BrainFectIN™ - *Results*

Major difficulties with gene transfer in the central nervous system is the weakness of non-viral gene carriers and the safety problems associated to the use of viral particles. Actually, cationic lipids as well as polymeric formulations have shown limited outcomes (Roessler and Davidson, Neurosc. Lett, 1994; Boussif *et al.*, PNAS, 1995), when viral particles have been recognized for their risk of excessive immune response or insertional mutagenesis (Somia and Verma, Nat. Rev. Genet. 2000).

BrainFectIN™ transfection reagent main benefits:

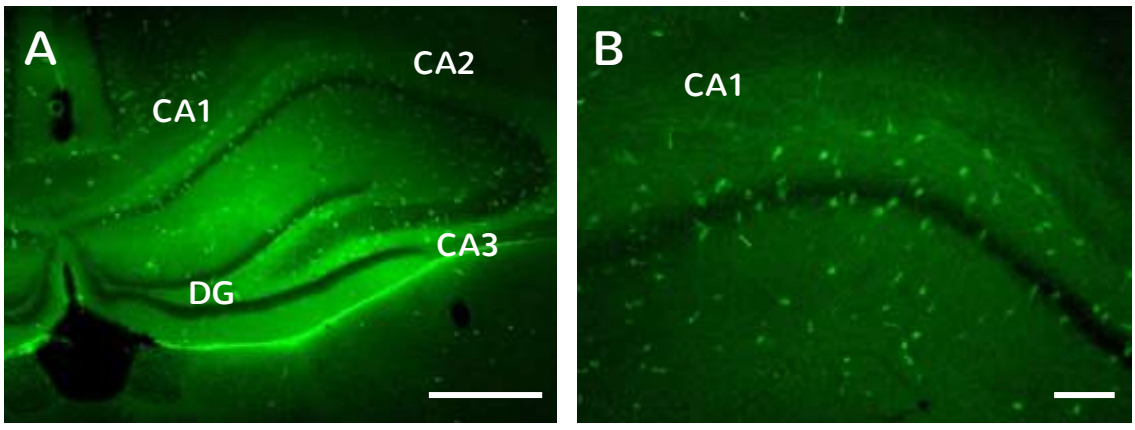
- Increased DNA transfection efficiency
- Targeting of specific regions (stereotaxic injection)
- Reduction of the injected volume
- Reduced DNA doses
- Minimized toxicity
- Low immunogenicity
- Rapid and long-term transgene expression

Applications

BrainFectIN™ is a new non-viral formulation that allows transfection of neural cells in specific brain area following stereotaxic injection.

Hippocampal neurons transfection with BrainFectIN™ following stereotaxic intra-hippocampal injection

Stereotaxic injection of **BrainFectIN™**/DNA complexes in hippocampus induced an efficient transfection rate of neural cells in all areas of hippocampus.



