

## Analysis of grafted Thy1-YFP-16 positive cells in mouse brains affected by ischaemic stroke

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### ABSTRACT

Stroke is an aggressive neurological disorder which may be haemorrhagic or ischaemic. Neural stem cells (NSCs) are a wide cell population which can differentiate into neurons and glia cells. In this study we induced stroke in mice by occlusion of the middle cerebral artery. Twenty-four hours post-surgery, NSCs were transplanted into the mouse brains. For purpose of this study two mouse strains were used, wild type and transgenic B6.Cg-Tg(Thy1-YFP)16Jrs/J (Thy1 YFP-16) kept on the same background. In total nine embryos and twelve adult animals were used: two pregnant females, five males for middle cerebral artery occlusion (MCAO), and five males for the control group. Thy1 YFP-16 cells express YFP in approximately 22% neurons under the influence of Thy1 promoter. The main goal of this study was to analyse the grafted cells eight weeks post transplantation in these two groups of animals. A significant difference in TUBB3 positive neurons ( $P=0.0340$ ) and GFAP positive astrocytes ( $P=0.0004$ ) between MCAO and the control group was found. Moreover, a significantly higher percentage of Thy1-YFP cells was found in the control group of animals ( $P=0.0003$ ). Pearson's coefficient of colocalization showed colocalization between Thy1-YFP and TUBB3 positive neurons, but there was no colocalization between Thy1-YFP and GFAP positive astrocytes. On the basis of our data we can conclude that Thy1-YFP cells may serve for transplantation and cell tracing studies.

**Key words:** Thy1-YFP-16; neural stem cells; cell transplantation; graft; stroke

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### Introduction

Stroke is one of the greatest medical problems worldwide and occurs as a result of disturbance in brain circulation which lasts at least 24 hours, leading to a loss of neurological function and/or death (HANKEY, 2017). In terms of aetiology, it is

classified into ischemic stroke, which accounts for more than 80% of stroke cases, and haemorrhagic stroke (ZHANG et al., 2019). Ischemic stroke occurs due to arterial occlusion causing reduced blood flow in the brain (CAMPBELL and KHATRI,

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2020) and resulting in local nervous tissue hypoxia and ischemia (ZHANG et al., 2019). In recent years, cell therapy has been gaining significance as a potential treatment for many diseases and conditions, including stroke (LIAO et al., 2019).

Neural stem cells (NSCs) are pluripotent cells which can differentiate into neurons, astrocytes and oligodendrocytes (LIAO et al., 2019; ZHANG et al., 2019). Transplantation of NSCs after a stroke can stimulate axonal growth, neurogenesis and angiogenesis (LIAO et al., 2019; ZHANG et al., 2019), as has been shown by several preclinical studies (CHEN et al., 2016; KALLADKA et al., 2016). Mouse NSCs (ALIĆ et al., 2016), as well as human NSCs (ISLAM et al., 2021), can be used for transplantation into a mouse brain affected by stroke. In this study, we used Thy1-YFP-16 cells isolated from E14.5 mouse telencephalon. Multiple Thy1 mouse strains have been described (FENG et al., 2000) and have been used successfully to study neuronal differentiation during development (PORRERO et al., 2010), visualizing cell projections and neuronal fate (ALIĆ et al., 2016), cell tracing (MITREČIĆ et al., 2017), and even tumours, inflammation and wound-healing processes (JÓSVAY et al., 2014). There are two main reasons why we used these cells for transplantation into the brains affected by stroke: (1) transplanted NSCs can replace and repair damaged brain cells after ischaemic injury, and (2) Thy1-YFP positive cells can serve as a toll to describe cell fate in the host tissue affected by ischaemic stroke, as well as in a healthy brain.

In this study, NSCs isolated from the Thy1-YFP-16 mouse E14.5 embryos were transplanted into three-month-old wild type mice of the same background, divided into two groups - the stroke-affected and the healthy control. The main goal of our study was graft analysis eight weeks post transplantation.

## Materials and Methods

*Animals and housing.* B6.Cg-Tg(Thy1-YFP)16Jrs/J (Thy1 YFP-16) and C57Bl/6NCrl (wild type) mice (The Jackson Laboratory, Bar

Harbor, ME, USA) were used in this study. The animals were kept in the animal facility of the Croatian Institute for Brain Research at a temperature of  $22 \pm 2^\circ\text{C}$ , with  $55\% \pm 10\%$  humidity, and 12/12 h light/dark cycle, while water and pelleted food (Mucedola 4RF21) were given *ad libitum*. All experiments were approved by Internal Review Board of the Ethical Committee of the School of Medicine, University of Zagreb (04-77/2010-238), and the Faculty of Veterinary Medicine (251/61-01/139-13-4) in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes. For this study, 12 adult animals and nine E14.5 embryos were used: two pregnant females, five males for middle cerebral artery occlusion (MCAO), and five males for the control group.

*Neural stem cells and cell culture.* Two week pregnant Thy1 YFP-16 females (n=2) were used for isolation of NSCs. NSCs were isolated as previously described (ALIĆ et al., 2016; MITREČIĆ et al., 2017; KOSI et al., 2018). Briefly, NSCs were isolated from the embryonic telencephalon (n=9, E14.5) and expanded in a proliferation supporting media. NSCs were grown as neurospheres, and passaged once before transplantation into the mouse brains. Before transplantation, the cells were validated and checked for NSCs specific markers such as Nestin and SOX2 (ALIĆ et al., 2016).

