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Direct Reprogramming of Glial Cells into Interneurons

Background: There are many neurodegenerative diseases that for most of human history have been deemed incurable. Certain neurological, and some neuropsychiatric disorders, can arise due to the dysfunction of neurons such as interneurons in the brain.

Previous studies have looked at the possibility of combatting these diseases with one of the newest technologies in the field, *in vivo* direct reprogramming of somatic cells into interneurons (1). Using adeno-associated viruses (AAVs) with certain transcription factors that allow for reprogramming and expression of GFP in reprogrammed neurons that is driven by the neuronal promoter synapsin as opposed to, for positive control, the targeted glial cells or astrocytes can be visualised by using AAVs with GFP under the ubiquitous chicken B actin (CBA) promoter (2). This is important to see the neurons that are specifically reprogrammed from the NG2/PDGFRα positive glial cells, and GFAP positive astrocytes.

Methods: The following methods were performed prior to *in vivo* reprogramming to determine the genotype of the animals and choosing the transgenic cre-positive animals that are to be used in the experiments.

Genotyping. The KAPA Mouse Genotyping Kit (KK7301) was used. The master mix was made with Polymerase Chain Reaction (PCR) grade water, 10X KAPA Express Extract Buffer, 1 *U/μL* KAPA express Extract Enzyme, and added to the mouse tissue to extract DNA. Lysis was performed at 75°C for 10 minutes before the enzyme was inactivated at 95° for 5 minutes. Then a PCR master mix was prepared by combining PCR-grade water, 2X KAPA2G Fast Genotyping Mix with dye, a forward and a reverse primer for the internal control as well as a forward and a reverse primer for cre along with DNA. The PCR was performed with the TouchDown cycling protocol as outlined in the *Supplementary table 1*.

Gel electrophoresis. An agarose gel was prepared and loaded with a gel ladder and samples including positive and negative controls. The gel electrophoresis was run at 120 *V* and 700 *mA* for 45 min.

Surgery: The animals were injected with Ascl1, Lmx1a and Nurr1 (ALN) reprogramming factors along with Syn-GFP and either a positive (CBA-GFP) or negative control (Syn-GFP) on either side of the striatum. Following methods were performed 4 weeks following *in vivo* reprogramming.

Immunohistochemistry. The animals were transcardially perfused with 4% paraformaldehyde (PFA) and their brains were post-fixed in PFA overnight. Following this, the brains were put in a solution of 25% sucrose for 12 hours and stored at 4°C until cutting them on a microtome. The brains were frozen with dry ice and cut at a thickness of 35 μm and were collected in 8 series. They were stored in antifreeze before being stained according to standard protocol found in *Supplementary figure 1*.

Microscopy. The fluorescence microscopy was used to look at the co-localisation of GFP and neuronal markers.

Results: Two sets of animals were genotyped. A gel electrophoresis was done to determine the Cre positive transgenic mice. 9 out of 14 NG2 animals and 1 out of 4 PDGFRα animals were cre-positive in one set and 8 out of 16 of GFAP animals and 3 out of 6 of PDGFRα animals were cre-positive in the other set (*Figure 2*).

Experiments with CBA-GFP show that cre is expressed in PDGFRα glial cells as the GFP co-localises with the PDGFRα glial marker (*Figure 3*). The ALN + Syn-GFP experiments show reprogramming of glial cells into interneurons (*Figure 4*). In the NG2 strain, there are few cre-expressing glial cells and a low targeting efficiency. For the GFAP strain, there is unspecific expression of cre in neurons, not just astrocytes.

Conclusion: The expression of GFP in the CBA-GFP injected PDGFRα animals show that this is a good candidate for further experimentation as cre is specifically expressed in glial cells. Moreover, this is further proven by the ALN + Syn-GFP experiments where some of the targeted glial cells seem to become neurons. The NG2 strain proved difficult to provide regular and extended reprogramming and by the leakage in the GFAP lineage, it is deemed unwise to continue with these strains in further experiments.

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There is still a lot that needs to be learnt about reprogramming of somatic cells into neurons. Though somewhat reliable methods are being developed, there still needs to be more optimisation and studies done on how to improve this field of research.