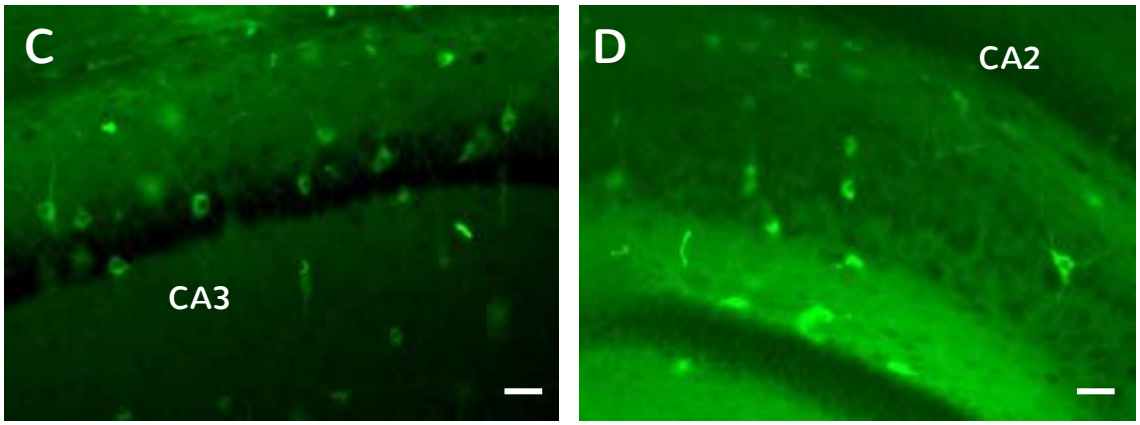


Fig. 1: GFP expression in hippocampus of rat (p11) 48h after **BrainFectIN™**/pGFP injection (ratio 1:1.5 – 1µg of pGFP was mixed with 1.5µL of **BrainFectIN™**). Scale bar = 100µm. **BrainFectIN™**/pGFP (1µL) was injected through a nanofil needle into the hippocampus at the following coordinates: 2mm posterior to the Bregma, 3mm lateral to the midline, and 4mm ventral to the skull surface. Brain were harvested by intra-cardiac paraformaldehyde perfusion and sliced at 100 µm thickness using a vibratome. Immunohistochemistry with an antibody directed against GFP was performed in order to increase the signal. GFP+ cells are located in Dentate Gyrus (A) as well as other hippocampal areas such as CA1 (A,B,C), CA2 (A) and CA3 (A,D). Negative controls have been done with a stereotaxic injection of DNA alone in the same conditions, and shows few transfected cells (data not shown).

