae and fat as intact as possible

Calcium Indicator Procedure:

1. Prepare Fluo-3 AM
2. Dilute to ~5–10 μM in Ringer's solution
3. Apply it to the exposed tissue and incubate for 30-60 min in the dark
4. Wash gently with Ringer's for 15-30 minutes to remove excess dye

Electrical Stimulation Procedure Local and Global:

1. Use the Roboroach software and spiker box to electrically stimulate the leg (local) and brain (global) at three different levels of electrical stimulation (low, medium, high)
2. Stimulate the leg or brain for around 1 minute and record the electrical output using the Spiker box or RoboRoach

3. Capture video or time-lapse images during electrical stimulation to track motor output

Calcium Dynamics Visualization Procedure:

1. Set up a fluorescence stereomicroscope
2. Record calcium imaging using the fluorescence stereomicroscope

Glutamate Application Procedure Local and Global:

1. Fill a glass micropipette with glutamate solution (around 1-10 mM)
2. Apply short pressure pulses of glutamate near the tissue in the leg (local) and brain (global) with a syringe setup
3. Start with low concentrations to avoid excitotoxicity

Procedure: Local and Global Application:

Part 1: Cockroach Preparation (see local procedure above)

Part 2: Applying Calcium Indicator (see above procedure)

Part 3: Applying Glutamate (see above procedure)

Part 5: Electrical Stimulation, test at the three levels (low, medium, high) (see above local procedure)

Part 6: Record Calcium Dynamics (see above procedure)

Procedure: Local and Global Control Groups

Part 1: Preparing the Cockroaches for testing (see above local procedure)

Part 2: Applying Calcium Indicator (see above procedure)

Part 3: Electrical Stimulation, test at the three levels (low, medium, high) (see above local procedure)

Part 4: Record Calcium Dynamics (see above procedure)