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# Rotarod Forced Locomotion Assay During Chronic Electrophysiological Recording

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

This protocol describes a custom-built rotarod assay used to impose brief, time-locked bouts of forced locomotion during chronic in vivo electrophysiological recordings. The assay was designed to compare neuronal activity during reverse (backward stepping requirement) and forward locomotion in separate experimental blocks.

Each recording session included: (1) baseline arena recording, (2) optotagging for neuronal identification, (3) acclimation sessions on the rotarod, (4) a rest period in the home cage, and (5) forced locomotion recording blocks.

## Materials

- Custom-built rotarod: Cylinder diameter: 5 cm.
- Adjustable lateral side walls.
- Side walls adjusted to ~1 cm clearance per flank.
- Angular speed during bouts:  $2.00 \text{ rads}^{-1}$ .
- Surface speed (5-cm diameter):  $5 \text{ cms}^{-1}$ .

## Session Structure

- 1 Connect the implanted mouse (optrode-implanted) with the headstage and patch cord.  
Confirm neural signal quality.  
Confirm TTL synchronization between rotation bouts, optotagging pulses, recording system, and behavioral video.
- 2 Place the mouse in a 20 × 30 cm arena.  
Record spontaneous baseline activity.  
Deliver short light pulses (1 ms pulses; wavelength and power depending on opsin) for optotagging.  
Record neural activity and TTLs.
- 3 Perform two consecutive acclimation sessions (3 minutes each):  
One session with forward rotation bouts.  
One session with reverse rotation bouts.  
Acclimation used the same bout structure as the main experiment.
- 4 Return the animal to its home cage for 15 minutes before beginning the main forced locomotion recording block.
- 5 Bout duration: 2 seconds.  
Angular velocity: 2.00 rads<sup>-1</sup>  
Surface speed: 5 cms<sup>-1</sup>  
Inter-bout interval: pseudo-random 4–35 seconds (per-mouse mean 9–14 seconds).  
Reverse and forward sessions were run in separate blocks.  
Forward and reverse trials were not interleaved.

## Data Acquisition and Synchronization

- 6 Neural data acquired continuously.  
Rotation bout onsets recorded via TTL signals.  
Optotagging TTLs recorded during initial arena phase.

## Critical Steps

- 7 Maintain consistent side-wall spacing (~1 cm per flank).  
Keep angular velocity constant across sessions.  
Complete optotagging before forced locomotion blocks.