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The Roundup Active® induced alterations in sensory neurons in a Neotropical fish (*Piaractus orinoquensis*)

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Abstract

Glyphosate-based herbicides (GBHs) are widely used agrochemicals frequently detected in aquatic ecosystems, and their potential effects on the peripheral nervous system of fish remain poorly understood. This study evaluated the histopathological, ultrastructural and morphometric effects of sublethal concentrations of the GBH (Roundup Active®) on sensory neurons of the dorsal root ganglia (DRGs) of juvenile *Piaractus orinoquensis*. A 30-day exposure bioassay was conducted using three treatments: T1: control 0 mg/L GP, T2: 0.1 mg/L GP, and T3: 1 mg/L glyphosate (GP). Histopathological analyses were performed by high-resolution optical microscopy (HROM), transmission electron microscopy (TEM), and morphometric evaluation of sensory neurons. Two neuronal subpopulations were identified in DRGs: large type A neurons and smaller type B neurons. The most frequent histopathological alteration was the presence of cytoplasmic vesicles, which increased in fish exposed to Roundup Active®, particularly in type A neurons. Ultrastructural analyses demonstrated that part of these vesicular alterations corresponded to mitochondrial damage, mainly affecting the mitochondrial cristae. Morphometric analyses revealed significant reductions in the area, perimeter, and diameter of type B neurons in exposed treatments. Mast cells were detected exclusively at the highest concentration T3 (1 mg/L GP), located adjacent to sensory neurons and in blood vessels associated with DRGs. This finding represents the first report of mast cells in the peripheral nervous system of fish exposed to a GBH. These results demonstrate that sublethal exposure to Roundup Active® induces structural and ultrastructural alterations in DRG sensory neurons, suggesting the peripheral neurotoxic potential of GBHs in tropical freshwater fish.

Introduction

Glyphosate (GP) is the active component (N-(phosphonomethyl) glycine) found in several herbicides used worldwide (Sánchez et al., 2021). In Colombia, this herbicide is extensively applied under the brand name Roundup® for weed control in crops, grain drying, and sugar cane maturation (Riaño et al., 2020). Glyphosate has been detected polluting bodies of water, affecting non-target species such as fish (Gill et al., 2018), with reported concentrations reaching up to 1.7 mg/L (Guilherme et al., 2010). However, most research has focused on glyphosate-based herbicides (GBH), which are reported to be more harmful due to the surfactants or additives used (Szepanowski et al., 2018). Additionally, farmers primarily use these commercial formulations, resulting in contamination of natural water bodies that adversely affect native fish populations (Menéndez-Helman et al., 2025). The white cachama (*Piaractus orinoquensis*), a commercially important fish species in Colombia, inhabits regions where GBH are used for agricultural or illicit crop weed control (Gomez-Ramirez et al., 2025).

Previous research has indicated diverse neurological effects of glyphosate on fish species, including alterations in cholinesterase activity (Braz-Mota et al., 2015; Dey et al., 2016), which could impair neurological development, cell proliferation, and neuronal apoptosis (Karczmar, 2010). Furthermore, studies have reported structural changes in the brain, including degeneration of Purkinje neurons, proliferation of glial cells and reduced expression of key developmental genes (Erhunmwunse et al.,

2014; Roy et al., 2016). Specifically, exposure to Roundup® has been associated to significant histopathological changes in *P. orinoquensis*, including brain lesions indicative of neurodegenerative processes and neuronal death (Eslava-Mocha et al., 2019). Moreover, sublethal concentrations of glyphosate in Roundup Active® formulations have been associated with impairments in olfactory bulb development in fry stages of *P. orinoquensis* (Castañeda, 2012). Despite these findings, the impact of Roundup Active® on the peripheral nervous system, particularly dorsal root ganglion (DRG) sensory neurons, remains understudied.

Dorsal root ganglia are critical components of the peripheral nervous system, housing sensory neurons that play a pivotal role in transmitting touch, temperature, pain, and nociception signals to the central nervous system (CNS) (Haberberger et al., 2019). These neurons exhibit diverse morphological and functional characteristics, categorized into distinct subpopulations based on their physiological responses (Jaramillo et al., 2009; Kitao et al., 2002). Histologically, DRGs consist predominantly of neurons, fibroblasts, satellite cells, and Schwann cells (Haberberger et al., 2019). DRG sensory neurons are commonly categorized into type A neurons (large, lightly stained, with large rough endoplasmic reticulum aggregates) and type B neurons (small, deeply stained, with compact Nissl substance) (Kitao et al., 2002). Juvenile *P. orinoquensis* has shown to be a suitable model for studying DRG sensory neurons, offering insights into the qualitative and quantitative effects of environmental contaminants on neuronal morphology and function (Jaramillo et al., 2009).

Based on the ecological relevance of this Neotropical fish and its potential implications for aquatic ecosystems, this study aims to investigate histopathological and morphometric alterations in the dorsal root ganglion (DRG) sensory neurons of *P. orinoquensis* exposed to environmentally relevant concentrations of glyphosate present in Roundup Active®.

