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14. ABSTRACT

Purpose: The main focus of the project is a development of recombinant cell-based therapy for chronic pain. **Scope:** The reduction in the GABA signaling and its relation to the development of chronic pain has been described after spinal cord and peripheral nerve injuries. Transplantation of GABAergic neuronal cells may restore the inhibitory potential in the spinal cord and replace dysfunctional interneurons. Grafted cells may also release additional analgesic peptides by means of genetic engineering to further enhance the benefits of this approach. Conopeptides are ideal candidates for recombinant expression using cell based strategies. The goal of the project is to develop transplantable recombinant GABAergic cells releasing MVIIA that can alleviate pain-like behavior in the models of neuropathic pain after peripheral and spinal cord injury. **Major findings:** We have engineered and characterized the GABAergic progenitors expressing MVIIA. Cells were intraspinally injected into animals in the model of peripheral nerve injury. Small number of animals with spinal cord injury also received the graft. Although the experiments are still undergoing, we have observed beneficial effect of the grafted cells in reducing hypersensitivity on animals after peripheral nerve injury. The presence of the graft in the tissue have been confirmed. Several other physiological markers that might be affected by the treatment are currently analyzed.

15. SUBJECT TERMS
neuropathic pain, nerve injury, spinal cord injury, cell therapy, GABAergic cells, conopeptides, MVIIA, animal models

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1. INTRODUCTION

Chronic pain is clinically challenging conditions often associated with the development of tolerance and addiction to analgesic drugs. Targeted therapy might overcome these issues and improve the management of chronic pain. One the key events underlying development of chronic pain is reduced inhibition in the spinal cord, causing misinterpretation of the incoming signal from the periphery. Dysfunctional signaling of GABA as an inhibitory neurotransmitter is suggested as the major cause of neuronal hyperexcitability. Pharmacological targeting of GABA receptors is insufficient to rebalance the spinal signaling due to widespread location of GABAergic receptor throughout the CNS. Transplantation of GABAergic cells showed reduction of chronic pain and partial restoring of the inhibitory balance in the spinal cord. To improve the analgesic outcome of this approach, cells may be engineered to produce additional analgesic peptides. The benefits of using recombinant cells are that it allows targeting multiple pain-processing pathways, to rebalance inhibitory signaling and to replace dysfunctional neurons at the same time. In this proposal, as a recombinant peptide produced by GABAergic cells, conotoxin MVIIA is investigated in the animal models of peripheral and central neuropathic pain. Conotoxin MVIIA is FDA approved therapeutic peptide for the treatment of chronic neuropathic pain. However, due to its poor penetration through blood brain barrier it must be delivered via intrathecal catheters. MVIIA produced by grafted cells might provide more targeted pain control and improve the quality of life of affected patients.

2. KEYWORDS

neuropathic pain, nerve injury, spinal cord injury, cell therapy, GABAergic cells, conopeptides, MVIIA, animal models

3. ACCOMPLISHMENTS

What were the major goals of the project?

Major Task 1: IACUC and ACURO approvals
Timeline: Month 1-3
Completion: 100%

Major Task 2: Engineering of recombinant cells
Timeline: Month 3-6
Completion: 98%

Major Task 3: Induction of peripheral and central chronic pain
Timeline: Month 7-15
Completion: 50%

Major Task 4: Histochemical and biochemical evaluation of the therapy
Timeline: Month 9-17
Completion: 30%

What was accomplished under these goals?

<u>Major Task 1: IACUC and ACURO approvals</u>

Major activities:
Subtask 1: Prepare a protocol and obtain IACUC and ACURO approvals
Subtask 2: Design the workflow of the experiment (ARRIVE standards) and order animals
Subtask 3: Recruit Research Assistant

Specific objectives:
1) All animal procedures need to be approved by IACC and ACURO before the actual start of the research project

2) All experiments need to be pre-planned to meet the space requirements of core facilities and availability of personnel.
3) The scope of the project and time-consuming methods required to hire additional personnel to facilitate successful accomplished of project goals within the given time frame

Results:
1) All necessary protocols have been approved within first 3 months of the official start date of the project.
2) Experimental workflow has been created to minimize interruption in the project
3) An opportunity for Research Assistant position was posted on the University of Miami Recruitment site. Several candidates were interviewed. Ms. Melissa Hernandez has been recruited based on her experiences and overall fit into our lab environment.

Major Task 2: Engineering of recombinant cells

Major activities:
Subtask 1: Train hired personnel for cell culture techniques
Subtask 2: Harvesting E14 cell, culturing, transformation with lenti-MVIIA
Subtask 3: Evaluation of recombinant cell survival and phenotype
Subtask 4: Quantification of MVIIA production by recombinant cells and optimization of culture environment

Specific objectives:
1) Training of the new personnel, including safety rules and general lab methods, together with specific training and IACUC approval on animal protocols are prerequisite for participation on the research projects.
2) Cells from medial ganglionic eminence at E14 stage of rat embryos are harvested to obtain a population rich on GABAergic cells. Cells are transfected with lentiviral vector encoding MVIIA to engineer recombinant cells.
3) To evaluate cell survival and possible phenotypic changes induced by transfection or culture environment.
4) To evaluate the stability and proper folding of recombinant MVIIA peptide and abilities of cells to produce and release MVIIA.