*Middle cerebral artery occlusion.* Ischaemic stroke was induced by transient left middle cerebral artery occlusion (MCAO) in the wild type mice, as previously described (ALIĆ et al., 2016; KOSI et al., 2018). Briefly, the surgery was performed in inhalation anaesthesia mixture of 2% isoflurane in 100% O<sub>2</sub>. Surgery was performed under a dissection microscope (Zeiss, Stemi DV4 Spot). The intraluminal filament was inserted through the common carotid artery into the internal carotid artery up to the origin of the middle cerebral artery and left for 90 min. After 90 min the intraluminal filament was withdrawn to restore circulation and the animal received analgetic buprenorphine intraperitoneally (0.03 mg/kg).

*Stereotaxic transplantation of NSCs into the mouse brains.* NSCs were prepared for transplantation into the mouse brains affected by stroke, as well as the control group of animals, as previously described (ALIĆ et al., 2016; MITREČIĆ et al., 2017; KOSI et al., 2018). The hippocampal stereotaxic coordinates (AP -1.3, ML +2.0 and DV -1.5) were determined according to the stereotaxic atlas (HOF et al., 2000), and injections were performed using KOPF stereotaxic apparatus (900LS) and a 2 $\mu$ L Hamilton syringe needle (7002KH, ga25/70mm/pst2). Stereotaxic transplantation was performed 24h after MCAO. In total, 1x10<sup>6</sup> cells in 1  $\mu$ L proliferation supporting media were injected into the mouse brain. The animals were anaesthetised using Avertin injected intraperitoneally (0.5 g/kg).

*Animal sacrifice, perfusion, fixation and tissue isolation.* The animals were anesthetized using Avertin and then perfused transcardially with PBS, and subsequently with 4% paraformaldehyde in PBS (pH 7.4). The brains were isolated and further fixed by immersion in the same fixative at 4°C overnight. Tissues were cryoprotected by 10% sucrose for 24h, followed by 30% sucrose at 4°C. Twenty  $\mu$ m thick frontal sections were prepared by cryostat and stored at -20°C.

*Immunohistochemistry.* Immunohistochemistry was performed as previously described (ALIĆ et al., 2016; MITREČIĆ et al., 2017; KOSI et al., 2018). Tissues were immunolabelled with specific primary antibodies as follows: for neurons TUBB3 was used (rabbit polyclonal ab, diluted 1:1000, Biologend, 802001) while for astrocytes GFAP was used (chicken polyclonal ab, diluted 1:250, Abcam, ab4674). Onwards, tissues were stained with Nestin (mouse monoclonal ab, diluted 1:200, Millipore, MAB353) and SOX2 (rabbit polyclonal ab, diluted 1:200, Novus Biologicals, NB110-37235) (not shown). Primary antibodies were diluted in 0.2% Triton X-100 in PBS and 3% goat serum at 4°C overnight. On the following day the primary antibodies were rinsed and incubated with fluorescent secondary antibodies as follows: Alexa Fluor 647 donkey anti-rabbit (ThermoFisher Scientific, A-31573) and Alexa Fluor 633 goat

anti-chicken (ThermoFisher Scientific, A-21103), diluted 1:500 in 0.2% Triton X-100 in PBS. Secondary antibodies were rinsed with PBS and counterstained with DAPI. Finally, tissues were rinsed in PBS and mounted with Dako Fluorescent Mounting Medium. Fluorescent analysis was performed with a confocal microscope Olympus FV 3000.

*Image Analysis and statistics.* Fluorescent images were captured using an Olympus FV 3000 confocal microscope. In total, 5-8 figures were captured per animal/condition and analysed by IMARIS 9.9.1 (BITPLANE, An Oxford Instruments Co., Zurich, Switzerland) software and Fiji 2.9.0 software (SCHINDELIN et al., 2012). Quantification was performed blinded to the condition, using IMARIS 9.9.1 “surface” and “spot” features, as well as Pearson’s coefficient of colocalization. Pearson’s coefficient was calculated by IMARIS 9.9.1 software, on the basis of the automatically calculated threshold. The total intensity of TUBB3 and GFAP was analysed per field of view, and normalised by the DAPI surface. The percentage of Thy1-YFP positive cells was calculated per field of view, and normalised by the DAPI positive nuclei. Statistical analyses were carried out using the standard procedures and guidelines in GraphPad Prism Software (v.8.4.2). The specific tests used are noted in the figure legend for each set of data. The following tests were used in this study: Shapiro-Wilk normality test, followed by the unpaired t-test. Statistical significance was defined as P>0.05 (ns), P<0.05 (\*), P<0.01 (\*\*), P<0.001 (\*\*\*), P<0.0001 (\*\*\*\*).

