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# Ex Vivo Whole-Cell Patch Clamp Recording and Pharmacological Isolation of Light-Evoked $GABA_A$ Currents

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol describes whole-cell patch clamp recordings from VTA neurons in acute horizontal slices. Light-evoked synaptic currents are recorded at -60 mV and pharmacologically isolated. $GABA_A$ receptor mediation is confirmed by picrotoxin sensitivity.

## Materials

**Recording aCSF (mM): NaCl 126, NaHCO3 24, glucose 10, KCl 2.5, CaCl2 2.4, NaH2PO4 1.2, MgCl2 1.2. Equilibrate with 95% O2 - 5% CO2, pH 7.3. Maintain at 32 C.

**Internal Solution (mM): 125 KCl, 10 HEPES, 10 EGTA, 5.2 CaCl2, 4 ATP-Mg2, 0.3 GTP-Na3, 10 phosphocreatine-Na2. Adjust pH to 7.3 with KOH.

## Step-by-Step Procedure

1 Transfer one recovered slice to the recording chamber and perfuse with recording aCSF at 32 C and 2.5-3.0 ml/min.

2 Identify VTA dopamine neurons based on anatomical location.

3 Verify slow or absent spontaneous firing (< 5 Hz) in cell-attached mode.

4 After whole-cell access, confirm presence of Ih using hyperpolarizing voltage steps.

5 Use 4-6 MOhm pipettes filled with internal solution and hold the cell at -60 mV.

6 Add CNQX 10 µM and D-AP5 50 µM to block glutamatergic transmission.

7 Add TTX 0.5 µM and 4-AP 100 µM to isolate monosynaptic release.

8 Deliver TTL-triggered light pulses (480 or 560 nm) using CoolLED system.

9 Record light-evoked inward synaptic currents.

10 Apply picrotoxin 100 µM to confirm GABAA receptor mediation.

11 Filter signals at 3-4 kHz and digitize at 10 kHz.