Results:
1) Ms. Hernandez has been trained for all necessary procedures involving cells culture, animal surgeries and behavioral testing and lab techniques.
2) E14.5 fetal neocortical tissue from Sprague-Dawley rats was microdissected into Hank's balanced salt solution and a cell suspension created via mechanical trituration. Cells were plated at an initial concentration of $5x10^5$cells/ml of the culture media containing 10ng/ml of human recombinant basic fibroblast growth factor (FGF-2; Sigma) in 75 cm^2 treated cell culture flasks (Corning). 24 hours post-harvest cells were transduced with lentivector encoding MVIIA at $1x10^{11}$ viral particles/ml for 4 hours. Media was changed and cell were replated into 70cm2 culture flasks.
3) Transfection partially reduced the viability of cells as expected, but still within the range suitable for the grafting. The viable cells were able to develop into GABAergic phenotype.
For initial characterization of cell survival and phenotype, cells were cultured for 3-4 days and their viability was estimated using Trypan blue solution and hemocytometer. The average viability at 3-4 days post lentiviral transduction was 78.3% which is suitable for grafting procedures.
Cells were then plated into 12 well plates or 8 well chambers coated with poly-L-ornithine/fibronectin at concentration $5x10^5$/well and incubated at 37°C for 2-3 days. Cells were fixed with 4% paraformaldehyde, washed and incubated in 5% normal goat serum for 2 hours and overnight in primary antibodies (GABA, 1:200, Sigma; betaTubulin 1:1000, Sigma; Doublecortin 1:200, Sigma; VGAT, 1:500, Sigma; MVIIA, 1:50, 21st Century Biochemicals) followed by incubation with appropriate secondary antibodies (Alexa Fluor 305, 488, 594, anti-rabbit, anti-mouse, 1:250, Invitrogen). After final wash the upper structure of the chamber was carefully removed and cells were coverslipped (VectaShield, Vector). The transduction efficiency was estimated based on number of $MVIIA^+$ cells out of β $tubulin^+$ cell. Using β tubulin positive cells as a marker can assure those cells are accessible to the staining and provide better estimate of the transduction rate. β tubulin was also selected based on the observation that $MVIIA^+$ signal was almost exclusively detected within the cells that express β tubulin.
Transduction efficiency was estimated at 83.6% of β tubulin+ cells (Fig. 1). No MVIIA signal was detected in non- β $tubulin^+$ cells. In general, no significant morphological changes were detected between recombinant and

non-recombinant cells, as shown on Fig.1. Detail of GABA^{+} recombinant cells are shown in Fig. 2. Cells from both groups shows GABAergic phenotype and form neurospheres.

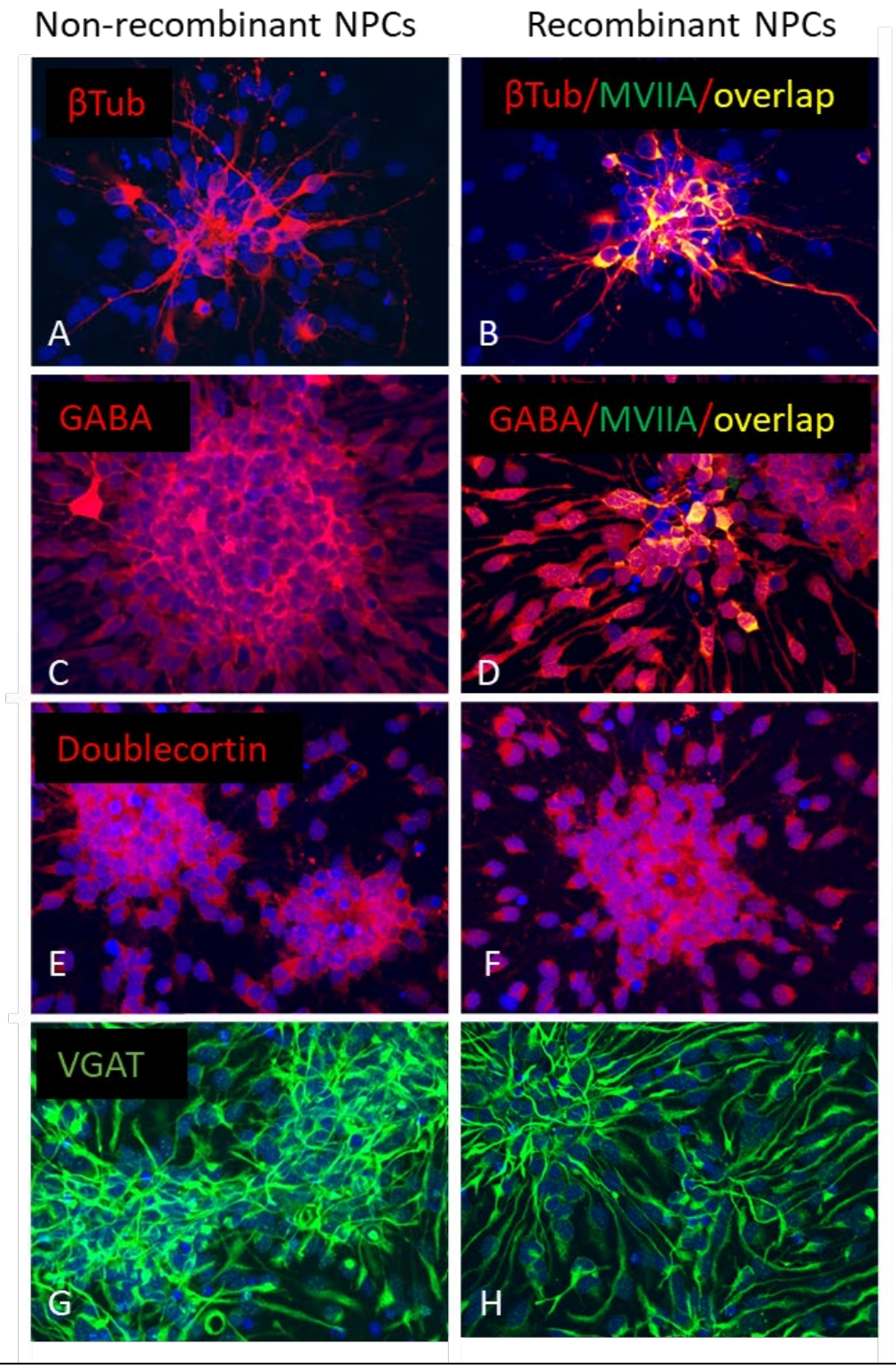


Fig. 1: Immunocytochemical detection of proneuronal markers and recombinant peptide MVIIA in E14.5 neuronal progenitor cells (NPCs). A) B tubulin indicate proneuronal phenotype of cells. B) Overlap between red βtubulin and green MVIIA creates yellow color in co-expressing cells. C-D) GABAergic phenotype was confirmed in both non-recombinant and recombinant cultures. E-F) Doublecortin is another proneuronal marker. Single staining was performed due to cross-reaction with MVIIA antibody. Morphology of the cells was not affected by the transfection. G-H) VGAT is an early marker of GABAergic phenotype. Single staining due to cross-reaction. Similar cells morphology between cultures.