Figures:

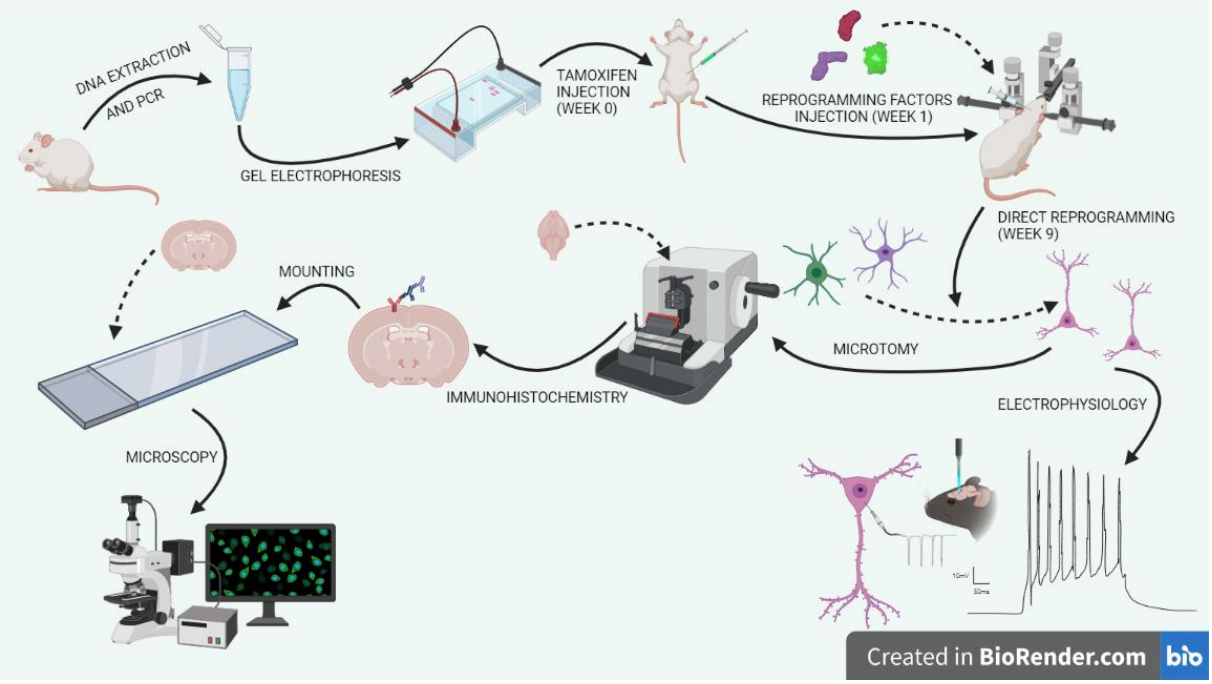

Figure 1. Graphical abstract outlining the complete work of the lab. The basic steps include genotyping, surgery leading to direct reprogramming of glial cells into neurons, and electrophysiology or immunohistochemistry.

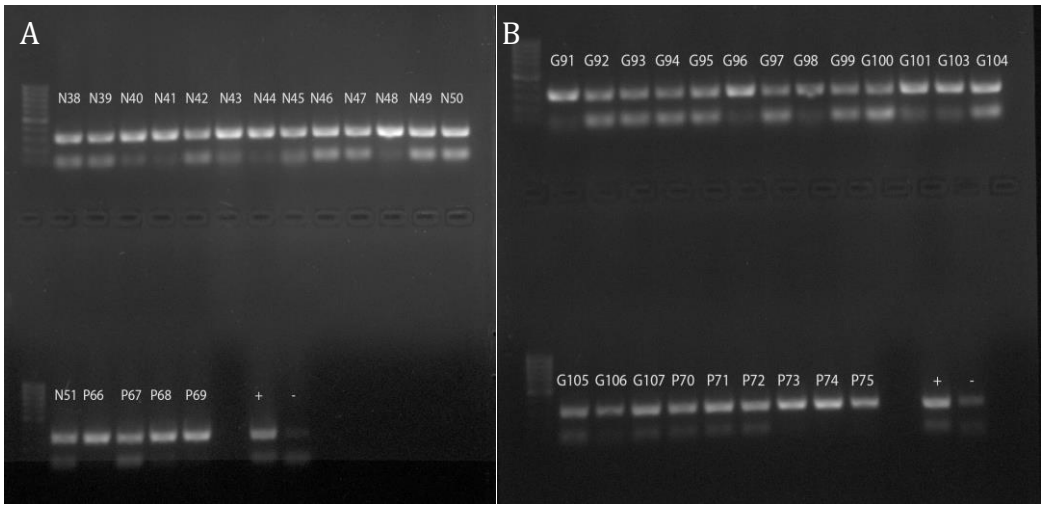

Figure 2. Gel electrophoresis looking at the cre-positive animals that can be used in further studies on the reprogramming of glial cells into neurons. The upper row shows the internal control to make sure the samples have been prepared properly and the lower row determines if the animal is cre-positive and thus apt for reprogramming. The strands of mice used in the experiments are N, NG2 (premature oligodendrocytes), P, PDGFRα, and G, GFAP. The cre-positive samples were determined to be N38, N39, N42, N45, N46, N47, N49, N50, N51, P67 (Panel A), P70, P71, P72, G92, G93, G94, G95, G97, G99, G100, G104, and G105 (Panel B).

