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Tracking acute response of C elegans to cocaine

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Protocol status: Working

We use this protocol and it's working

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Abstract

Our aim is to study addiction in C elegans with cocaine being the drug choice of abuse. This short experiment is done to check how the worms react instantaneously upon exposure to this drug for the first time.

Preparing worms

- 1
 - Chunk WT N2 on 3 maintenance plates
 - Bleach the plates after 2 days following the protocol for *Bleach synchronization of c elegans*
 - Refeed the starved L1s on 4 different plates that are OP50 seeded, 72hr prior to the experiment day

Preparing Imaging plates

- 2
 - Prepare and pour Low peptone NGM on 35mm imaging plates following the protocol for *Making low peptone NGM and Plate pouring*
 - Store the plates at 4C till in use
 - Take 6 plates for control and 6 for cocaine (Or more plates as required)
 - Prepare cocaine solution to have a final concentration of 50uM when 35ul is added to a plate with 3.5ml agar (Use DI water)- So 5mM need to be prepared as it will get diluted 100X when added to the imaging plate
 - Dissolve 2.5mg of cocaine hydrochloride in 1471 ul DI water= 5mM cocaine hydrochloride
 - Pipette 35ul of the cocaine solution to the plates and let dry (1-2hrs) under a hood
 - Similarly, pipette the 35ul of water on the control plates and let them dry for the same time

Imaging on the multi worm tracker

- 3
 - Pre-Imaging set-up**
 - Clean the rectangular glass plate with ethanol and lint free tissue
 - Switch ON the rig
 - Align the movable part till desired position and lock it by pressing ON (If you want to move it again press EMO, to lock again twist the EMO knob and press ON again)
 - Log in to the 3 PCs (Password: BehavGenom709 (BehavGenom710 for PC 3)
 - Change the screen lock time to 20 mins
 - Set up destination folder (PC → Data Part 1 → Run script (init_exp.ps1) → Name the folder with the date as (year month date e.g. 20181011)
 - Turn on the software GECKO on each pc and check the following:
 - i) Recording mode: 15 mins
 - ii) Video format: hdf5
 - iii) Output folder: Select the folder created earlier
 - iv) Press the drop-down arrow beside the RECORD icon to make sure ALL CAMERA option is selected if you want both the cameras in use otherwise select CURRENT CAMERA option

Actual Imaging

- With a hair pick, pick 5 adult worms onto each imaging plates, place the plates on the rectangular glass plate underneath the cameras
- Place the worm plates agar side up with the lids off
- Check the focus on each camera of each PC
- Record immediately

Post Imaging

- Turn OFF the rig and close GECKO
- Run the script to copy the data to the network (PC → Data Part 1 → Run script (move_files_anyPC.ps1))
- Change back the lock screen time to 1 minute
- Discard the plates
- See the protocol for video analysis to proceed with the analysis