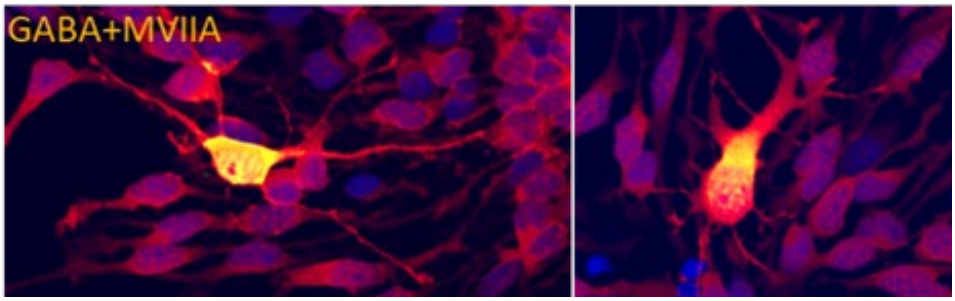
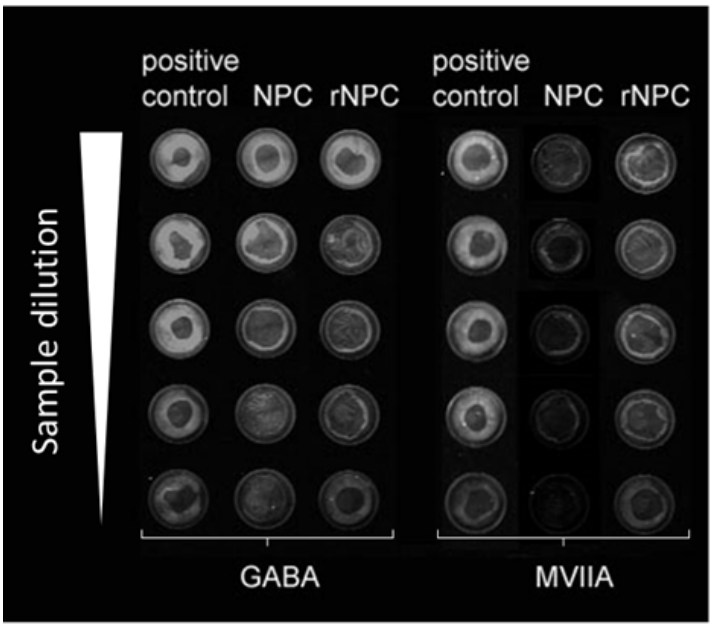
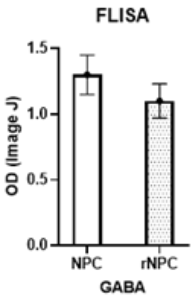


Fig. 2: Details of GABAergic cell expressing MVIIA. Characteristic morphology with long processes or more pronounced axon with dendrites indicate the overall "healthy" conditions of the recombinant cells.

4) To detect recombinant MVIIA peptide, FLISA analysis and immunoblotting were used. For FLISA, culture supernatants or peptides were coated onto wells in a 96-well format, incubated with anti-MVIIA and anti-GABA antibodies according to manufacturer's protocol. Wells were probed with anti-mouse and anti-rabbit antibody conjugated to IRDye 700 and 800CW and read using Odyssey Infrared Imaging System. For immunoblotting, samples were loaded on gradient gel, run at 100V for 1.5h, and transferred on PVDF membrane (Immobilon PSQ; Millipore). Blots were incubated in MVIIA, mRFP and β-actin primary antibodies (rabbit anti-mRFP 1:1000 Chemicon; mouse anti-beta-actin 1:1000 Abcam; anti-MVIIA monoclonal antibody 1:1000, 21st Century Biochemicals), followed by horseradish peroxidase (HRP) conjugated secondary antibodies (1:10000 goat anti-rabbit HRP; Santa Cruz; 1:5000 goat anti-mouse IgG-HRP) and detected by chemiluminiscence (Perkin-Elmer) with BioRad FluorS scanner equipped with Quantity One software. For western blot, samples were loaded onto agarose gel, run at 100V for 2 hours and transferred to membrane. Membrane was incubated in the primary MVIIA and GAPDH.
Results of FLISA analysis shows the presence of GABA in the non-recombinant and recombinant cells as expected and the presence of MVIIA peptide in the supernatant of recombinant cells (Fig. 3). Immunoblot analysis was under the detection range of the scanner and the procedure is modified to increase the signal strength. The size of the MVIIA peptide might also by an issue with the immunoblot detection. RNA analysis will be used for further identification of MVIIA in the cell culture and in the tissue of transplanted animals.





