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Genetic expression suppressor screen

In 1 collection

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We use this protocol and it's working

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Abstract

This protocol describes a low-throughput genetic screen to identify glycolytic enzymes that improve neuronal function under hypometabolic conditions.

Materials

Media Supplies

- 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Alomone Labs, Cat. No. C-141)
- Calcium Chloride (Millipore-Sigma, Cat. No. C3881)
- DL-2-Amino-5-phosphonovaleric acid (APV; Millipore-Sigma A5282)
- Glucose (Millipore-Sigma, Cat. No. G7021)
- HEPES (Millipore-Sigma, Cat. No. H3375)
- Magnesium Chloride (Millipore-Sigma, Cat. No. M0250)
- Potassium Chloride (Millipore-Sigma, Cat. No. P9333)
- Sodium Chloride (Millipore-Sigma, Cat. No. S5761)

Stock solutions

APV (25mM Stock)

1. Add 20L ddH2O to whole bottle of APV
2. Sonicate at ~55C for 30 minutes.
3. Make 1mL aliquots and store at -20C.

CNQX (10mM Stock)

1. Add 10 mL of ultrapure H2O to the whole bottle of CNQX.
2. Vortex until dissolved.
3. Make 1 mL aliquots and store at -20 oC.

Media solutions

0.1mM glucose tyrodes (119mM NaCl, 2.5mM KCl, 0.1mM D-Glucose, 54.9mM HEPES, 2mM $CaCl_2$, 2mM $MgCl_2$, 50uM APV, 10uM CNQX)

NH_4Cl tyrodes (119mM NaCl, 2.5mM KCl, 5mM HEPES, 50mM NH_4Cl, 2mM $CaCl_2$, 2mM $MgCl_2$, 50uM APV, 10uM CNQX)

Equipment

- 40x, 1.3 N.A Objective (like Zeiss-Fluar)
- 488nm Laser (like Coherent-OBIS 488nm LX Laser)
- CO2 Incubator (like VWR Symphony)
- EMCCD Camera (like Andor iXon Ultra 897)
- Epifluorescence Microscope (like Zeiss ObserverA1)
- Filter cube set for GFP excitation and emission (like Chroma filters)
- Laser speckle reducer (like Optotune LSR 3005)
- Laser/stage/stimulation-Master board controller (like a custom-designed **Arduino board**)
- Light transmission microscope (like Olympus CK40)
- Mercury arc lamp (like Zeiss HBO 100)
- Perfusion system (like Automate Scientific Valvelink 8.2)
- Microscope XYZ Stage (like Applied Scientific Instrumentation stage)
- Stimulus isolator (like World precision Instruments A385)

- Temperature controller for Objective (like Minco)

Plasmids

- vGLUT1-pHluorin
- mTagBFP2-N1
- hSyn HK1 (Addgene plasmid #220915; http://n2t.net/addgene:220915; RRID:Addgene_220915)
- hSyn AldoA (Addgene plasmid #220916; http://n2t.net/addgene:220916; RRID:Addgene_220916)
- hSyn AldoC (Addgene plasmid #220917; http://n2t.net/addgene:220917; RRID:Addgene_220917)
- hSyn GAPDH (Addgene plasmid #220918; http://n2t.net/addgene:220918; RRID:Addgene_220918)
- hSyn PGK1 (Addgene plasmid #220919; http://n2t.net/addgene:220919; RRID:Addgene_220919)
- hSyn PKM1 (Addgene plasmid #220920; http://n2t.net/addgene:220920; RRID:Addgene_220920)

Neuronal culture and transfection

1 Use cultured rodent primary hippocampal neurons, transfected with vGlutI-pH, mTagBFP2 and a construct expressing a single glycolytic enzyme, as explained in **dx.doi.org/10.17504/protocols.io.ewov1qxr2gr2/v1**.

Live-cell imaging

25m

2 Remove the cells from the incubator and bathe them in Tyrodes containing 0.1 mM glucose.

3 Mount the coverslips with the cells on the imaging chamber and image as explained in **dx.doi.org/10.17504/protocols.io.q26g7pn4qgwz/v1**.

4 Locate cells in an unbiased manner using the mTagBPF2 channel and center on their axon, judging by their morphology.

5 After a total of 00:05:00 that the cells have been in the 0.1 mM glucose tyrode buffer, start the live-cell imaging experiment in the vGlutI-pH channel. 5m

6 Stimulate the cells with 100 APs delivered at 10 Hz every 00:01:00, for a total of 10 rounds of activity. 1m

7 At the end of the experiment, perfuse cells with Tyrodes containing 50 mM NH_4Cl.

8 Analyze the collected data accordingly.

Protocol references

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