Materials and methods

This study was conducted at the Universidad Militar Nueva Granada embryology laboratory and the Universidad de Los Llanos pathology laboratory. Ethical procedures for fish management were followed (Underwood and Anthony, 2020).

Animals.

180 juveniles (30 days after hatching) of *Piaractus orinoquensis* were obtained from the Instituto de Acuicultura de los Llanos (IALL) fishery and acclimatized on 500 L plastic containers with constant aeration. The fish used in this study had a weight of 2.28 ± 0.57 g and a length of 5.06 ± 0.42 cm, and were fed three times a day (9:00, 12:00 and 16:00 h) with commercial pellets of 35% crude protein at 5% of total biomass. A 12:12 h (light/darkness) photoperiod was used. The physicochemical parameters were maintained within the appropriate ranges for the species (Gonzales, 2001): dissolved oxygen 7.0 ± 0.5 mg/L, pH 7.1 ± 0.2, temperature 26.6 ± 0.8°C (Extech® DO 700 probe), and total ammonia nitrogen 0.38 ± 0.21 (TAN) were measured using Multy Espectroquant Merck®.

Experimental design and exposure

A semi-static toxicity bioassay was performed using the herbicide Roundup Active®, which is a mixture of 363 g/L of acidic glyphosate, equivalent to 446 g/L of potassium salt of N-(phosphonomethyl)glycine, and an unspecified surfactant at an unreported concentration (Riaño et al., 2020; Bernal-Rey et al., 2020). To avoid environmental pollution, discarded test solutions were stored in plastic containers for 30 days to allow herbicide transformation (Soso et al., 2007).

Three treatments with three replicates were evaluated (3x3): T1 (0 mg/L GP), T2 (0.1 mg/L GP) and T3 (1 mg/L GP)I rather use the concentrations instead of T1, T2, T3. If you want to maintain it, T1, T2, T3 must be defined in all figures and tables, prepared by dilution from the initial concentration of glyphosate acid equivalent (a.e.)/L present in the product Roundup Active®. Twenty fish per replicate were transferred (40 L aquarium) and exposed for 30 days. At the end of the exposure time, fish were anesthetized and euthanized following ethical criteria, with a benzocaine (0.1 g/L) overdose (Gomez-Ramirez et al., 2025).

Sample processing for histological and image analysis

Nine fish per treatment were collected for histopathological description by high resolution optical microscopy (HROM n = 6) and transmission electron microscopy (TEM n = 3). Tissue samples were fixed for 2 hours in a mixture of 2% formaldehyde and 2.5% glutaraldehyde prepared in phosphate buffer pH 7.2–7.4, followed by three ten minutes washes in phosphate buffer. Tissues were post-fixed for two hours with 2% osmium tetroxide, dehydrated in ethanol 70%, three-five minutes wash, 90% one five minutes wash, 95% two ten minutes wash and 100% two ten minutes washes. After dehydration, samples were embedded in Poli/Bed®812 resin. The samples were polymerized at 60°C for 16 hours. Transversal and longitudinal ultrathin sections 1µm were obtained using a glass knife on a rotatory microtome (CUT SLEE 4060®), and stained with toluidine blue (Gomez-Ramirez et al., 2025).

For TEM analysis, ultrathin sections we made with ultramicrotome Leica® EM UC6 at a thickness of 130 nm, the samples were collected on naked copper-meshed grids and contrasted with uranyl acetate and lead citrate. The DRGs were examined in an electron microscope of transmission Jeol® JEM 1400 Plus using an accelerating voltage of 120 kV. Photographs and morphometric measurements were carried out with a GATAN® camera attached to this equipment and GATAN® Digital Micrograph 1.80.70 program.

2.4. Morphometry of DRG sensory neurons

Six DRGs of the anterior region of the fish per treatment were analyzed. Images were captured and processed to improve contrast and diminish background noise using ImageJ software (http://rsbweb.nih.gov/ij/). Using digitized and processed images, ImageJ software was calibrated, and area, diameter and perimeter from A and B soma and nuclei from differentiated sensory neurons were measured (Jaramillo et al., 2009).

Histopathological alterations such as cytoplasmic vesicles (CV), migrated nuclei (MN) and double nucleoli (DN) were estimated as a percentage by neuronal type (A and B) of the total of neurons evaluated. The area of cytoplasmic vesicles in the neuronal soma (A and B) was also determined and expressed as a percentage.

2.5. Statistical analysis

Data were tested for normality using the Shapiro Wilk test and homogeneity of variance using Levene's test. Multivariate analysis of variance (MANOVA) was used to evaluate the effect of different concentrations of glyphosate on the diameter, perimeter and area of sensory neurons and their nuclei. The MANOVA test generates ANOVA and individual Tukey tests for each variable at the 5% significance level ($P < 0.05$ type I error) (Zar, 2006). A Kruskal-Wallis test was used to determine the differences in the percentage of cytoplasmic vesicles in the total neuronal area, as well as for the percentages of the histopathological alteration variables (eccentric nucleus EN and two nucleoli DN). The statistical package R version 2.15.1 was used (http://www.r-project.org/).