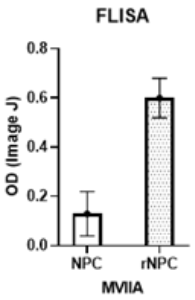


Fig. 3: FLISA analysis of cell culture supernatantS for the presence of GABA and MVIIA. GABA was detected in both non-recombinant and recombinant cells cultures, MVIIA signal was significantly different from the background in recombinant cells supernatant samples

Major Task 3: Induction of peripheral and central chronic pain

Major activities:
Subtask 1: Surgeries for chronic constriction injury (CCI) model, train personnel for behavioral testing and animal handling
Subtask 2: Transplantation of recombinant and nonrecombinant cells, saline injections
Subtask 3: Behavioral evaluation of the treatment in CCI model
Subtask 4: Surgeries for spinal cord injury (SCI) model
Subtask 5: Transplantation of recombinant and nonrecombinant cells, saline injections
Subtask 6: Behavioral evaluation of the treatment in SCI model

Specific objectives:
1) To induce chronic pain after peripheral nerve injury using specific animal model and ensure personnel ability to follow the procedures.
Completion: 70%, n=6 for control group, 8 for NPC group, 8 for rNPC group, 22 animals total
2) As a proposed therapy to alleviate chronic pain, recombinant cells are grafted into the spinal cord in animal models. Non-recombinant cells and saline injections serve as controls.
3) Evaluation of the analgesic effect of the grafted cells in the CCI model
4) To induce chronic pain after spinal cord injury for the evaluation of cell therapy.
Completion: 20%, n=6 for saline control group, n=2 for non-recombinant cell group
Notes: The progress has been temporarily interrupted due to the Covid 19 restrictions.
5) To develop the model for evaluation of analgesic propertied of grafted cells in SCI animals.
6) Evaluation of the analgesic effect of the grafted cells in the SCI model.

Results:
1) Ms. Hernandez has previous experiences with the model, a brief training was introduced to assure she can perform all surgical and behavioral procedures according to the approved protocols. To induce CCI, adult male Sprague-Dawley rats weighing 200-300 g were used. Animals are anesthetized with 2-3% isoflurane in O_2, and the common sciatic nerve exposed on one side at the mid-thigh level using aseptic surgical techniques. Four 4-0 chromic gut ligatures spaced about 1 mm apart are loosely tied around the sciatic nerve proximal to the trifurcation. Following surgery, the musculature is sutured in layers, and the skin closed with wound clips.
2) For transplantation, cells were pelleted (1500 rpm/3 min) and resuspended in Hanks media at a concentration of 50,000 cells/µl. Cells were transplanted at 1 week post CCI to target early stages of chronic pain development in this particular model. Cells were injected in the ipsilateral superficial dorsal horn at L3-L5 with 10 µl Hamilton syringe attached to a pulled glass pipet (diameter ~50 µm). A small puncture was made in the meninges and 1.0 µl of cell suspension (~5 $x10^4$ cells) was stereotactically injected. Following transplantation, the area was covered with elastic sheathing, the overlying musculature sutured, and the skin closed with clips. Control animals received saline injection. Animals with grafted cells received daily cyclosporine injections starting 2 days prior the surgery.
3) CCI induced development of hypersensitivity to mechanical and thermal stimulation within the first week post injury. Alleviation was observed in animals with grafts.

Tactile hypersensitivity (Fig. 3A) : The threshold level to an innocuous mechanical stimulus was measured with calibrated von Frey hairs ranging from 0.4 to 15 g. Animals were placed beneath an inverted clear plastic cage on an elevated wire mesh floor. Calibrated von Frey filaments were applied to the plantar skin of the hind paw with increasing force. The withdrawal threshold was taken as the lowest force (g) that evokes a brisk hind paw withdrawal response, with vocalization, head turns towards stimulus. Saline injected animas (controls) developed signs of hypersensitivity that persisted throughout the experiment. In CCI model there is a spontaneous regeneration observed after 2 months post injury and animals might show less sensitivity. Cells were grafted at 1 week post CCI when the hypersensitivity was already developed. Animals with non-recombinant graft showed mild attenuation of hypersensitivity, with significant difference from the control group starting at 3 weeks post grafting (week 3) till the week 9 ($p<0.05$). Animals with recombinant cells showed better outcomes, when the effect was significantly different from the non-recombinant graft at weeks 7-12 ($p<0.05$). The withdrawal threshold of animal in this group reached almost the pre-injury levels at the end the experiment.

Cold hypersensitivity (Fig 3B): Sensitivity to a non-noxious cooling stimulus was evaluated using acetone. 100 µl of acetone was dropped onto the lateral margin on the hind paw from a blunted 22 ga needle attached to a syringe.

