RESTAURATION OF AGE RELATED MOTOR IMPAIRMENT: ROLE OF IGF-1 BASED GENE THERAPY AND MICROGLIAL ACTIVATION.

Falomir Lockhart, Eugenia*; Millan J; Anesetti-Nelli S; Hereñú, CB y Bellini, MJ.
Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP- UNLP-CONICET)
*eugefalomir@gmail.com

INTRODUCTION

Microglial cells play an important role in healthy and diseased brain removing apoptotic neurons, establishing transient connections with neuronal synapses and producing neurotrophic factors that modulate neurogenesis during embryogenesis and adulthood. These cells are essential for ensuring neuroprotection in the normal and pathological condition of central nervous system as they are an important sources of neurotrophic factors. It has been described that aging reduces the ability of microglia to provide neuroprotection.
It is well known that IGF-1 plays a physiological role in neuroprotection. In situations involving cytotoxic damage, the microglia increases the production of IGF-1. Previous studies of our group described that intracerebroventricular (ICV) IGF-1 gene therapy induced a significant improvement in motor performance in aged rats.

HYPOTHESIS

WE PROPOSE THAT RESTORATIVE EFFECTS OF IGF-1 ON MOTOR SKILLS COULD BE MEDIATED BY GLIAL CELLS.

CONCLUSION

Our results suggest that microglia could be involved in the maintenance of motor skills and offer a novel approach for reversing age-associated motor and exploratory performance in rats.

RESULTS

We demonstrated that IGF-1 restored motor coordination and forelimb grip strength in aged rats **(Figura 1)**. Also, we found that microglia (Iba-1+) cell number was significantly increased for at least 17 days after treatment with IGF-1 (Xm-senil-IGF-I=556.2±30.50 vs Xm-senil-DsRed= 359.9±18.08; p<0.001) **(Figura 2)**, while astrocytes (GFAP+) showed not changes **(Figura 3)**. The analyze of microglial morphology showed that after treatment with IGF-1 there is an increase in the proportion of microglia with a reactive phenotype **(Figura 4)**.

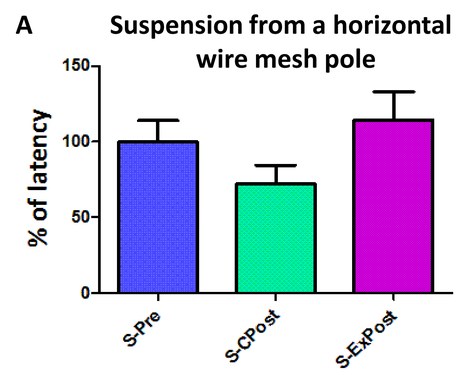
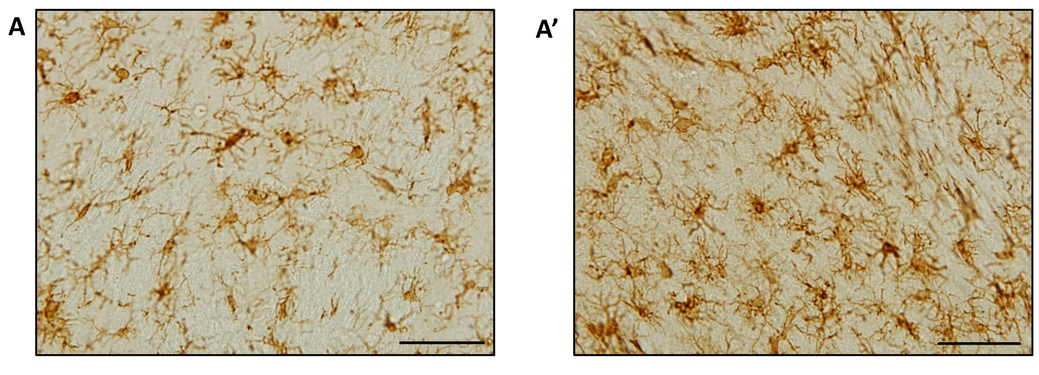


Figure 1: IGF-1 gene therapy restorative effect on the motor performance. Panel **(A)** shows the resistance of rats to fall from a horizontal pole from which they hang. Panel (B) shows the ability of rats to remain on a ramp set at 70° to the horizontal. Panel (C) shows the resistance of rats on top of a rotating platform. Panels represent percentage of change from corresponding vector preinjection values. [% change = (postinjection value–pre injection value)100/pre injection value]. Error bars represent SEM. * p < 0.05.



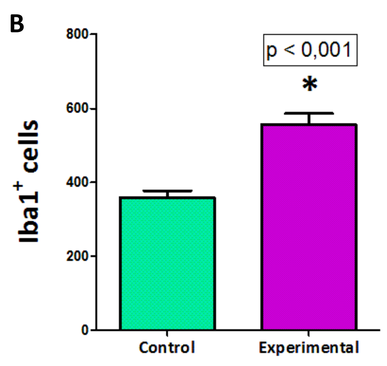


Figure 2: Representative images of Straitum showing immunoreactivity for Iba1. **(A)** and **(A')** Immunohistochemistry for Iba1 of control and experimental rat brain slides, respectively. Bars: 50 µm **(B)** Quantification of Iba1+ cells.