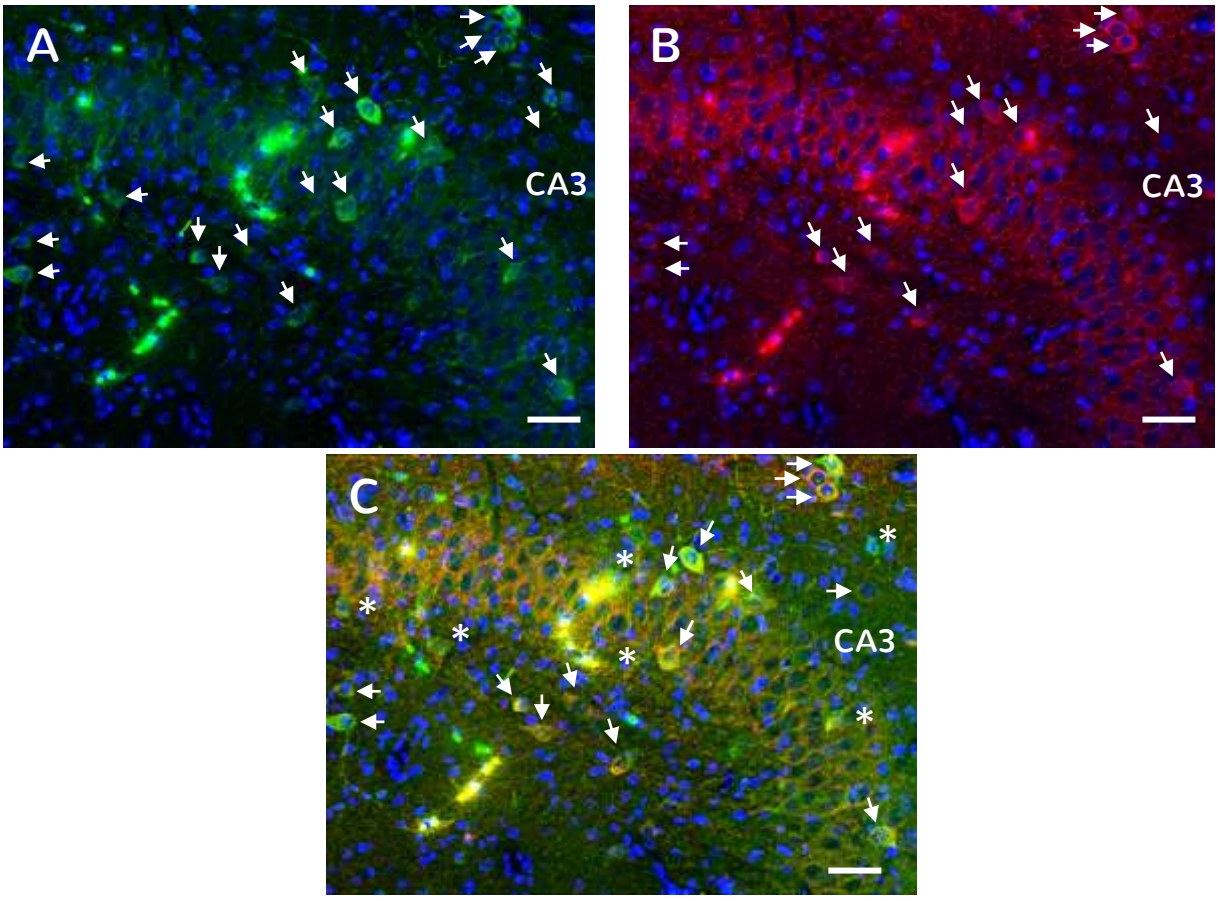


Fig. 2: GFP and GAD 65/67 expression in hippocampus of rat (p11) 48h after **BrainFectIN™**/pGFP injection (ratio 1:1.5). Scale bar = 50µm. **BrainFectIN™**/pGFP (1µL) was injected through a nanofil needle into the hippocampus at the following coordinates: 2mm posterior to the Bregma, 3mm lateral to the midline, and 4mm ventral to the skull surface. Brain were harvested by intra-cardiac paraformaldehyde perfusion and sliced at 100 µm thickness using a vibratome. Double immunofluorescence staining was performed on slices. Transfected cells are GFP+ (A, arrows), and interneurons are labelled with GAD 65/67 (B, arrows), nuclei are counterstained with Hoechst 33342 (A,B,C). Merge shows that we are able to transfect GABAergic interneurons (C, arrows). By exclusion, every other cells GFP+ are pyramidal cells (C, asterix). As Granular cells are also visible into the dentate gyrus, altogether these data show that **BrainFectIN™** allows to transfected at least 3 different neural cell types after intra-hippocampal injection.

Hippocampal neurons transfection with BrainFectIN™ following stereotaxic cortical injection

The stereotaxic injection of **BrainFectIN™**/DNA complexes in the cortical area induces the expression of transgene in cluster of cortical cells.

