ESTUDIO DE LA NEUROTOXICIDAD DEL HERBICIDA GLUFOSINATO DE AMONIO DURANTE EL DESARROLLO EN MAMÍFEROS.

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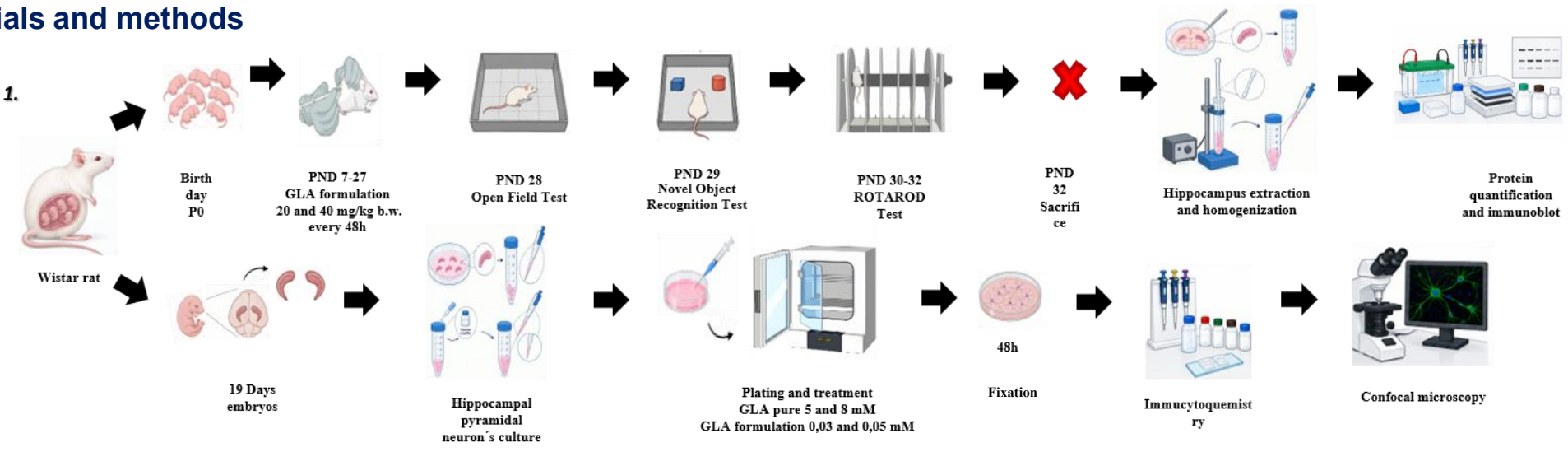
Palabras clave: glufosinato de amonio, neurodesarrollo, neurotoxicidad.

Introduction

The use of herbicides is a widespread agricultural practice worldwide, particularly for weed control in genetically modified crops. In Argentina, herbicides are extensively used, including glufosinate-ammonium (GLA), an irreversible inhibitor of glutamine synthetase. Following the emergence of weed resistance to other widely used herbicides, such as glyphosate, the use of GLA has escalated. Acute exposure to GLA has been associated with neurological manifestations including seizures and memory impairment, raising concerns about its potential neurotoxic effects. Moreover, commercially available GLA products contain additional components that may potentiate the effects of the active ingredient. Given the particular vulnerability of the developing nervous system to environmental toxics, understanding the effects of GLA exposure during development is of particular relevance. Therefore, we studied the neurotoxic effects of ammonium glufosinate and a commercial GLA formulation during development using complementary in vivo and in vitro approaches.

Materials and methods

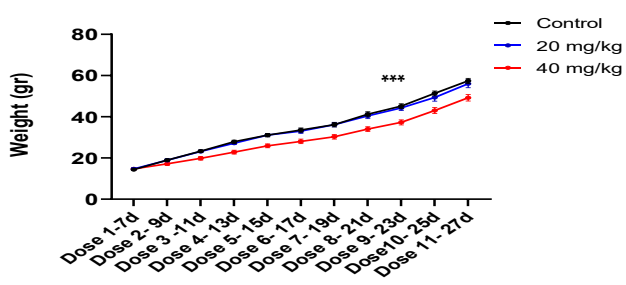
Figure 1.



Results

Development of juvenile rats is affected by GLA formulation

Figure 2. Weight evolution was carried out every 48h from PND 7 to 27. From PND 11, animals exposed to 40 mg/kg had a significantly lower weight than the control and 20 mg/kg b.w. groups, differences that were maintained until PND27.



