**Substance:** CNO

**Date:** 160317

**Zebrafish DOB:** 160307 (10 dpf)

**Aim:** Dose response CNO/ dark

**Volume in wells:** 1 ml + 20 µl

**Stock CNO solution:** 10 mM in DMSO (50 µl)

**Dilute to:** **500 µM** (50 µl 10 mM CNO + 950 µl E3)

**150 µM** (300 µl 500 µM CNO + 700 µl E3)

**50 µM** (100 µl 500 µM CNO + 900 µl E3)

**10 µM CNO:** (20 µl 500 µM CNO) / **3 µM CNO:** (20 µl 150 µM CNO) / **1 µM CNO:** (20 µl 50 µM CNO)

**Control:** 20 µl 5% DMSO/E3 (0.2% in wells)

**Stock Apomorphine solution:** 10 mM in DMSO (50 µl)

**Dilute to: 2500 µM** (50 µl 10 mM Apomorphine + 150 µl E3)

Plate 1:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| A | CNO 10 µM | CNO 10 µM | CNO 10 µM | CNO 10 µM | CNO 10 µM | CNO 10 µM |
| B | CNO 3 µM | CNO 3 µM | CNO 3 µM | CNO 3 µM | CNO 3 µM | CNO 3 µM |
| C | CNO 1 µM | CNO 1 µM | CNO 1 µM | CNO 1 µM | CNO 1 µM | CNO 1 µM |
| D | Control | Control | Control | Control | Control | Control |

Plate 2:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| E | CNO 10 µM | CNO 10 µM | CNO 10 µM | CNO 10 µM | CNO 10 µM | CNO 10 µM |
| F | CNO 3 µM | CNO 3 µM | CNO 3 µM | CNO 3 µM | CNO 3 µM | CNO 3 µM |
| G | CNO 1 µM | CNO 1 µM | CNO 1 µM | CNO 1 µM | CNO 1 µM | CNO 1 µM |
| H | Control | Control | Control | Control | Control | Control |

File

**Add CNO**

**Record during 13x5 min in total darkness**

After 0 min: recording during 5 min 103913

After 5 min: recording during 5 min 104415

After 10 min: recording during 5 min 104916

After 15 min: recording during 5 min 105418

After 20 min: recording during 5 min 105919

After 25 min: recording during 5 min 110421

After 30 min: recording during 5 min 110922

After 35 min: recording during 5 min 111424

After 40 min: recording during 5 min 111925

After 45 min: recording during 5 min 112427

After 50 min: recording during 5 min 112929

After 55 min: recording during 5 min 113431

After 60 min: recording during 5 min 113933

**Add Apomorphine (50 µM) to all wells**

**Record during 13x5 min in total darkness**

After 0 min: recording during 5 min 114915

After 5 min: recording during 5 min 115417

After 10 min: recording during 5 min 115918

After 15 min: recording during 5 min 120420

After 20 min: recording during 5 min 120922

After 25 min: recording during 5 min 121423

After 30 min: recording during 5 min 121925

After 35 min: recording during 5 min 122427

After 40 min: recording during 5 min 122928

After 45 min: recording during 5 min 123430

After 50 min: recording during 5 min 123932

After 55 min: recording during 5 min 124434

After 60 min: recording during 5 min 124936

**Other conditions:** Cover blanket, in total darkness (IR-light only)

Exposure: 1350 µsec, 300 s per recording

Thermostat: 31.5°C

Water temperature: in bath: 30.5°C in wells: 28.0°C

After experiment: all larvae terminated on ice