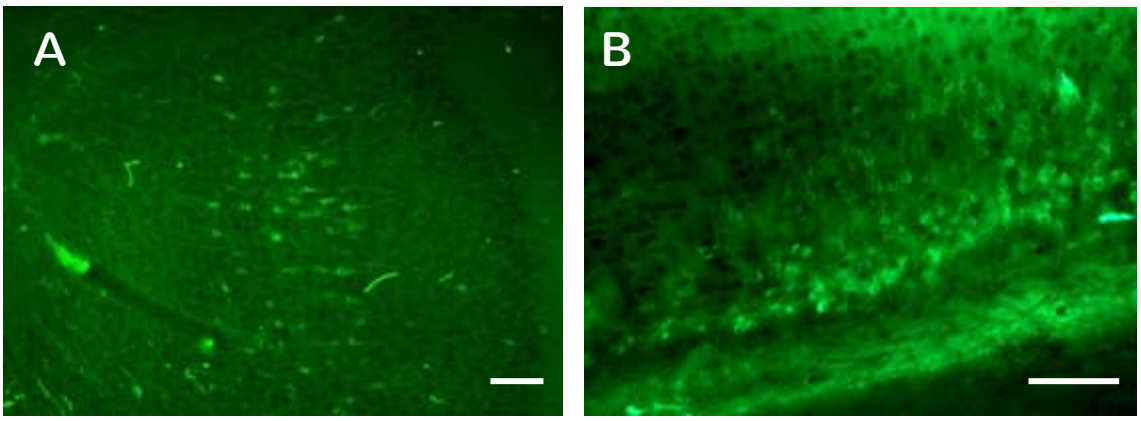


Fig. 3: GFP expression in hippocampus of rat (p12) 48h after **BrainFectIN™**/pGFP injection (A, ratio 1:1,5; B, ratio 1:1). **BrainFectIN™**/pGFP (1µL) was injected through a nanofil needle onto cortex at the following coordinates: 2mm posterior to the Bregma, 3mm lateral to the midline, and 3mm ventral to the skull surface. GFP+ cells are located as cluster in the cortex. Scale Bar = 100µm.

BrainFectIN™/DNA complexes diffusion after intra-hippocampal injection

After injection, **BrainFectIN™**/pGFP complexes can spread into the whole hippocampus structure from rostro-caudal to lateral direction.