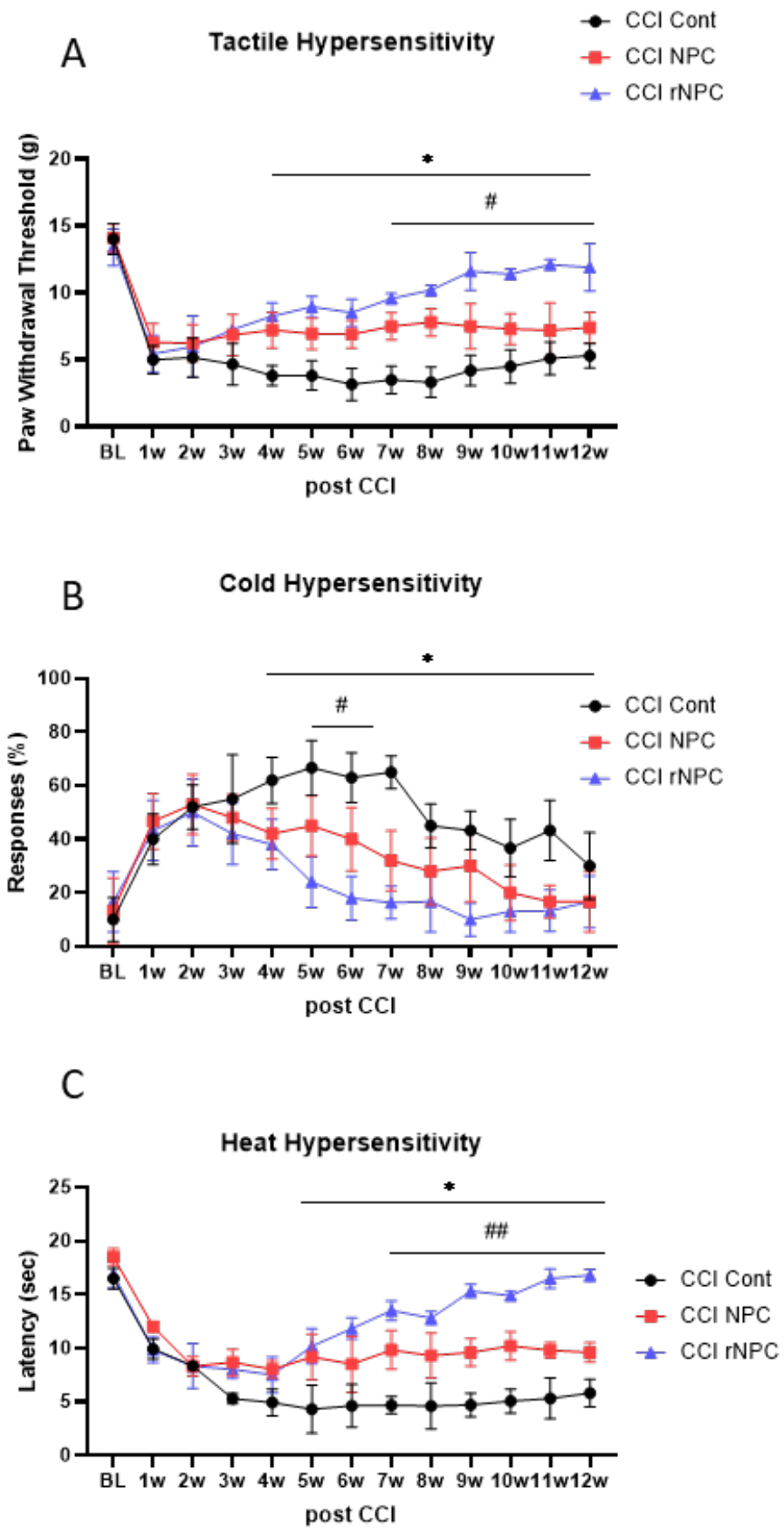
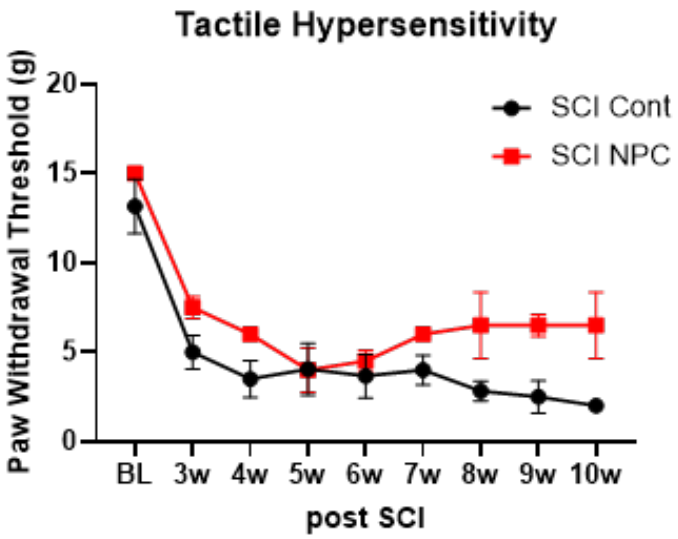
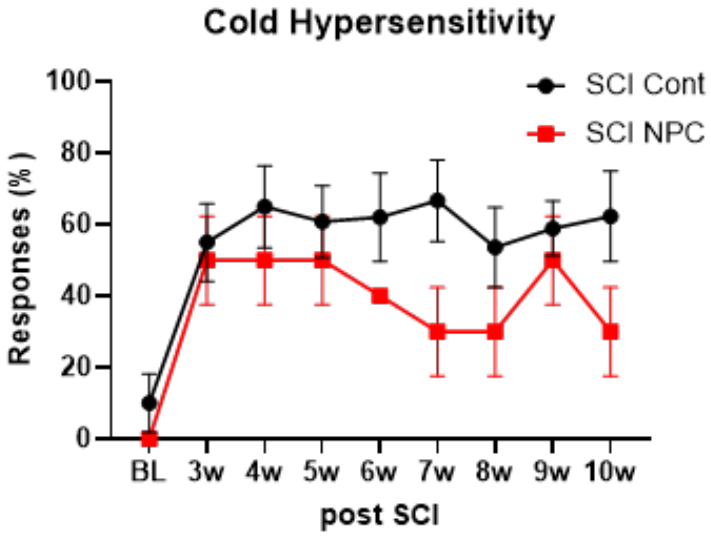


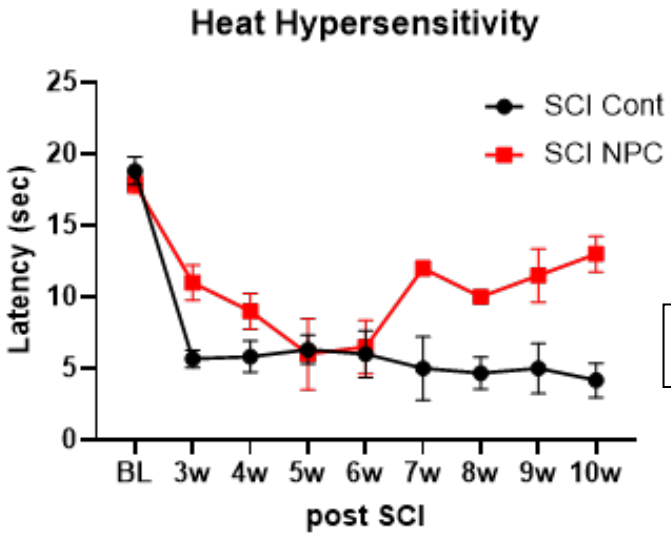
Fig. 3. Behavioral evaluation of the cell therapy in CCI model. *p<0.05 vs control; #p<0.05 between groups.