Results

Histopathology of DRG and its neurons

DRGs are paired structures located adjacent to the spinal cord. Afferent sensory fibers enter the DRGs via the dorsal horn of the spinal cord. Two neuronal subpopulations were identified by HROM based on their staining properties. Large and clear type A neurons, and smaller B neurons, with darker cytoplasm due to Nissl substance. Both neuronal types present stained nucleus, with one or two strongly stained nucleoli. Satellite glial cells were identified around the sensory neurons of the DRGs (Fig. 1). The main histopathological alteration was the presence of cytoplasmic vesicles, which were more abundant in treatments exposed to Roundup Active® (T2 and T3) and predominantly present in type A neurons (Fig. 1) .

Another histopathological alteration identified exclusively in T3 was the presence of mast cells associated with both neuronal types (A and B), located adjacent to nerve fibers and abundantly present in blood vessels irrigating the DRGs (Fig. 2, 3).

The ultrastructure of sensory neurons of the dorsal root ganglia enabled the identification of two distinct neuronal populations: large neurons exhibiting a predominantly electron-lucent cytoplasm (Type A), and smaller neurons characterized by increased electron density (Type B), attributable to the abundant presence of Nissl substance (rough endoplasmic reticulum aggregates). The neuronal soma is characterized by a relatively large nucleus occupying a substantial portion of the cell volume. The nucleus is predominantly euchromatic, with finely dispersed chromatin and one or occasionally two prominent nucleoli, consistent with high transcriptional activity. In some neurons, slight nuclear eccentricity or marginal chromatin condensation may be observed.

The rough endoplasmic reticulum is abundant and organized into parallel stacks of cisternae forming prominent Nissl bodies distributed mainly in the perinuclear cytoplasm. Numerous free ribosomes and polyribosomes are dispersed throughout the cytosol. The Golgi complex is conspicuous, composed of multiple stacks of flattened cisternae accompanied by numerous transport vesicles and small vacuoles, typically located adjacent to the nucleus.

Mitochondria are distributed throughout the cytoplasm, and more abundant in type A neurons exhibiting elongated or ovoid profiles with well-defined cristae. The neuronal surface is tightly enveloped by satellite glial cells forming a discontinuous but closely associated sheath around the soma. These glial cells display a thin rim of cytoplasm with sparse organelles and nuclei containing condensed heterochromatin.

The predominant ultrastructural alteration was the presence of cytoplasmic vesicles, which were more abundant in type A neuron. In some cases, these vesicles were associated with mitochondrial damage, particularly affecting the mitochondrial cristae. It is noteworthy that all mast cells exhibited intact cell membranes, and no evidence of degranulation or release of vesicular contents toward neurons or surrounding support cells, such as satellite glial cells, was detected.

DRG neuronal morphometry

No significant differences were observed in type A neurons and nuclei area, perimeter and diameter, but type B neurons from fish exposed to T2 and T3 are significantly smaller than those from fish exposed to T1 (Table 1).

Table 1. Morphometry of DRG neurons and their nuclei. Each value represents the mean +/- standard error. Different letters in the columns indicate significant difference between treatments ($p < 0.05$).

The frequency of nuclei migration (NM) and double nucleoli (DN) was not affected by glyphosate exposure. In both neuronal types, CV represented the most frequent alteration across treatments, with a clear increase from T1 to T3 (Fig. 4A-4B).

The distribution of neurons varied according to the percentage of cytoplasmic vesicles in both neuronal types (A and B). For type A neurons, T1 showed the highest proportion within the lowest vesicle category (0–1%), followed by a progressive decline as vesicle percentage increased. In contrast, T2 and T3 exhibited lower frequencies in this category and a relative increase in the intermediate ranges (1–5% and 5–10%). Similarly, type B neurons were predominantly concentrated in the 0–1% vesicle range across treatments, particularly in T1 and T2, whereas T3 displayed a lower proportion in this category and a marked increase in the 1–5% interval. In both neuronal types, cells presenting vesicle percentages above 10% occurred at low frequencies across treatments, generally remaining below 10%. Overall, T2 and especially T3 showed a displacement in neuronal distribution toward higher cytoplasmic vesicle percentages compared to T1, suggesting increased cytoplasmic vesiculation in sensorial neurons of fish

exposed to sublethal concentrations of Roundup Active® (Fig. 4C-4D) Fig. 4 must be placed after this description.

Discussion

DRG sensory neurons

The primary histopathological alteration identified in sensory neurons was the presence of cytoplasmic vesicles, which were more abundant in type A neurons in the treatments exposed to active Roundup (T2 and T3). In contrast, type B neurons exhibited this alteration less frequently and showed differences only at the highest Roundup Active® concentration (T3). The presence of cytoplasmic vesicles may have different causes, but precise mechanisms are not known (Greaves, 2007). Vacuoles are observed in rat DRG apoptotic neurons, after axotomy by ligation or crushing (Atlasi et al., 2009), or in mouse embryos spinal and brain neurons at early synaptogenesis (Oppenheim et al., 2001), in caspase mediated neurodegenerative processes, in necrotic processes after Paclitaxel administration in the L5 DRGs of rats (Jamieson et al., 2003) and in brain neurons in mice exposed to high levels of ethanol (Young et al., 2005).