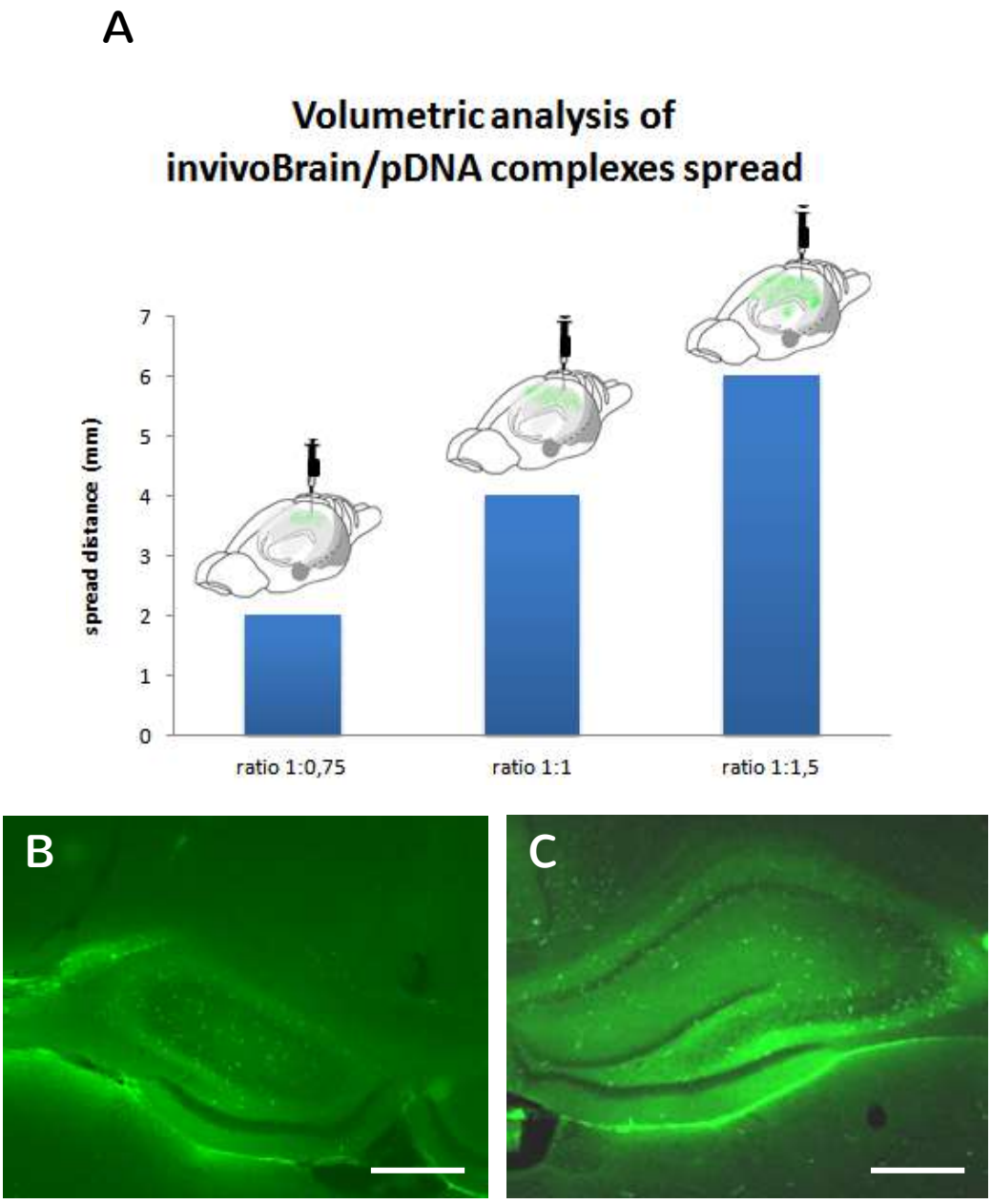


Fig. 4: Quantitative (A) and immunofluoscence microscopic (B, C) analysis of **BrainFectIN™**/DNA spread into the rat hippocampus (p12). **BrainFectIN™**/pGFP (ratio 1:1.5, 1µL) was injected through a nanofil needle into the hippocampus at the following coordinates: 2mm posterior to the Bregma, 3mm lateral to the midline, and 4mm ventral to the skull surface. PFA-perfused brains were sliced at 70µm from -4.8 mm to +0.6 mm relative from Bregma. Immunochemistry with an antibody directed against GFP was performed in order to increase the signal. Pictures were done at different levels of the hippocampus and cells were then counted manually. GFP+ cells are localized in frontal gyrus (B) as well as areas CA1, CA2, CA3 and dentate gyrus of hippocampus (C). These data show that the complexes **BrainFectIN™**/pGFP can diffuse in a rostro-caudal direction in a ratio-dependant manner.

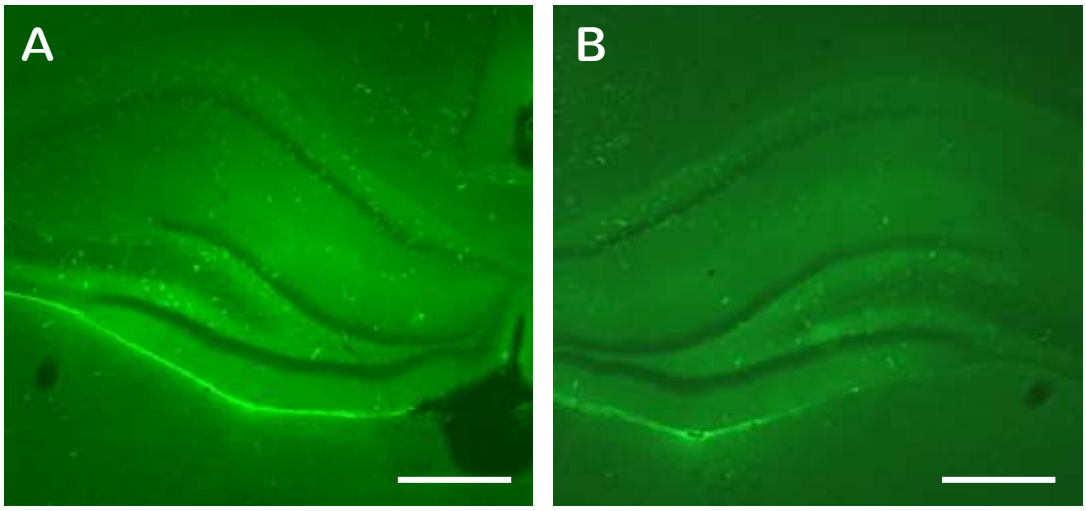


Fig. 5: GFP expression in the ipsilateral (A) and contralateral (B) rat hippocampus (p11) 48h after **BrainFectIN™**/pGFP injection (ratio 1:1,5). **BrainFectIN™**/pGFP (1µL) was injected through a nanofil needle into the hippocampus at the following coordinates: 2mm posterior to the Bregma, 3mm lateral to the midline, and 4mm ventral to the skull surface. Immunochemistry with an antibody directed against GFP was performed in order to increase the signal on PFA-perfused brains. These data show that the complexes **BrainFectIN™**/pGFP can also diffuse in a lateral direction.