Locomotor activity may be altered by exposure to glufosinate

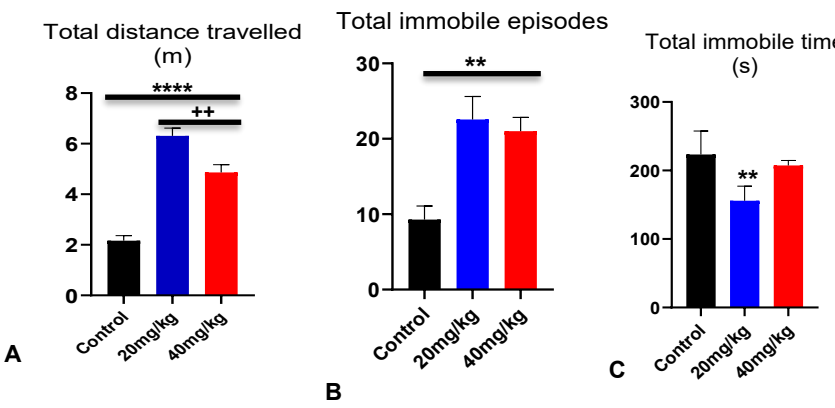


Figure 3. **A-** The total distance travelled increased in treated groups compared to the control. **B-** Both doses were associated with increased number of immobility episodes with no significant differences among the treated. **C-** There is difference between conditions regarding total immobility time. Exposure was linked to an increase in locomotor activity and the frequency of immobility episodes, accompanied by a reduction in total immobility time at the 20 mg/kg dose.

Early exposure to GLA impairs cognitive function

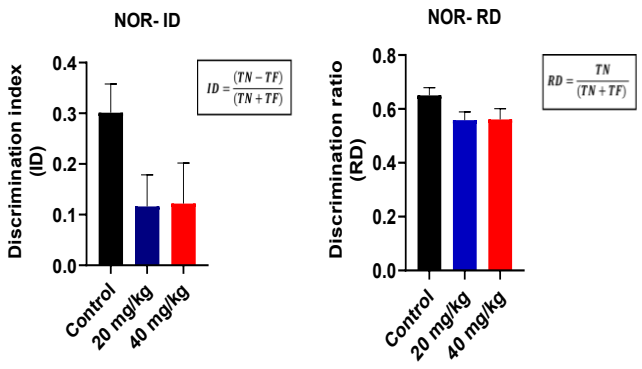
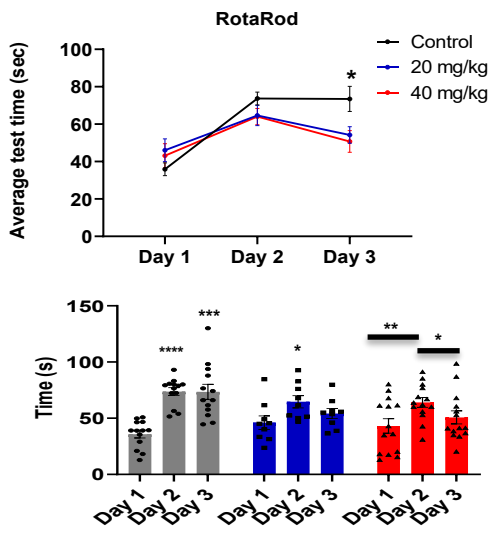


Figure 4. Recognition memory was evaluated by NOR test. Results showed the ratio of time spent beside the new object. The treated animals had a tendency to spent less time exploring the new object.

Glufosinate ammonium effect on motor coordination and learning

Figure 5. Exposure to GLA affects coordination and balance ability in early-exposed pups. **A.** Rats in the control group stayed longer on the rotating cylinder than the exposed to higher doses of the formulation at the end of the assay. **B.** From the second day of the trial, the time spent by the controls on the cylinder begins to match and exceed the time of the treated ones. By the third day, the differences are significant. Motor learning process and the ability to maintain coordination would be impaired in animals exposed to the herbicide.