These alterations may be caused by a process of necrosis or apoptosis, or even both may occur. Necrosis is characterized by cell disintegration and swelling, and vesicles may be formed due to a passive and poorly regulated organelle disintegration, and cell disassembly (Proskuryakov et al., 2003). Later events may include immune system activation by cytoplasmic products, resulting in macrophage arrival and local inflammation (Martin et al., 2019).

Apoptosis is an active process, with early chromatin condensation, followed by DNA cleavage, cytoskeleton reorganization, and organelle redistribution. It has been described in DRG neurons after peripheral nerve lesions, with an expression of *caspase 3* and TUNEL positivity (Wiberg et al., 2018, Martin et al., 2019). Oppenheim et al. (2001) and Borsello et al. (2002) suggest that these neuronal alterations are consistent with type 3B apoptosis characterized by the presence of cytoplasmic vesicles around the neuronal soma, conserved nuclei, subsequent granulation and chromatin accumulation, with nuclear deformation and evagination. Despite this, Borsello et al. (2002) indicated that an unequivocal distinction between type 3B apoptosis and necrosis remains difficult to establish. Moreover, exposure to different xenobiotics may lead to either apoptosis or necrosis, and both processes share common messengers, activators, and inhibitors (Formigli et al., 2000; Liao and Lieu, 2005).

However, other research supports the hypothesis that-what alteration? not clear it is a process of apoptosis, due to an increase in caspase-3 immunoreactivity in the hypothalamus in adult rats that had been exposed to glyphosate and Roundup Active® in their drinking water (Duque-Diaz et al., 2022). Similarly, Cardona (2023) found an increase of Caspase-3 positive cells in the brain of tadpole *Dendrosophus molitor* exposed to 1 and 3 mg/L of glyphosate present in the Roundup Active® formulation. Therefore, we suggest that sensory neurons in DRGs may be undergoing a process of cell

apoptosis similar to that reported in neurons of the CNS. Nevertheless, additional studies with different markers of apoptosis, neuronal proliferation and glial cells are needed to establish the mechanisms that are affecting the sensory neurons of DRGs when exposed to these xenobiotics.

Paclitaxel is used as a chemotherapeutic agent for mammary, ovary, and lung cancer. However, a harmful effect on the peripheral nervous system has been described, with early cytoplasmic vesicle appearance, followed by neuron degeneration (Postma et al., 1995). This compound is a clear example of a necrosis or apoptosis triggering agent (Liao and Lieu, 2005). Paclitaxel induces alterations in axoplasmic transport by disrupting normal microtubule polymerization (Authier et al., 2000). Other effects are observed, mainly in type A neurons, with a nuclear enlargement (Jamieson et al., 2003; Jamieson et al., 2007), increased eccentricity, and neuronal multinucleolation, which according to Jamieson et al., (2007) are clear neuropathy symptoms. Nevertheless, in our study no differences were observed in migrated nuclei, which is a measure of nuclear eccentricity, nor in double nucleoli in either neuronal type (A and B) among the evaluated treatments. The absence of observable differences in these histopathological alterations may be associated with the use of juvenile *P. orinoquensis*, a species with indeterminate growth. Studies by Jaramillo et al. (2009) support this interpretation, reporting that in this species it is common to observe sensory neurons in the DRG exhibiting double nucleoli and migrated nuclei, which are considered indicators of neuronal immaturity or neurogenesis.

According to events during embryonic DRG neurons development in mammals (Jackman and Fitzgerald, 2000) and birds (Eide and Glover, 1997), an apoptotic process is more in agreement than a necrosis one. In tetrapods these neurons present oscillatory development mediated by apoptosis. In DRG, type A neurons develop first, followed by type B neurons, as A neuron precursor cells suffer a mitotic arrest earlier than B neuron precursor cells (McGraw et al., 2012). However, this oscillatory process has not been demonstrated in fish. In mammals *neurogenin 2* is responsible for a first neurogenesis wave given rise to large mechanoreceptive and proprioceptive neurons (predominantly type A neurons), while *neurogenin 1* is responsible for a second wave generating mechanoreceptive and nociceptive neurons (primarily type B neurons). In addition, a third wave controlled by *Krox20* cap cells leads to more nociceptive neurons (type B neurons). By contrast, in zebrafish (*Danio rerio*) only one neurogenic gene is present (*neurogenin 1*), which is upregulated by the interaction of *Sorbs3* with *ErbB* genes (Malmquist et al., 2013). Therefore, it is necessary to determine which genes regulate the proliferation and development of sensory neurons in the DRGs of fish, to understand why differential damage occurs in the sensory neurons of an indeterminate growth species such as *P. orinoquensis*. Additionally, it is essential to evaluate cellular apoptosis biomarkers such as genes of the caspase family, as well as cell proliferation proteins such as PCNA and S100 in the DRGs of fish, and to determine whether sensory neurons indeed exhibit an oscillatory developmental pattern.