Acetone was applied to the hind paw 5 times, with about 1-2 min between applications. The total number of positive responses out of five were converted to a percent response frequency. Saline injected animals developed cold hypersensitivity with the peak between weeks 4-7 post injury. Later the slow drop in the sensitivity was observed as animal reach the stage of spontaneous regeneration observed in this model. Non-recombinant graft prevented development of severe hypersensitivity, with significantly lower response rate compared to control animals starting at 3 week post grafting and lasting till the end of experiment ($p<0.05$). Recombinant graft further attenuated ongoing hypersensitivity, reaching almost the pre-injury level at 8 weeks post grafting. The overall responses were lower than in control group starting at 2 weeks post grafting and compared to the non-recombinant group, between weeks 5-7 ($p<0.05$).

Heat hypersensitivity (Fig. 3C): Rats were placed beneath an inverted clear plastic cage on an elevated glass floor and a radiant heat source beneath the glass was aimed at the plantar hind paw which activates a timer. Withdrawal latencies are the length of time between the activation of the heat source and the hind paw withdrawal from the glass (normal baseline ~10 sec). To avoid tissue damage in the absence of a withdrawal, the cutoff was set at 20 sec. The average latency was calculated from 3 trials with 30 sec apart. Saline injected animal progressively developed hypersensitivity to heat stimulation that remained also stable thought the experiment. Non-recombinant graft attenuated heat hypersensitivity starting at 2







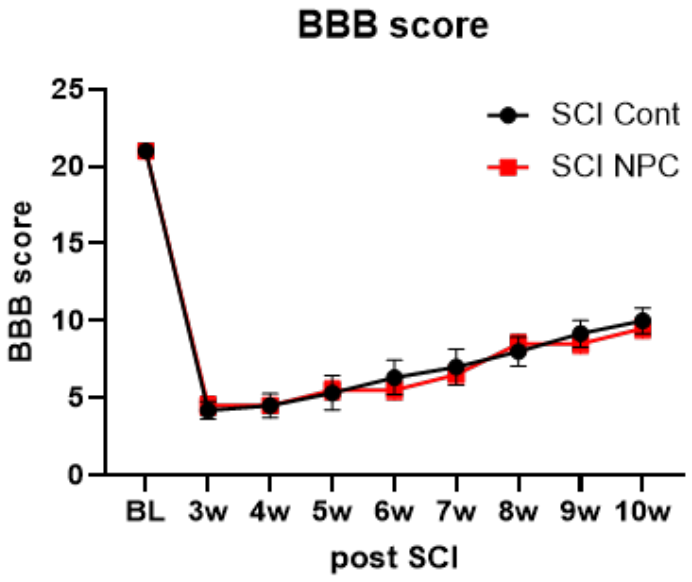
weeks post grafting with statistically higher latency compared to the control animals thought the experiment ($p<0.05$). Recombinant graft attenuated hypersensitivity starting at 2 weeks post grafting with significantly better outcome compared to both control ($p<0.01$) and non-recombinant ($p<0.05$) grafted groups starting at week 7.

4) Animals underwent surgery for spinal cord injury. Male Sprague-Dawley rats (Harlan, IN) were anesthetized with 4-5% isoflurane in O_2 and maintained on 2-3% isoflurane/O_2. 2-3 thoracic vertebrae were exposed, and a laminectomy was performed to exposed spinal cord segments T6-T8. An aneurism clip 1 mm wide (20 g compression force; Harvard Apparatus) was oriented in a vertical position and a spinal segment in the area between T6-T7 was compressed for 60 sec. The clip was then removed, and the wounds closed. Following spinal compression, the bladder was expressed twice daily for 7-10 days, or until voiding was regained. Animals with grafted cells received daily cyclosporine injections starting 2 days prior the surgery.

5) Animals underwent surgery for grafting of the cells, following the same procedure as stated for the CCI model (Subtask 2). Injection was bilateral.

6) Due to the Covid-19 restrictions, only a small number of animals have been used for SCI model. Animals were evaluated using tactile, heat and cold hypersensitive test, as stated for CCI model (Subtask 3). The preliminary results show development of hypersensitivity in all 3 tests observed by week 3 post injury (Fig. 4), when partial recovery of hind lib paralysis is observed. Control animals shows presence of hypersensitivity throughout the experiment, animals with non-recombinant graft shows partial attenuation of hypersensitivity.

Fig. 4: Evaluation of analgesic effect of grafted cells in SCI model.



Open field test (Fig. 5): The Basso-Beattie-Bresnahan test was used for evaluation of motor behavior. Rats were placed in the center of an open-field area with a 4 foot diameter, and the behavior of the animals is observed for a 4 min test period by two individuals blinded to the treatment. The scale was designed to reflect motor rating scores, ranking from zero which indicates complete paralysis without joint movement to 21 which indicates normal locomotion with full coordination and proper gait, movement at all joints, full weight support, and appropriate limb, body and tail positioning. BBB score was not affected by the treatment, as expected.

Major Task 4: Histochemical and biochemical evaluation of the therapy

Major activities:
Subtask 1: Perfusion, tissue harvesting and processing for immunostaining
Subtask 2: Biochemical and histochemical evaluations

Specific objectives:
1) To harvest spinal cord tissue from the experimental animals after behavioral test are finalized and to prepare the tissue for biochemical or histochemical analysis.
Completion: 20%
Note: The progress was partially slowed down due to Covid- 19 restrictions and limited availability of core facilities

2) To evaluate the distribution, survival and the physiological effect of graft in the spinal cord tissue.

Results:
1) All animals from CCI group have been perfused or sacrificed and tissue was harvested for immunohistochemical and biochemical evaluation. Animals were deeply anesthetized and intracardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Spinal cords were removed and post-fixed overnight, followed by incubation in 30% sucrose for 48 hours. Serial sections cut on cryostat at 40 µm are collected either as slide mounted or free floating. For biochemical analysis, tissue samples were collected from the anesthetized animals after decapitation, frozen on dry ice and stored at -80C.