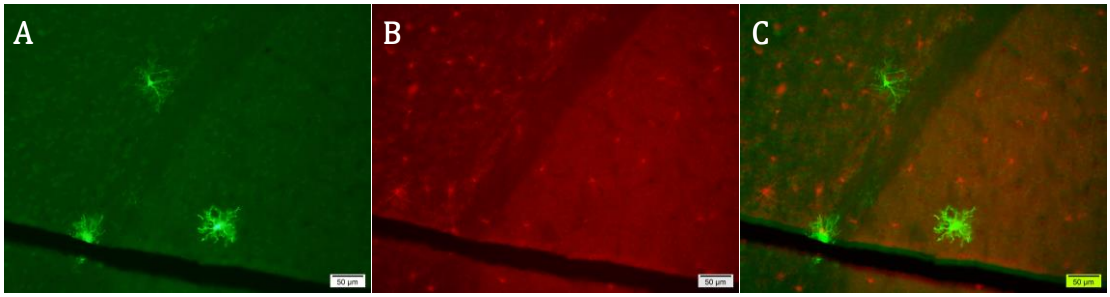

Figure 3: Fluorescence microscopy of GFP co-localising with PDGFRα in CBA-GFP injected animal. The AAV injections were performed 21 days post the tamoxifen treatment. Panel A shows GFP positive glial cells that only expresses GFP in the presence of Cre and Panel B shows the glial marker and marks all PDGFRα glial cells. As the cells in Panel A co-localises with cells in Panel B (see Panel C for merged image), it can be determined that the GFP positive cells indeed express Cre and co-localises with oligodendrocyte progenitor cells which PDGFRα is a marker for. The scale bar of all panels is 50 µm.

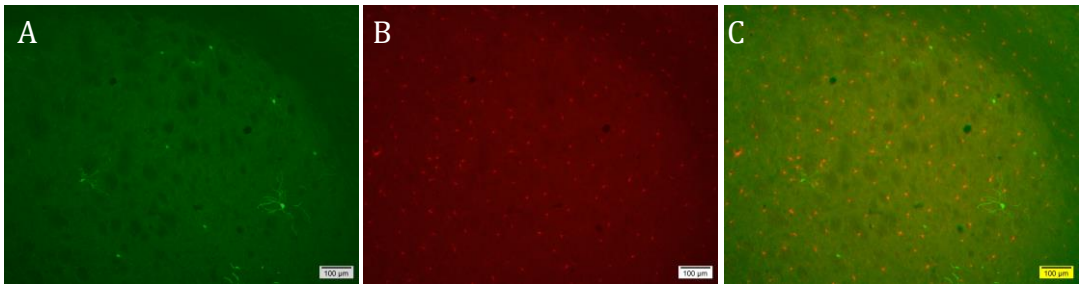

Figure 4: Fluorescence microscopy done on an ALN + Syn-GFP injected PDGFRα animal. The AAV injections were performed 21 days post tamoxifen. ALN is the reprogramming factors the animals are injected with in order to have their glial cells reprogrammed into interneurons. The GFP staining (Panel A) show cre expressing neuronal cells that are not co-localising with the glial PDGFRα marker (Panel B) in Panel C. This shows that the neuronal cells have been reprogrammed from the oligodendrocyte progenitor cells. The scale bar of all panels is 100 µm.

Supplementary figures and tables: https://1drv.ms/w/s!AkZu3Z4teGI38h3oTbRPQyfV33TL

References:

1. Pereira M, Birtele M, Rylander Ottosson D. Direct reprogramming into interneurons: potential for brain repair. Cell Mol Life Sci. 2019;76(20):3953–67.

2. Torper O, Ottosson DR, Pereira M, Lau S, Cardoso T, Grealish S, et al. In Vivo Reprogramming of Striatal NG2 Glia into Functional Neurons that Integrate into Local Host Circuitry. Cell Rep. 2015 Jul 9;12(3):474–81.