As adult zebra fish may have more than 100 neurons per DRG, most of these cells must appear during larval and adult stages (McGraw et al, 2012). New neurons arise from progenitor cells associated with DRG and are regulated by Notch signaling (McGraw et al, 2012). Jaramillo et al (2009) observations are compatible with such a mechanism. In addition, *P. orinoquensis* is an indeterminate growth species, and

most probably both neurogenesis and apoptosis are present throughout the individual whole life, to provide sensorial innervation to newly formed muscular, cutaneous and visceral tissues.

Transmission electron microscopy provided evidence that part of the cytoplasmic vesicles is due to mitochondrial damage, especially in the mitochondrial crests of type A and B neurons, with type A being more pronounced at T3 (1 mg/L). This is the first report of histopathological mitochondrial alterations in sensory neurons of DRGs caused by a GBH such as Roundup Active®. These findings are consistent with those reported by Nerozzi et al., (2020) in rat sperm mitochondria and with those described by Riaño et al., (2020) for cytoplasmic vesicles in tadpole hepatocytes. Apparently, glyphosate and adjuvants present in Roundup Active® cause cellular oxidative stress that affects the electron transport chain and the enzyme succinate dehydrogenase located in the mitochondrial crests (Peixoto, 2005, Riaño et al., 2020). These mitochondrial alterations could lead to decreased ATP, cellular respiration and increased intracellular hydrogen peroxide which if sustained over time potentially cause neuronal apoptosis (Bailey et al., 2018).

Neuronal morphometry revealed a reduction in the area, perimeter, and diameter of type B neurons when exposed to sublethal concentrations of glyphosate present in Roundup Active®. These size alterations are a sign of neuropathy in neurons exposed to toxic agents. These findings suggest that another possible way of entry for Roundup Active® could be through the skin, taste buds, neuromasts, and other epithelial cells. Type B sensory neurons primarily innervate cutaneous targets and some internal organs such as viscera, therefore these neurons might be expected to be more sensitive than type A neurons, which mainly innervate muscles (Jaramillo et al., 2009). Hu and McLachlan (2003) provide clear evidence on B neuron selective vulnerability after rat L5 peripheral nerve lesion. Progressive death and diameter decrease are reported due to loss of growth factors from cutaneous targets. In addition, Lopez-Florez (2023) found more evident skin damage in tadpoles exposed for 31 days to 1.4 mg/L of GP as Roundup Active®. This suggests that sublethal Roundup Active® concentration may damage cutaneous cells, indirectly affecting the morphology and size of type B neurons in DRG.

Sensory afferent diameter and conduction speed depends on neuronal size (Martínez et al., 2000). Larger type A neurons exhibit myelinated fibers, while smaller B neurons present thin myelin sheets or are unmyelinated, with slower conduction speed (Jaramillo et al., 2009). Physiological effects of Paclitaxel are related to diminished conduction speed and an increase in latency time for peripheral sensory nerves, mainly form type A neurons (Jamieson et al., 2007). On the other hand, alterations in type B sensory neurons may lead to a reduction in sensory conduction velocity, resulting in disturbances in cutaneous sensitivity and affecting the perception of pressure, vibration, or mechanical stimuli, thereby reducing the ability to detect environmental variables in the water (Jaramillo et al., 2009).

A type of neuron degeneration may lead to ataxia and muscular sensitivity loss, pain, and hyperesthesia, with long-term nerve fiber degeneration and ability loss (Sghirlanzoni et al., 2005). If fishes are exposed to low concentrations of xenobiotics present in natural water basins, a progressive decrease in conduction speed may result, altering predator avoidance responses as well as food capture (Castañeda,

2012). In addition, these two conditions are more relevant for fry or juvenile organisms, as adults are less affected by predators and are able to explore and use larger foraging areas (Riaño et al., 2020).

Mast cells were observed exclusively in fish exposed to Roundup Active® at the highest concentration (T3, 1 mg/L), becoming the first report of this cell type in sensory neurons of the DRGs. Mast cells were found near both A and B neurons and in capillaries and arteries associated with DRGs.

Mast cells have been the most recurrent histopathological alteration reported in the brain of tropical fish such as *P. orinoquensis* and the ornamental fish *Paracheirodon axelrodi* following exposure to sublethal concentrations of Roundup Active®. These cells have been detected in several brain structures, including the olfactory bulbs (Castañeda, 2012), the telencephalic hemispheres and the preoptic area (Gomez-Ramirez et al., 2025). However, this is the first report describing mast cells in the peripheral nervous system, specifically affecting the sensory neurons of the dorsal root ganglia (DRGs). Mast cells in the nervous system may indicate ongoing processes of neurodegeneration, apoptosis, and neuronal remodeling (Riaño et al., 2020). Accordingly, these cells are frequently observed surrounding neurons undergoing damage or in adjacent vascular tissues, where they may act as mediators that facilitate or accelerate these processes (Eslava-Mocha et al., 2019).