## Results

Thy1-YFP-16 NSCs were transplanted into the mouse brains using a stereotaxic frame, 24h after MCAO. In total 1x10<sup>6</sup> cells/1  $\mu$ L were injected into the mouse brains. For the purpose of this study, cells were injected into the penumbra surrounding the stroke area. Moreover, as this model leads to a cortico-striatal stroke, cells were also transplanted into the hippocampal region. The same coordinates were used for the stroke affected group of mice (n=5) and the control mice (n=5). Eight weeks

post transplantation the animals were sacrificed, the brains were isolated, cryoprotected, sliced, and histologically analysed.

All stroke affected brains had a large cortical scar on the left hemisphere and a dramatically smaller left hemisphere compared to the contralateral hemisphere, as well as compared to the brains in the control group of animals. For histological analysis, the whole brains were sliced into 20µm thick slices. In total, from the stroke affected mice we obtained 10 slides with grafted cells; there were ten to twelve 20µm thick sections on each slide. Finally, the graft volume in the stroke affected mice was around 2000-2500µm. Grafted cells migrated toward the stroke core and completely incorporated their processes into the host tissue. On the other hand, in the control group the graft was 50% smaller than in the stroke affected group; the grafted cells did not migrate from the graft.

The histological slides were stained with a pan-neuronal marker, TUBB3 (Fig. 1 and 3) and an astrocytic marker, GFAP (Fig. 2 and 3). For the purpose of this study, we analysed the total intensity of both markers in the graft. Our data showed a difference in tissue architecture and cell number. Moreover, in the MCAO animals compared to the control group a smaller number of grafted DAPI positive nuclei were observed. This result was expected because in the MCAO animals the brain tissue went through massive cell death and inflammation caused by the stroke which also affected the transplanted cells. Our data showed a significant difference between the MCAO and the control animals stained with TUBB3 ( $P=0.0340$ ), with higher expression in the control group (Fig. 1 and 3a). This result may be explained by the massive cell death present in the MCAO animals. However, regardless of the differences observed, in both groups Thy1-YFP differentiated to mature neurons which were completely incorporated into the host tissue and formed a strong neuronal network.

The adjacent slides were stained with GFAP and they showed a significantly higher difference between the MCAO and the control group

( $P=0.0004$ ), with higher expression present in the MCAO group (Fig. 2 and 3b). In other words, apart from the host reactive astrocytes around the graft, the majority of transplanted cells differentiated into GFAP positive astrocytes. Moreover, the slides were stained with NSCs specific markers (Nestin and SOX2), and the result was negative since the majority of transplanted cells were differentiated into mature cells during the eight weeks post transplantation (not shown).

We also analysed the percentage of Thy1-YFP cells in both groups of animals. A significantly higher percentage of positive cells was observed in the control group compared to the MCAO group ( $P=0.0003$ ) (Fig. 3c). The reason for the huge difference in Thy1-YFP positive cells could be in the high ratio of cell death in the MCAO group.

Finally, we analysed colocalization between Thy1-YFP and TUBB3 positive neurons as well as GFAP positive astrocytes (Fig. 3d-e). Pearson's coefficient of colocalization showed colocalization between Thy1-YFP and TUBB3 positive neurons because Thy1 is specific for neurons, and is expressed in all cell compartments. On the other hand, there was no colocalization between Thy1 and astrocytes.

## Discussion

In our study we analysed Thy1-YFP-16 cells grafted into mouse brains affected by stroke. There are several reasons why we chose this mouse strain for NSCs isolation and transplantation into the mouse brain: (1) this mouse strain was described by FENG et al. (2000) as a transgenic mouse strain which expresses green fluorescent protein or one of its spectral variants, in our case yellow fluorescent protein (YFP); (2) YFP expression, under the influence of Thy1 promoter, is neuron specific (FENG et al., 2000); (3) approximately 22% NSCs express YFP (ALIĆ et al., 2016); (4) fluorescent protein is expressed in all cell compartments, including dendritic spines (ALIĆ et al., 2016; FENG et al., 2000; YANG et al., 2023a).