Stability of BrainFectIN™/DNA expression after intra-hippocampal injection

GFP expression is detected in ipsi and contra-lateral hippocampus of young rats injected with **BrainFectIN™**/pGFP complexes 3 weeks after injection.

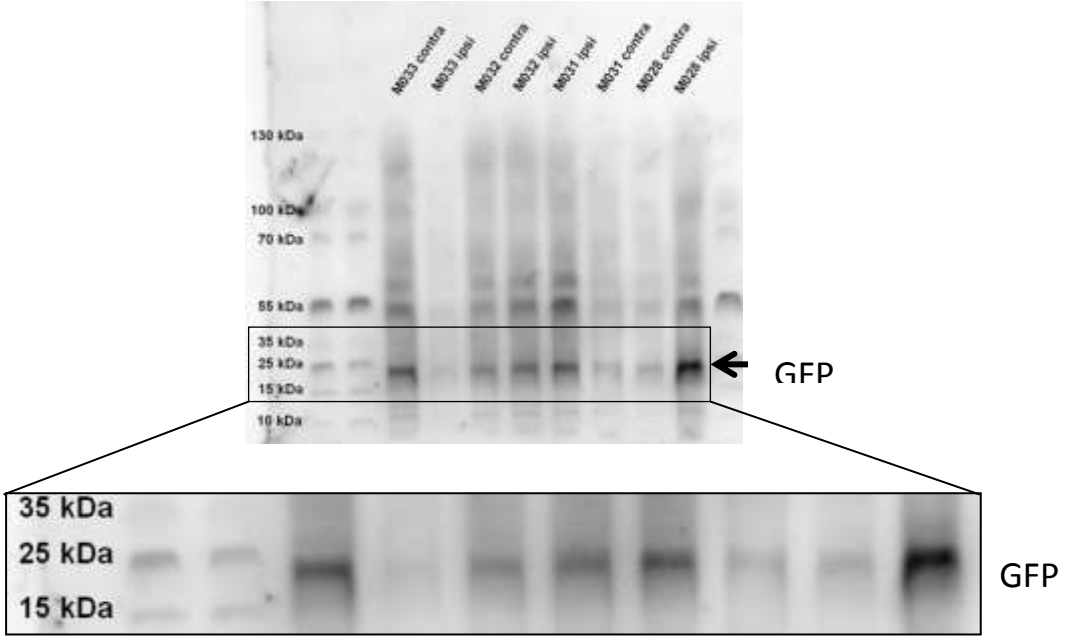


Fig. 6: Qualitative analysis of GFP expression in rat hippocampus (p11) transfected with **BrainFectIN™**/pGFP. 3 weeks after surgery, hippocampal dissection was performed, ipsi- and contra-lateral hippocampi were pulled apart, proteins were extracted with RIPA buffer, and quantified using Pierce BCA Protein assay kit. Western blot analysis was performed and GFP expression was detected (27 kDa).

Bibliography

Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr JP. A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: polyethylenimine. *Proc Natl Acad Sci U S A*. 1995; 92(16):7297-301.

Roessler BJ and Davidson BL. Direct plasmid mediated transfection of adult murine brain cells *in vivo* using cationic liposomes. *Neurosci Lett.* 1994;167(1-2):5-10.

Somia N and Verma IM. Gene therapy: trials and tribulations. *Nat. Rev. Genet.* 2000; 1, 91–99.