2) Immunohistochemical staining was used to detect the graft and to evaluate the levels of some of the pain-related markers. Sections were incubated in 5% normal goat serum, followed by overnight incubation in primary antibodies in 4°C (recombinant conopeptide MVIIA 1:200 (21st century Biochemicals); gama-ammino-butyric acid (GABA) 1:200 (Sigma); Neuronal N (NeuN) 1:1000 (Genetex); glial fibrilary acidic protein (GFAP) 1:1000 (Millipore); Iba-1 1:1000 (Wako), Calcitonine gene related peptide (CGRP, 1:1000, Millipore)) and incubations in secondary antibodies (anti-rabbit, anti-mouse, anti-guinea pig, 1:200, Alexa Fluor) in 5% NGS. Sections are glass mounted, air dried and coverslipped by VectaShield (Vector Labs). Analyses will be done with a Zeiss Axiovert 200M research microscope (Ludl Electronic Products), DVC cooled camera and multi-band fluorescent filters allowing for viewing single, double or triple fluorophores at the same time.

We have observed reduction in the amountof GABA+ cells in the spinal dorsal horn in the control CCI animals (Fig. 6A-C). Grafting of recombinant or non-recombinant cells partially restored the amount of GABA+ cells (white arrows in Fig. 6 A-C). We have analysed possibe changed in the level of CGRP, as changes in the expression of this pain-related neurtransmitter are documented in several studies. We have observed a reduction in the immunostaining in the middle dorsal horn area in the control animals but not significantly

different from the grafted groups (Fig. 6 D-F). Similarly, activation of microglial cells detected by Iba-1 imunostaining is observed in CCI model early post injury. In our case, 12 weeks post CCI, we havent found any significant differences between groups (Fig. 6 G-I). To further analyse possible changes, ELISA or western blot methods will be used.

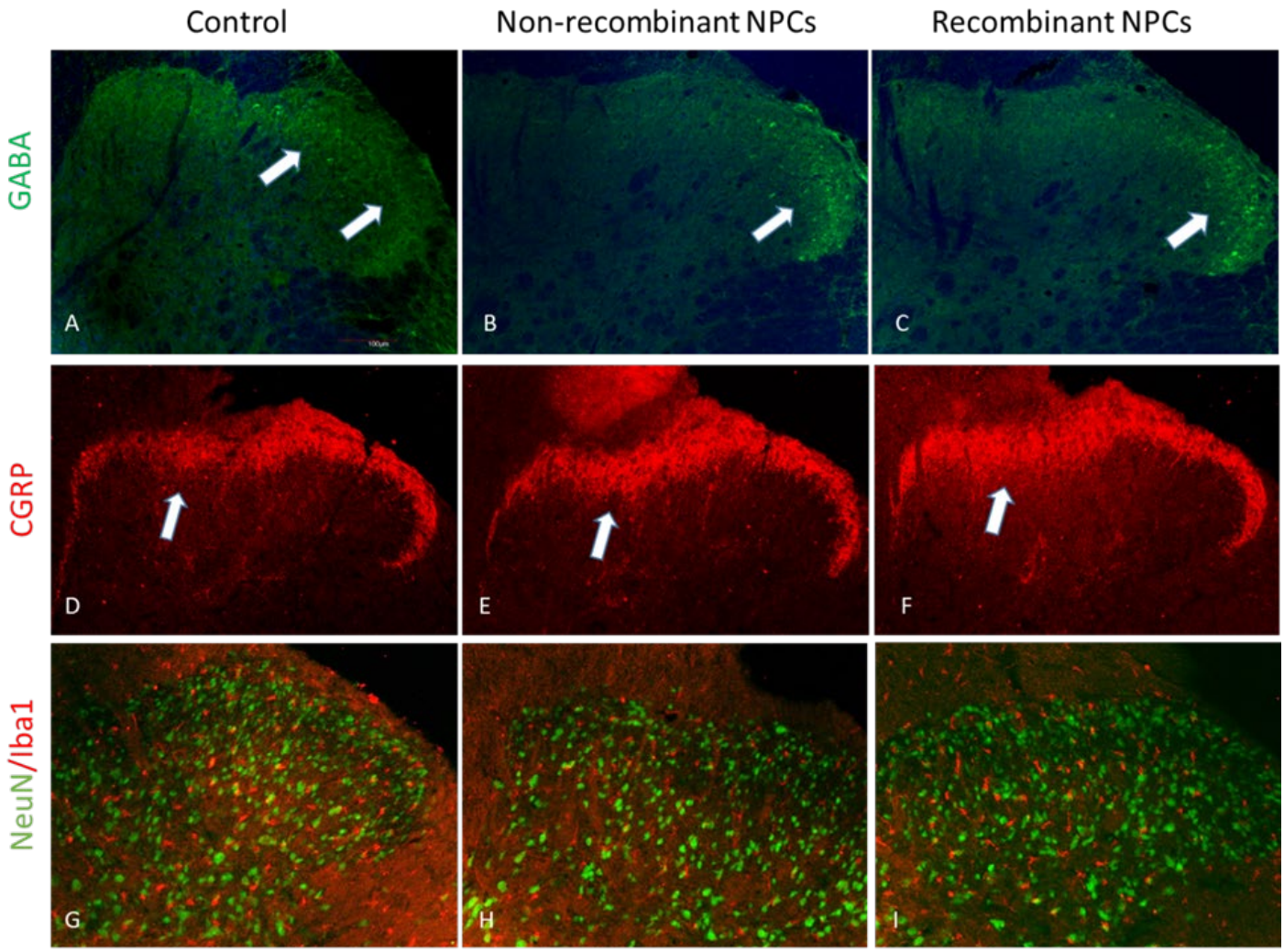


Fig. 6: Immunohistochemical detection of GABA (A-C), CGRP (D-E) and Iba1/NeuN (G-I) in the spinal dorsal horn of animals with intraspinal saline (control), non-recombinant and recombinant cell graft. Tissue was analyzed at the end of experimental period (12 weeks post CCI). Reduced amount of GABA+ cells observed in control CCI animals was partially restored after cell graft (A-D). Changes in the level of CGRP were only minimal. Microglial activation was similar between groups.