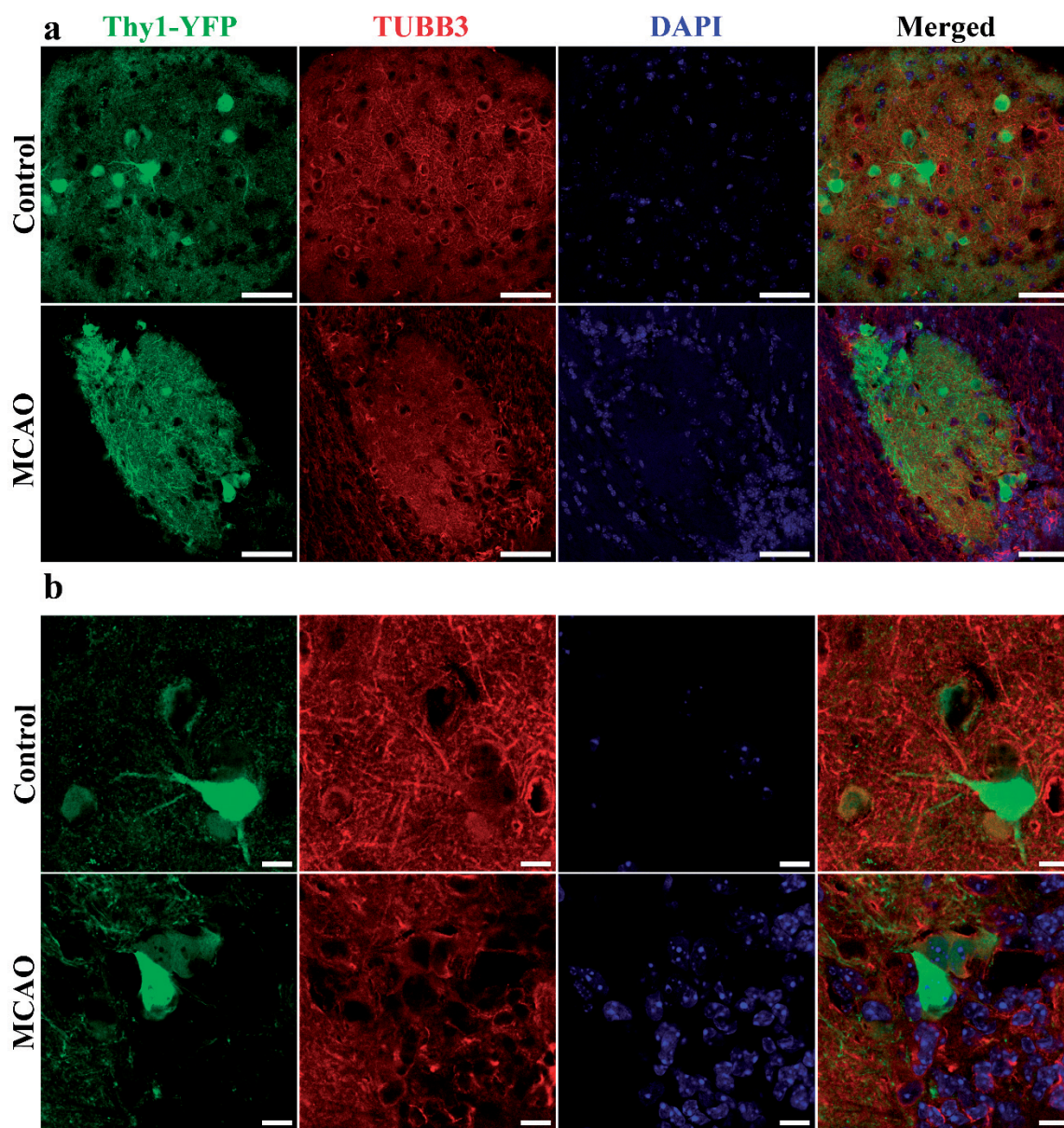


Fig. 1. Representative figures showing Thy1-YFP grafted cells into a mouse brain stained with pan-neuronal marker - TUBB3 (red) and DAPI counterstained (blue) on lower magnification (a, Scale bar: 50 $\mu$ m) and higher magnification (b, Scale bar: 10 $\mu$ m)

