Jul 16, 2024

Striatal sections immunofluorescence and analysis

DOI
dx.doi.org/10.17504/protocols.io.n2bvjnpmxgk5/v1

Chuyu Chen[1]

[1]Northwestern University, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815

ASAP Collaborative Rese… Parisiadou lab

Chuyu Chen
Northwestern University, Aligning Science Across Parkinson's...

DOI: dx.doi.org/10.17504/protocols.io.n2bvjnpmxgk5/v1

Protocol Citation: Chuyu Chen 2024. Striatal sections immunofluorescence and analysis. **protocols.io https://dx.doi.org/10.17504/protocols.io.n2bvjnpmxgk5/v1**

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

Created: July 16, 2024

Last Modified: July 16, 2024

Protocol Integer ID: 103539

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's [ASAP-020600] through the Michael J. Fox Foundation for Parkinson's Research (MJFF)
Grant ID: ASAP-020600

Abstract

Previous studies have shown that the phosphorylation of ribosomal protein S6 (p-rpS6) at S235/236 residues increases in iSPNs in response to haloperidol activation. This phosphorylation is cAMP/PKA dependent, which is the canonical pathway inhibited by D2R antagonism. We therefore explored whether LRRK2 inhibition interferes with this haloperidol-mediated phosphorylation event.

Immunostaining

1 Drd2-eGFP mice crossed with LRRK2-GS KI and control littermates were treated as indicated and perfused with 50 ml of PBS

2 Mice were perfused with 4% paraformaldehyde in PBS.

3 Brains were dehydrated with 30% sucrose in PBS for 48hrs and cut coronally (30 µm) by cryostat

4 Striatal sections were incubated with 5% goat serum in 0.2% Triton X-100 for 2 hrs

5 the sections were incubated in the same solution overnight at 4 °C with the primary antibodies anti-GFP (1:1000, Invitrogen) and anti-phospho-S6 Ribosomal protein (Ser236/236) (1:300, Cell Signaling Technology)

6 Sections were washed with PBS for 5mins at RT

7 Repeat the wash with fresh PBS

8 Incubate with the secondary antibodies Alexa Fluor™ 488 and Alexa Fluor™ 647 (both 1:600, Invitrogen) for 3 hrs.

9 Sections were washed with PBS for 5mins at RT

10 Repeat the wash with fresh PBS

11 Sections were mounted on ProLong Diamond Antifade Mountant

Confocal imaging

12 Fluorescence images were obtained with Nikon A1R microscope and were acquired with a 20x objective at 1,024 x1,024 pixel resolution

12.1 Stitched images of the whole striatum were automatically acquired with Nikon Element

Image analysis

13 GFP and phospho-S6 Ribosomal protein signal were measured using Imaris 10.1 software

14 Surface rendering function was used to segment Drd2-eGFP cells

14.1 Background subtraction was enabled

14.2 The diameter of the largest sphere was set at 15 µm and automatically thresholded

14.3 Smooth surface was set to 1.23 µm

14.4 Segments were filtered with an area ranging between 100 µm2 and 6,000 µm2

15 Mean intensity of phospho-S6 Ribosomal protein within Drd2-eGFP surface above 2 times of average phospho-S6 Ribosomal protein channel mean intensity was considered positive