Recombinant graft was detected in the lumbar spinal cord with minimal spreading from the injection site (about 0.8mm rostral and caudal, Fig. 7). Grafted cells shows predominantly neuronal phenotype, in the analysed tissue we haven not found overlap of the MVIIA sigal with the glial marker GFAP

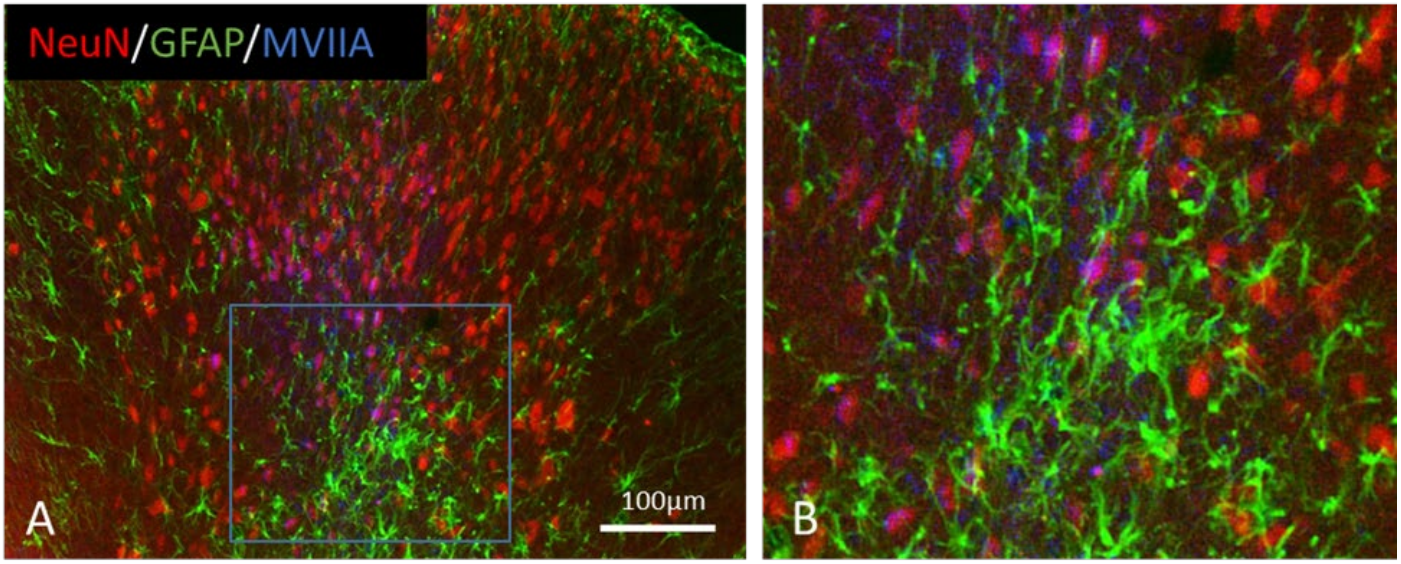


Fig. 7: A) Recombinant cells detected in the spinal dorsal horn. NeuN (red) label neuronal cells, GFAP (green) astrocytes and MVIIA (blue) label cells producing MVIIA protein. Overlap between NeuN and MVIIA is shown in magenta. No overlap between GFAP and MVIIA was observed. B) Enlarged area with the graft.

4. IMPACT

What was the impact on the development of the principal discipline of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on the technology transfer?

Nothing to report

5. CHANGES/PROBLEMS

Changes in the approach and reason for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Due to Covid-19 restrictions on animal orders, core facilities and personnel availability, progress on the project was partially slowed down. No-cost extension for this project has been requested to compensate for this interruption.

To reflect the new rules and regulations, the analysis that require core facilities, such as cryostat sectioning and imaging, will be planned in the most efficient way to follow the current restrictions. Data analysis will be conducted under “home office” when possible.

To evaluate the presence of MVIIA in the recombinant cells and the tissue, RNA analysis will be conducted, together with FLISA. Western blot did not prove to be effective method due to the size of the peptide and the sensitivity of the antibody.

Changes that had significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report. IACUC approval date 3/18/2019, ACURO approval date 8/15/2019.

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals
Nothing to report

Significant changes in use biohazard and/or select agents
Nothing to report

6. PRODUCTS
Publications, conference papers and presentations
Due to Covid 19, several planned conferences have been canceled. Poster presentation of the current results is planned for the upcoming 2020 Society of Neuroscience Meeting and 2021 Miami Winter Symposium. Draft of the manuscript will be prepared in the next period.

Websites or other Internet sites

Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Stanislava Jergova, PhD
Project Role: Principal Investigator
Researcher Identifier:
Nearest person month worked: 5
Contribution to Project: Dr. Jergova participated in the project design, training of personnel, recombinant cells engineering, tissue analysis, data management and preparation of reports
Funding Support : N/A

Name: Jacqueline Sagen,PhD
Project Role: Collaborator
Researcher Identifier:
Nearest person month worked: 1
Contribution to Project: Dr. Sagen managed animal protocols and supervised preparation of reports
Funding Support : N/A

Name: Melissa Hernandez, MS
Project Role: Research Associate I
Researcher Identifier:
Nearest person month worked: 9.5
Contribution to Project: Ms. Hernandez provided all surgeries and behavioral evaluation, participated on cell culturing and tissue processing
Funding Support : N/A

Name: Anjalika Eeswara, MS
Project Role: Research Associate I
Researcher Identifier:
Nearest person month worked: 5

Contribution to Project: Ms. Eeswara provided pre and post-surgical treatments, assisted with behavioral evaluation and tissue processing and managed data input
Funding Support : N/A

Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners?
Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Quad chart is attached

9. APPENDICES
Nothing to report