*The CPP experimental setup requires the use of Bonsai, a programming language with an intuitive interface, designed for data acquisition from multiple devices. In the CPP configuration presented below, Bonsai receives video streams from a video camera and controls optogenetic stimulation timing through an Arduino Uno (AU1). A secondary Arduino Uno (AU2) controls pulse parameters.*

1. Connect devices

Plug in a USB camera (e.g., webcam), AU1 and AU2.

Place a jumper wire from AU1 Pin 6 to AU2 Pin 6.

Connect your TTL cable to the laser on one side and the AU2 on the other side. Ground wire goes to a GND pin and coaxial wire to Pin 13.

(e.g., <https://www.digikey.com/products/en/test-and-measurement/test-leads-bnc-interface/625>)

2. Install programs

Download Bonsai from <http://bonsai-rx.org/> and install it. If you’re new to it, read the documentation and play example workflows first.

Download CPP\_stimulation.ino, CPP.bonsai and CPP.bonsai.layout from <https://github.com/wanglab-duke/craniofacial-specific-monosynaptic-circuit-for-affective-pain>

Load StandardFirmata in AU1 (get it from Files > Examples > Firmata).

Load CPP\_stimulation.ino into AU2.

Open CPP.bonsai workflow

Check that the video camera is detected in the VideoCaptureDevice module.

Check that the AU1’s COM port is accurate in the Trigger Arduino Pin 6 nested workflow

3. Run and test it

Run the code in Bonsai.

Adjust detection parameters in the Image Process nested workflow

TTL pulses will be generated when movement is detected (check the Boolean state).

The code will write a video recording and two csv files containing subject location data.