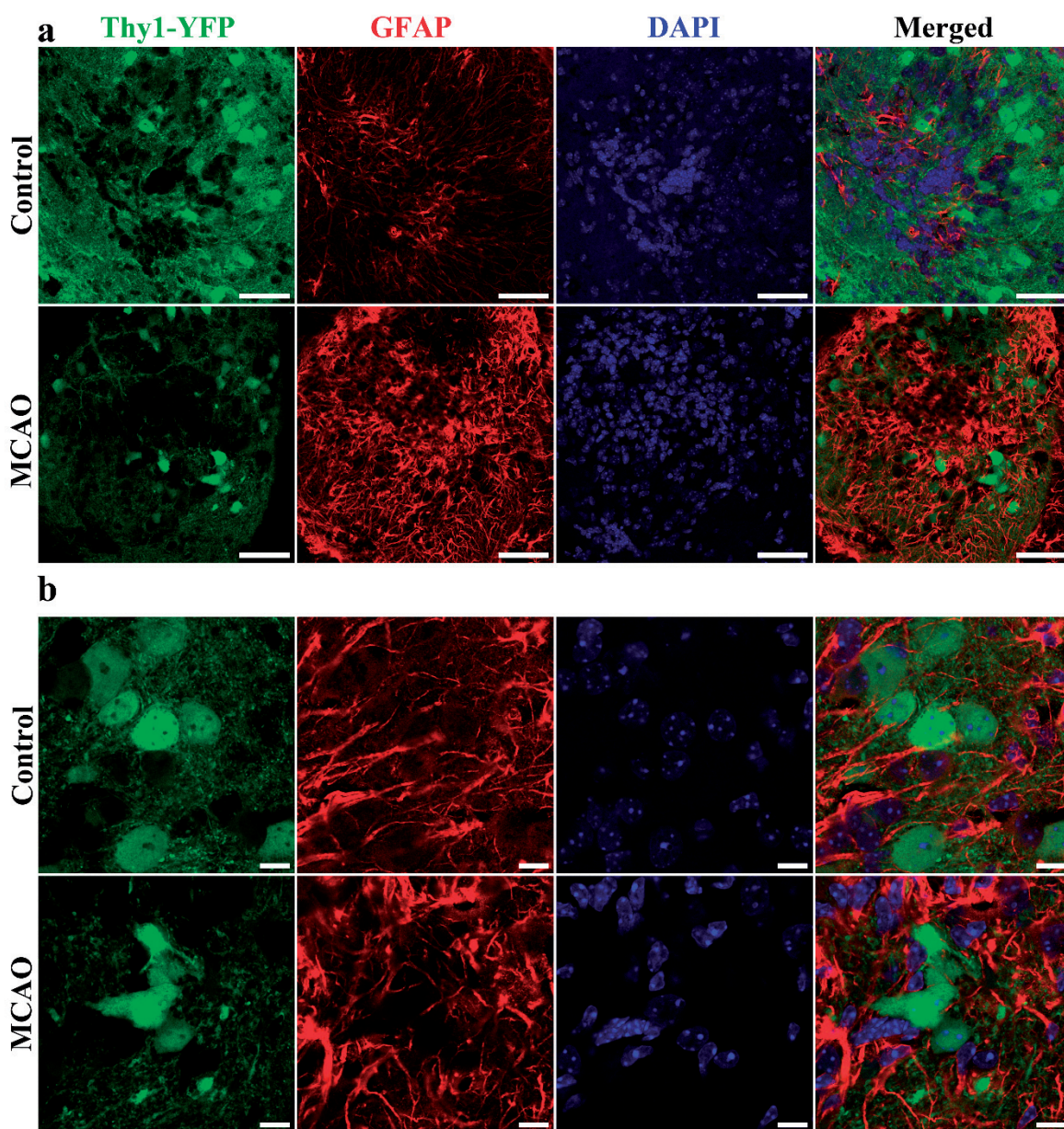


Fig. 2. Representative figures of Thy1-YFP grafted cells into a mouse brain stained with astrocytic marker - GFAP (red) and DAPI counterstained (blue) on lower magnification (a, Scale bar: 50 $\mu$ m) and higher magnification (b, Scale bar: 10 $\mu$ m)