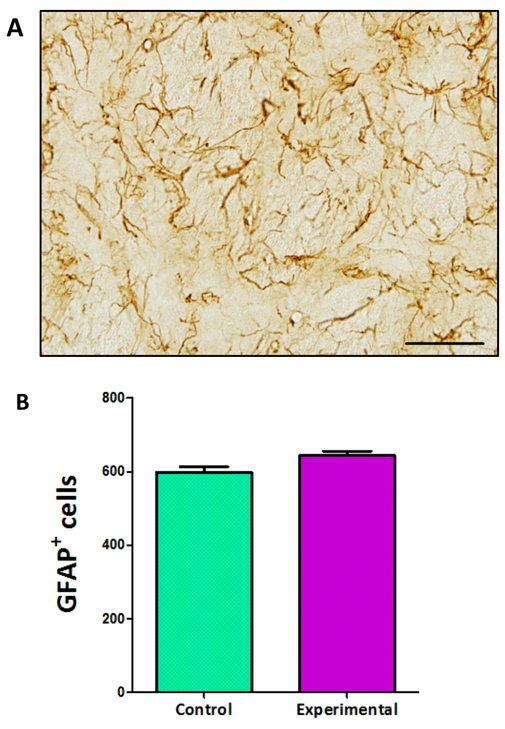


Figure 3: Representative images of Straitum showing immunoreactivity for GFAP. **(A)** and **(A')** Immunohistochemistry for GFAP of control and experimental rat's brain slides, respectively. Bars: 50 µm **(B)** Quantification of GFAP+ cells.

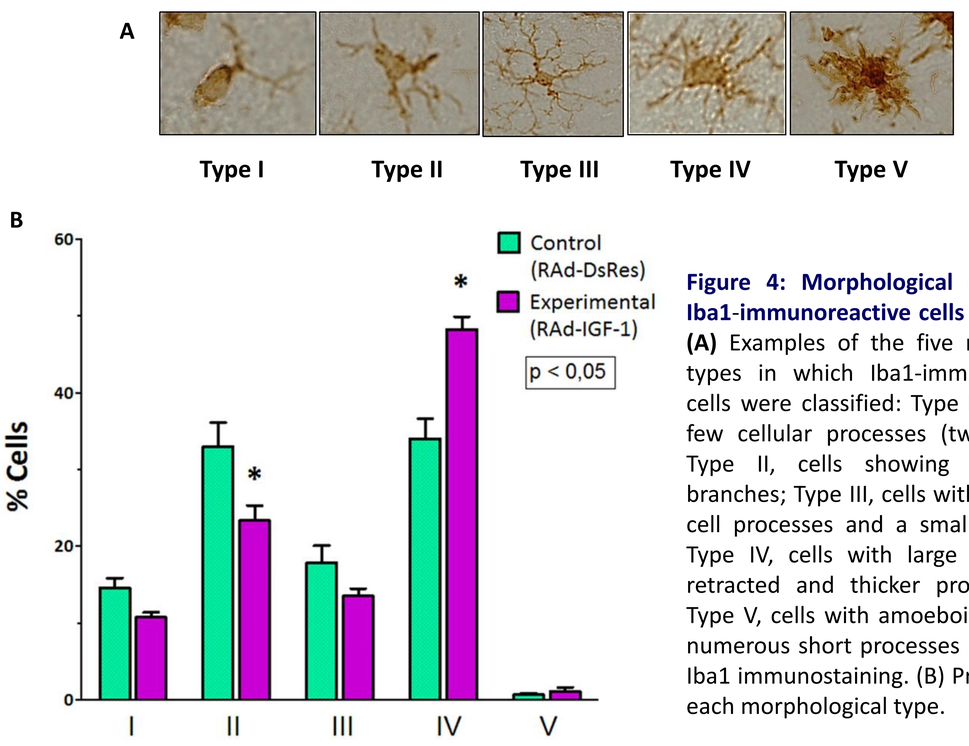
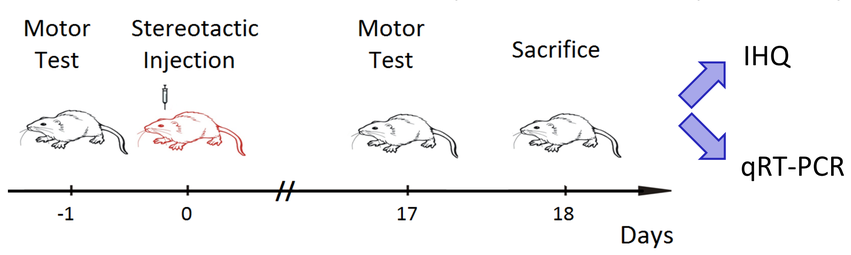


Figure 4: Morphological changes of Iba1-immunoreactive cells in Striatum **(A)** Examples of the five morphology types in which Iba1-immunoreactive cells were classified: Type I, cells with few cellular processes (two or less); Type II, cells showing four short branches; Type III, cells with numerous cell processes and a small cell body; Type IV, cells with large somas and retracted and thicker processes and Type V, cells with amoeboid cell body, numerous short processes and intense Iba1 immunostaining. (B) Proportion of each morphological type.

MATERIALS AND METHODS

Animals: Aged Sprague-Dawley female rats (28 month) were used. Animals were divided into two groups: Experimental group (E), treated with RAd-IGF-1, and Control group (C) treated with RAd-DsRed.

Experimental Protocol: On experimental day -1 (D-1) all the animals were performed for motor tests. On D0, we perform the stereotactic ICV injection with RAd-IGF-1 or RAd-DsRed. On D17, we performed again the motor tests in all the animals to evaluate the motor performance. Finally, all animals were sacrificed on D18 and their brains were removed and processed for IHQ or qRT-PCR analysis.



Immunohistochemistry: Immunohistochemistry was carried out on free-floating sections under moderate shaking. Non-contiguous brain slide were selected and processed with Iba1 or GFAP antibodies (diluted 1:1000) and tetraclorhydrate de 3,3-diaminobenzidine (DAB).

MOTOR TESTS

Suspension from a horizontal wire mesh pole

Performance on a wire mesh ramp

Performance on a rotating platform