Synaptic protein expression -Hippocampus homogenate-

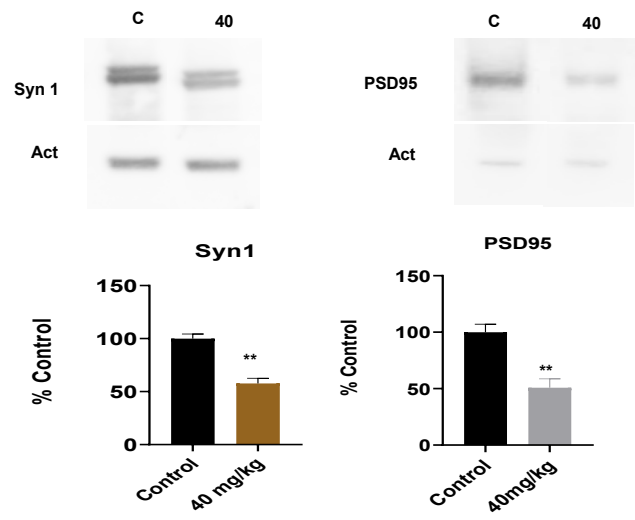
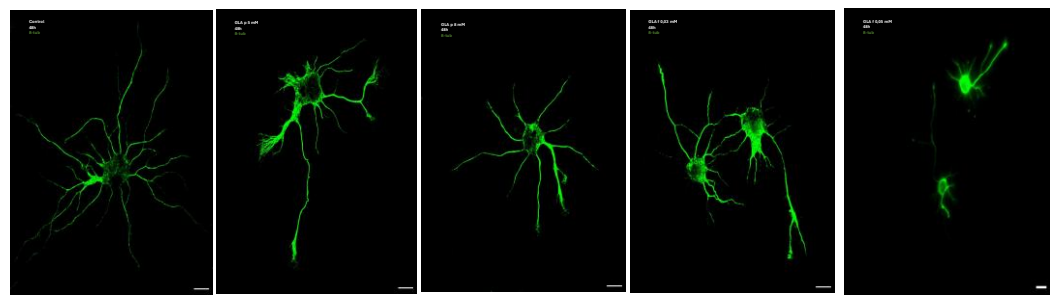


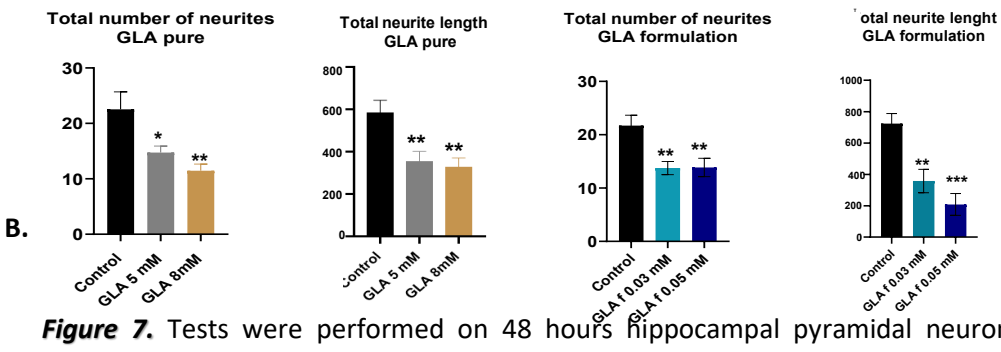
Figure 6. **A.** Exposure to GLA 40 mg/kg resulted in an approximate reduction of 42.3% of relative Synapsin-1 levels compared to the control group.

B. The treated group presented a decrease in the relative levels of PSD-95 compared to the control group (50.82% of the control value).

Hippocampal neuron culture



A.



B.

Figure 7. Tests were performed on 48 hours hippocampal pyramidal neuron cultures exposed to glufosinate ammonium both pure and a commercial formulation. **A.** Immunocytochemical techniques were applied for the analysis of the images by confocal microscopy. Scale bar 10 µm.
B. Preliminary results show a decrease within the number of neurites and the total neurite length in both cases at the doses of 5 and 8 mM of pure GLA, and 0,03 mM; 0,05 mM of the comercial formulation.

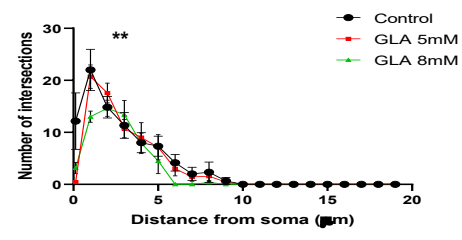


Figure 8. Sholl analysis was performed on 48h hippocampal neurons treated with pure GLA. It shows difference between the conditions, with a tendency to a diminished complexity of the dendritic tree of the highest dose.

Preliminary conclusions

- **GLA may impact growth, development and cognitive function by decreasing learning and memory performance.**
- **Glufosinate alters the development of the dendritic tree envolving a decrease within the number of neurites and the total neurite length.**
- **The treatment caused a decrease in the relative levels of synaptic markers suggesting a possible disruption in the organization and/or maintenance of synaptic structure, that could affect neuronal communication and plasticity.**
- **The presence of adjuvants in the formulations might increase glufosinate's toxicity.**

This work is founded by ANPCyT (PICT 2020-0125), ASaCTei (Santa Fe, PEIC 2022-107), UNR (BIO 587), and CONICET