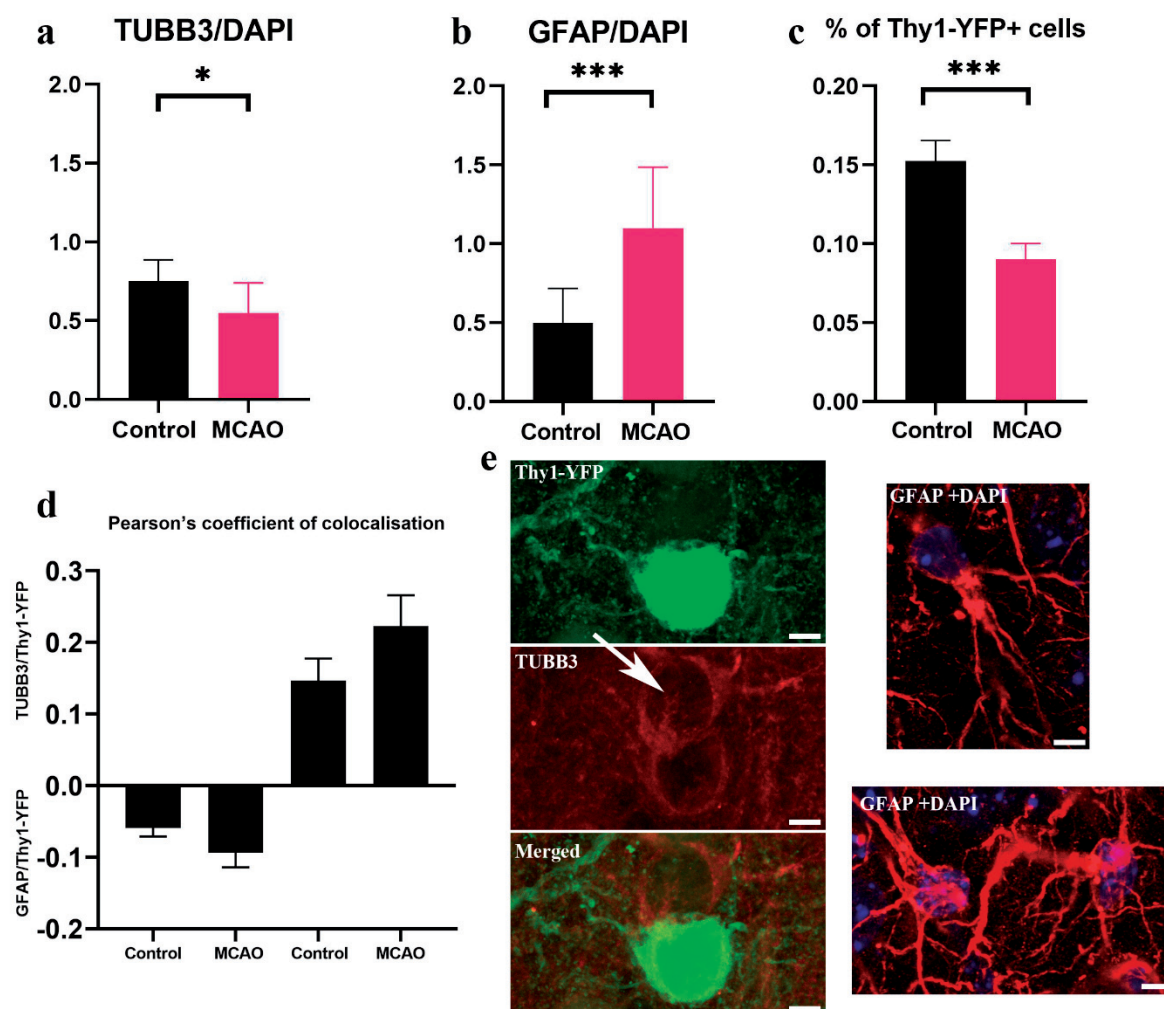


Fig. 3. Analysis of grafted Thy1-YFP cells

Surface analysis showed: a significant difference between control and MCAO groups of animals stained with TUBB3 ( $P=0.0340$ ) (a) and a significant difference when stained with GFAP ( $P=0.0004$ ) (b). Moreover, in the control group of animals a significantly higher percentage of Thy1-YFP cells were observed  $P=0.0003$  (c). Pearson's coefficient of colocalization showed colocalization between Thy1-YFP and TUBB3 positive neurons, but there was no colocalization between Thy1-YFP and GFAP positive astrocytes (d). Representative figures of single neurons as well as astrocytes (e). The white arrow shows a TUBB3 positive neuron but it is YFP negative. Shapiro-Wilk normality test was followed by the unpaired t-test. Scale bar:  $5\mu\text{m}$ .

The greatest advantage of this strain is the relatively small percentage, 22%, of YFP positive cells of total NSCs which allows us to visualize all cell projections. If all cells expressed the signal, it would not be possible to distinguish what belongs to which cell if we consider cell death, caused by stroke and inflammation, as well as proliferation of

transplanted cells. In this way, we were able to trace and describe individual cells inside the graft and describe cell morphology in Thy1-YFP-16 positive cells (Fig. 3e). A similar approach was used in the following studies: (1) HARRIS et al. (2022) used the Thy1-YFP-H mouse strain to study injury of the axon initial segment (AIS) in the 5<sup>th</sup> cortical

layer, (2) BOSCH et al. (2023) used the Thy-YFP strain to study Lewy Body Dementia (LBD) and Parkinson's disease (PD) and quantify the number of dendritic spines after local overexpression of alpha-Synuclein, (4) YANG et al. (2023a) analysed dendritic spines after ischaemic stroke in Thy-YFP mice and (5) YANG et al. (2023b) axonal injury after intracerebral haemorrhage in Thy-YFP mice.

NSCs are a wide cell population, isolated from telencephalon (ALIĆ et al., 2016; KOSI et al., 2018) or derived from induced pluripotent stem cells (iPSCs) (FINK et al., 2014), which have the ability of self-renewal, as well as symmetric or asymmetric division (McGEADY et al., 2014). There are several studies which demonstrate the protective influence of transplanted NSCs into the stroke affected brain (HRIBLJAN et al., 2018; KOSI et al., 2018; XU et al., 2023). In addition, transplanted cells can rebuild neural circuits, as a response to inflammation (PLUCHINO and NICAISE, 2021), which is possible with two main mechanisms: replacing and repairing damaged brain cells. In our study we analysed cells grafted in the brain affected by stroke, as well as in control group. We observed a two-fold increase in total intensity/volume per field of view of astrocytes, which is expected for neurodegenerative diseases. In other words, in the graft area there were the host's reactive astrocytes, as well as transplanted NSCs-differentiated astrocytes, which were YFP negative (Fig. 3e). Moreover, we observed significantly higher neuronal expression in the control group which we can explain by the cell death of the transplanted NSCs in the stroke affected brains and massive astrocyte differentiation. Finally, we observed a two-fold increase in the percentage of Thy1-YFP positive cells in the control group compared to the MCAO group.

### Conclusions

NSCs from the B6.Cg-Tg(Thy1-YFP)16Jrs/J (Thy1 YFP-16) mouse strain are an excellent tool for transplantation into the host, wild type, mice as we can trace and analyse single cell fate in grafted cells.

### Ethics approval

All experiments were approved by Internal Review Board of the Ethical Committee of the School of Medicine, University of Zagreb (04-77/2010-238), and the Faculty of Veterinary Medicine (251/61-01/139-13-4) in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

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### Author contribution

Ana Bekavac and Ante Plečaš contributed equally (joint first authors).

### Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

### References:

- ALIĆ, I., N. KOSI, K. KAPURALIN, D. GORUP, S. GAJOVIĆ, R. POCHE, D. MITREČIĆ (2016): Neural stem cells isolated from mouse strain Thy1-YFP are a valuable tool to monitor and evaluate cell differentiation and neuronal morphology. *Neurosci. Lett.* 634, 32-41.  
<https://doi.org/10.1016/j.neulet.2016.10.001>
- BOSCH, P. J., G. KERR, R. COLE, C. A. WARWICK, L. H. WENDT, A. PRADEEP, E. BAGNALL, G. M. ALDRIDGE (2023): Enhanced spine stability and survival lead to increases in dendritic spine density as an early response to local alpha-synuclein overexpression in mouse prefrontal cortex. *bioRxiv preprint 559765*.  
 doi: <https://doi.org/10.1101/2023.09.28.559765>
- CAMPBELL, B. C. V., P. KHATRI (2020): Stroke. *Lancet* 396, 129-142.  
[https://doi.org/10.1016/S0140-6736\(20\)31179-X](https://doi.org/10.1016/S0140-6736(20)31179-X)
- CHEN L., G. ZHANG, Y. GU, X. GUO (2016): Meta-Analysis and Systematic Review of Neural Stem Cells therapy for experimental ischemia stroke in preclinical studies. *Sci. Rep.* 32291.  
<https://doi.org/10.1038/srep32291>
- FENG, G., R. H. MELLOR, M. BERNSTEIN, C. KELLER-PECK, Q. T. NGUYEN, M. WALLACE, J. M.