Ultrastructural analysis and HROM revealed that all mast cells were in a non-degranulated state, whereas at the same concentration (1 mg/L GP) partial degranulation has been reported in the brain of *P. orinoquensis*. Nevertheless, the mere presence of mast cells already indicates immune activation and pro-inflammatory processes triggered by toxic agents or stressors, in this case Roundup Active® (Gomez-Ramirez et al., 2025).

Conclusion

The principal histopathological alterations were the presence of cytoplasmic vesicles in all treatments and the occurrence of mast cells in the DRGs and the surrounding vascular tissue at the highest concentration (T3). This represents the first report of this cell type in the peripheral nervous system of fish following exposure to the xenobiotic Roundup Active®.

Ultrastructural analysis provided evidence that some of the cytoplasmic vesicular alterations corresponded to mitochondrial damage, particularly affecting the mitochondrial cristae. Likewise, it enabled a detailed characterization of mast cells, with no evidence of degranulation processes.

This study suggests a differential effect on the two neuronal types. Type B neurons decreased in size when exposed to both concentrations (T2 and T3) and showed differences in cytoplasmic vesicles at T3. In contrast, type A neurons did not exhibit significant differences in size but showed an increase in the percentage of cytoplasmic vesicles in T2 and T3.

Declarations

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Authors' Contributions:

Edwin Gomez Ramirez: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing original draft preparation, Funding acquisition.

Pedro René Eslava Mocha: Methodology, Histopathological and ultrastructural analysis, Supervision, Writing, review and editing, Funding acquisition.

Bernardo Baldisserotto: Experimental design, Data analysis, Supervision, Validation, Writing, review and editing

Ethical Approval:

The study followed institutional and international animal care standards, including AVMA (2020) euthanasia guidelines. It was approved by the ethics committee of the Vice-Rectory of Research of the Universidad Militar Nueva Granada (INV-CIAS 552).

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Competing Interests

The authors declare that there are no conflicts of interest.

Data Availability Statement

The datasets used and analyzed during the current survey are available from the corresponding author on reasonable request.

Mandatory if your study involves humans and/or animals

Not applicable. This study did not involve human participants. All experimental procedures involving animals were conducted in accordance with institutional and international guidelines for the care and use of laboratory animals.

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Table

Table 1 is available in the Supplementary Files section.

