VALIDATION NEURONS

Which NeuN?

* Storks et al. 2023 made homogenates and then resuspended the pellet in a 1:100 dilution of **NeuN (ABN78C3, Millipore Sigma)** and incubated under agitation overnight in the dark at 4°C. We then centrifuged the nuclei, rinsed with PBS, and then resuspended in 200–400 uL for counting. Everything was fixed with 4% paraformaldehyde for at least three days. Check if fixing with our method may work.

What was the dissolvent in the NeuN dilution?

* Farrow et al. 2021 dissected brains and post-fixed for 1 h in 4 % paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4 before being placed in 1% NaN3 in 0.1 M PBS for a minimum of three days. Later, they resuspended the pellet in 100 μL of homogenization buffer containing a 1:100 dilution of mouse anti-NeuN monoclonal antibody (EMD Millipore, USA) for 30 min on ice. The brain tissue lysate was washed by adding 1 mL of ice-cold PBS (pH 7.4) and centrifuged at 300 g for 3 minutes and the supernatant discarded. The pellets were resuspended in 100 μl of homogenization buffer containing a 1:200 dilution of goat anti-mouse IgG-FITC antibody (EMD Millipore, USA) for 30 minutes on ice in the dark. Brain tissue lysate was washed by adding 1 ml of PBS (pH7.4) and centrifuged at 300 g for 3 minutes. The supernatant was discarded, and the cells resuspended in 100 μl of PBS (pH 7.4) and were stored on ice in the dark. Why this last steps? Is it related to the fluorophore conjugate for NeuN?

We may need to **fix the tissue 4% PFA** once the homogenates are done.

We may need to permeabilize the tissue. Are we doing that for DNA damage? Check our protocols with Dalton

We need a fluorophore conjugate for NeuN for the flow cytometer. Ask Mick and tell him about our plan.

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