- NERBONNE, J. W. LICHTMAN, J. R. SANES (2000): Imaging Neuronal Subsets in Transgenic Mice Expressing Multiple Spectral Variants of GFP. *Neuron* 28, 41-51.  
[https://doi.org/10.1016/S0896-6273\(00\)00084-2](https://doi.org/10.1016/S0896-6273(00)00084-2)
- FINK, K. D., J. ROSSIGNOL, M. LU, X. LÉVÊQUE, T. D. HULSE, A. T. CRANE, V. NERRIERE-DAGUIN, R. D. WYSE, P. A. STARKI, M. T. SCHLOOP, D. J. DUES, S. J. WITTE, C. SONG, L. VALLIER, T. H. NGUYEN, P. NAVEILHAN, I. ANEGON, L. LESCAUDRON, G. L. DUNBAR (2014): Survival and Differentiation of Adenovirus-Generated Induced Pluripotent Stem Cells Transplanted into the Rat Striatum. *Cell Transplant.* 23, 1407-1423.  
<https://doi.org/10.3727/096368913X670958>
- HAKEY, G. J. (2017): Stroke. *Lancet* 389, 641-654.  
[https://doi.org/10.1016/S0140-6736\(16\)30962-X](https://doi.org/10.1016/S0140-6736(16)30962-X)
- HARRIS, A. C., J. SUN, K. JACOBS (2022): Concussive head trauma perturbs axon initial segment function in axotomized and intact layer 5 pyramidal neurons. *bioRxiv preprint* 490079.  
<https://doi.org/10.1101/2022.04.29.490079>
- HOF, P. R., W. G. YOUNG, F. E. BLOOM, P. V. BELICHENKO, M. R. CELIO (2000): Comparative Cytoarchitectonic Atlas of the C57BL/6 and 129/Sv Mouse Brains. Elsevier, Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo.
- HRIBLIJAN, V., I. SALAMON, A. ĐEMAILI, I. ALIĆ, D. MITREČIĆ (2018): Transplantation of neural stem cells in the mouse model of ischemic brain stroke and expression of genes involved in programmed cell death. *Croat. Med. J.* 59, 203-212.  
<https://doi.org/10.3325/cmj.2018.59.203>
- ISLAM, R., S. DRECUN, B. V. VARGA, I. VONDERWALDE, R. SIU, A. NAGY, C. M. MORSHEAD (2021): Transplantation of Human Cortically-Specified Neuroepithelial Progenitor Cells Leads to Improved Functional Outcomes in a Mouse Model of Stroke. *Front. Cell Neurosci.* 15, 654290.  
<https://doi.org/10.3389/fncel.2021.654290>
- JÓSVAY, K., Z. WINTER, R. L. KATONA, L. PECZE, A. MARTON, A. BUHALA, G. SZAKONYI, Z. OLÁH, C. VIZLER (2014): Besides neuro-imaging, the Thy1-YFP mouse could serve for visualizing experimental tumours, inflammation and wound-healing. *Sci. Rep.* 4, 6776.  
<https://doi.org/10.1038/srep06776>
- KALLADKA, D., J. SINDEN, K. POLLOCK, C. HAIG, J. McLEAN, W. SMITH, A. McCONNACHIE, C. SANTOSH, P. M. BATH, L. DUNN, K. W. MUIR (2016): Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. *Lancet* 388, 787-796.  
[https://doi.org/10.1016/S0140-6736\(16\)30513-X](https://doi.org/10.1016/S0140-6736(16)30513-X)
- KOSI, N., I. ALIĆ, K. I. SALAMON, D. MITREČIĆ (2018): Stroke promotes survival of nearby transplanted neural stem cells by decreasing their activation of caspase 3 while not affecting their differentiation. *Neurosci. Lett.* 666, 111-119.  
<https://doi.org/10.1016/j.neulet.2017.12.040>
- LIAO, L.-Y., B. W.-M. LAU, D. I. SÁNCHEZ-VIDAÑA, Q. GAO (2019): Exogenous neural stem cell transplantation for cerebral ischemia. *Neural. Regen. Res.* 14, 1129-1137.  
<https://doi.org/10.4103/1673-5374.251188>
- McGEADY, T. A., P. J. QUINN, E. S. FITZPATRICK, M. T. RYAN (2014): Veterinarska embriologija. 1st Croatian Edition. Naklada slap. Jastrebarsko, pp. 153-183. (in Croatian)
- MITREČIĆ, D., I. ALIĆ, D. GORUP (2017): Stem cells and stroke-how glowing neurons illuminate new paths. *Neurogenesis (Austin)* 4, e 1304847  
<https://doi.org/10.1080/23262133.2017.1304847>
- PLUCHINO, S., A. M. NICAISE (2021): NSCs: Sentinel Cells of the Brain. *Cell Stem Cell* 28, 177-179.  
<https://doi.org/10.1016/j.stem.2020.11.016>
- PORRERO, C., P. RUBIO-GARRIDO, C. AVENDAÑO, F. CLASCÁ (2010): Mapping of fluorescent protein-expressing neurons and axon pathways in adult and developing Thy1-eYFP-H transgenic mice. *Brain Res.* 1345, 59-72.  
<https://doi.org/10.1016/j.brainres.2010.05.061>
- SCHINDELIN, J., I. ARGANDA-CARRERAS, E. FRISE, V. KAYNIG, M. LONGAIR, T. PIETZSCH, S. PREIBISCH, C. RUEDEN, S. SAALFELD, B. SCHMID, J.-Y. TINEVEZ, D. J. WHITE, V. HARTENSTEIN, K. ELICEIRI, P. TOMANCAK, A. CARDONA (2012): Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676-682.  
<https://doi.org/10.1038/nmeth.2019>
- XU, H., F. ZHAO, H. WANG, Q. LIU (2023): Neural stem cell transplantation improves neurological function in focal cerebral ischemia through GDNF/PI3K/AKT axis. *CMB* 69, 125-129.  
<https://doi.org/10.14715/cmb/2023.69.9.18>
- YANG, C., X. CHEN, C. ZHANG, X. LEI, Y. LU, Y. WANG, H. FENG, T. CHEN, Y. YANG (2023a): Acetylated  $\alpha$ -tubulin alleviates injury to the dendritic spines after ischemic stroke in mice. *CNS Neurosci. Ther.* 8, 2327-2338.  
<https://doi.org/10.1111/cns.14184>
- YANG, Y., K.-Y. ZHANG, X.-Z. CHEN, C.-Y. YANG, J. WANG, X.-J. LEI, Y.-L. QUAN, W.-X. CHEN, H.-L. ZHAO, L.-K. YANG, Y.-H. WANG, Y.-J. CHEN, H. FENG (2023b): Cyclophilin D-induced mitochondrial impairment confers axonal injury after intracerebral hemorrhage in mice. *Neural Regen. Res.* 4, 849-855.  
<https://doi.org/10.4103/1673-5374.353495>