Figures

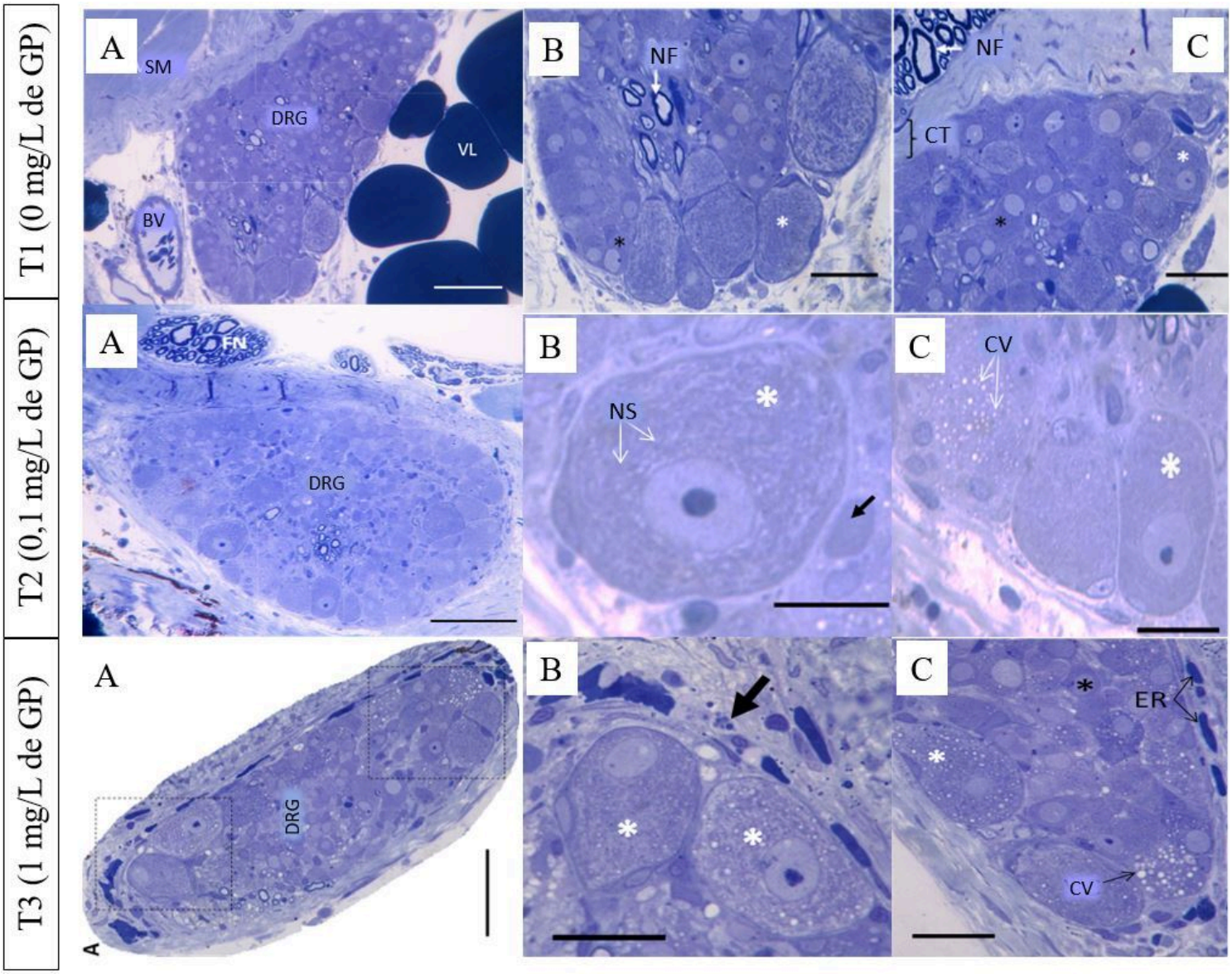


Figure 1

Dorsal root ganglia (DRG) from P. orinoquensis. A. Panoramic view. B and C. Details form different neurons of DRG. Dorsal root ganglia (DRG), skeletal muscle (SM), blood vessel (BV), nerve fibers (NF), connective tissue (CT), Nissl substance (SN), type A neurons (white *), type B neurons (black *), cytoplasmic vesicles (CV), mast cells (black arrow), erythrocyte (ER). T1: Bar A= 50 µm, bar B and C= 20 µm. T2: Bar A= 50 µm, bar B and C= 10 µm. T3: Bar A= 50 µm, B and C= 20 µm.