ZHANG, G.-L., Z.-H. ZHU, Y.-Z. WANG (2019): Neural stem cell transplantation therapy for brain ischemic stroke: Review and perspectives. *World J. Stem Cells* 11, 817-830. <https://doi.org/10.4252/wjsc.v11.i10.817>

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**BEKAVAC, A., A. PLEĆAŠ, I. ALIĆ, D. MITREČIĆ: Analiza transplantiranih Thy1-YFP-16 pozitivnih stanica u mozak miša zahvaćen ishemijskim moždanim udarom. *Vet. arhiv* 94, 313-322, 2024.**

#### SAŽETAK

Moždani udar je agresivan neurološki poremećaj koji može biti hemoragijski i ishemijski. Živčane matične stanice široka su stanična populacija koja se može diferencirati u neurone i glija stanice. U ovom istraživanju izazvali smo moždani udar u miševa privremenim zaustavljanjem protoka krvi kroz srednju moždanu arteriju. Dvadeset četiri sata nakon operacije živčane matične stanice transplantirane su u mozak miša. Za potrebe istraživanja korištena su dva soja miševa, divlji tip i transgenični B6.Cg-Tg(Thy1-YFP)16Jrs/J (Thy1 YFP-16) uzgojeni na istoj podlozi. Ukupno je korišteno 9 embrija i 12 odraslih životinja: dvije gravidne ženke i pet mužjaka za izazivanje moždanog udara te pet mužjaka za kontrolnu skupinu. Thy1 YFP-16 stanice izražavaju YFP u približno 22% neurona pod utjecajem Thy1 promotora. Glavni cilj ovog istraživanja bila je analiza presađenih stanica osam tjedana nakon transplantacije u dvije skupine životinja. Naši rezultati pokazali su znakovitu razliku u TUBB3 pozitivnim neuronima ( $P=0,0340$ ) i GFAP pozitivnim astrocitima ( $P=0,0004$ ) između dvije skupine životinja. Štoviše, utvrđen je znakovito veći postotak Thy1-YFP stanica u kontrolnoj skupini životinja ( $P=0,0003$ ). Pearsonov koeficijent kolokalizacije pokazao je kolokalizaciju između Thy1-YFP i TUBB3 pozitivnih neurona, ali ne postoji kolokalizacija između Thy1-YFP i GFAP pozitivnih astrocita. Na temelju naših podataka možemo zaključiti da se Thy1-YFP stanice mogu koristiti za transplantaciju i istraživanja koja za cilj imaju praćenje stanica.

**Ključne riječi:** Thy1-YFP-16; živčane matične stanice; transplantacija stanica; graft; moždani udar

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