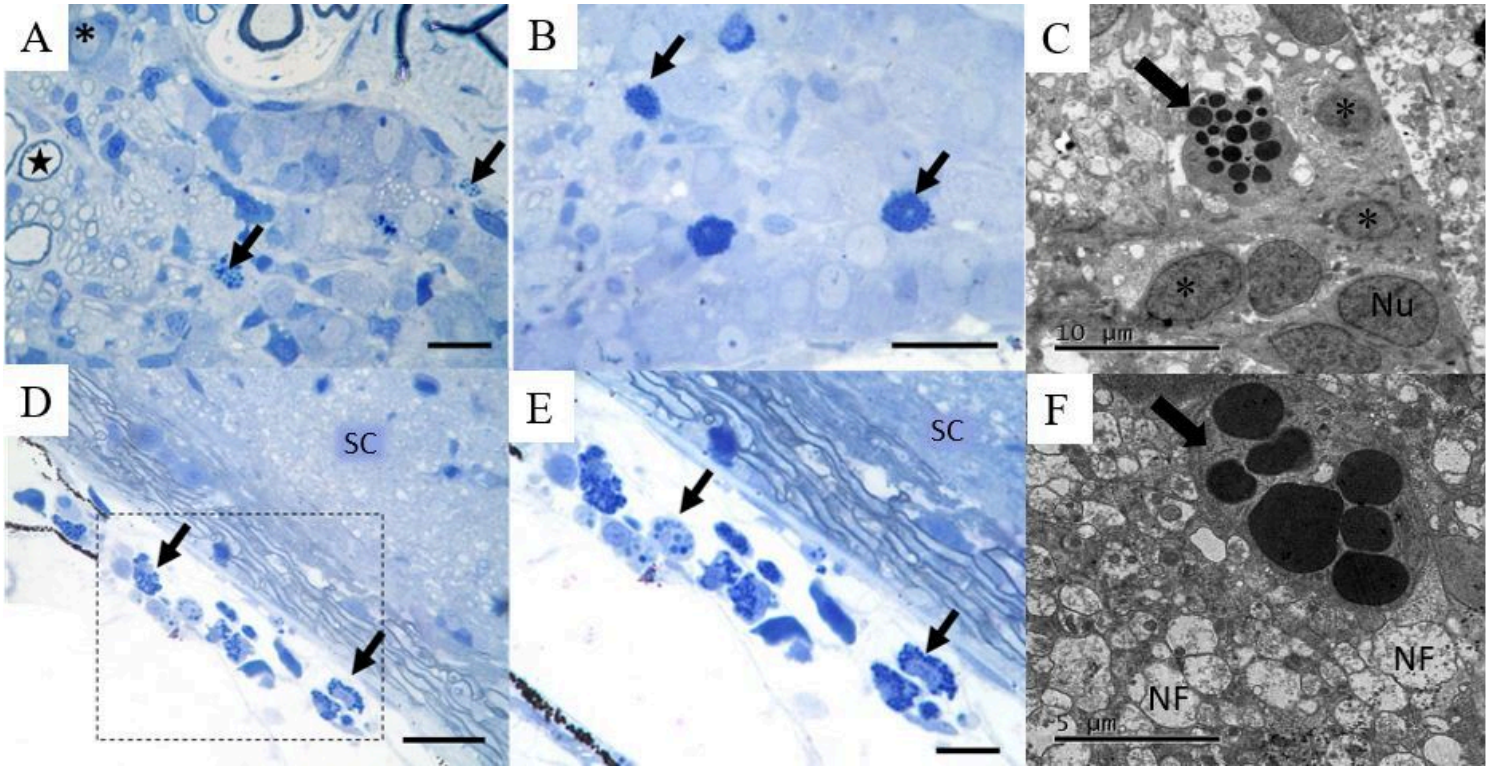


Figure 2

Mast cells in the dorsal root ganglia (DRG) and associated with blood vessels of P. orinoquensis exposed to treatment 3. A and B, mast cells in the dorsal root ganglia. D and E, mast cells associated with blood vessels. C and F, mast cell ultrastructure. Mast cells (black arrow), type B neurons (black *), Spinal cord (SC), Nucleus (Nu), nerve fibers (NF). Bar A, B= 20 µm. Bar D= 50 µm, E= 20 µm.

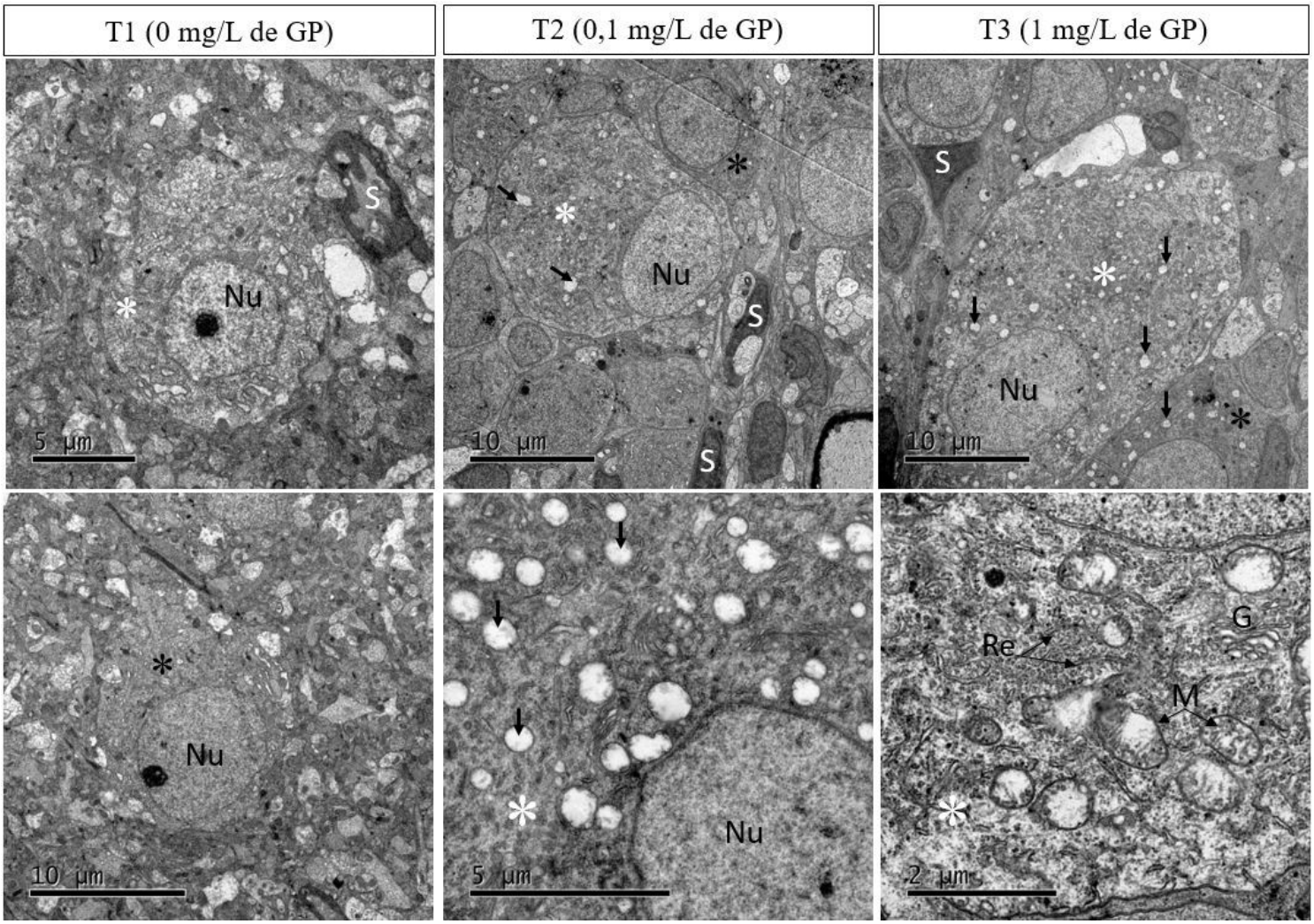


Figure 3

Ultrastructure of sensory neurons of DRG from P. orinoquensis. Type A neurons (white *), type B neurons (black *), Satellite glial cells (S), cytoplasmic vesicles (black arrow), Nucleus (Nu), rough endoplasmic reticulum (Re), Mitochondria (M), Golgi complex (G).

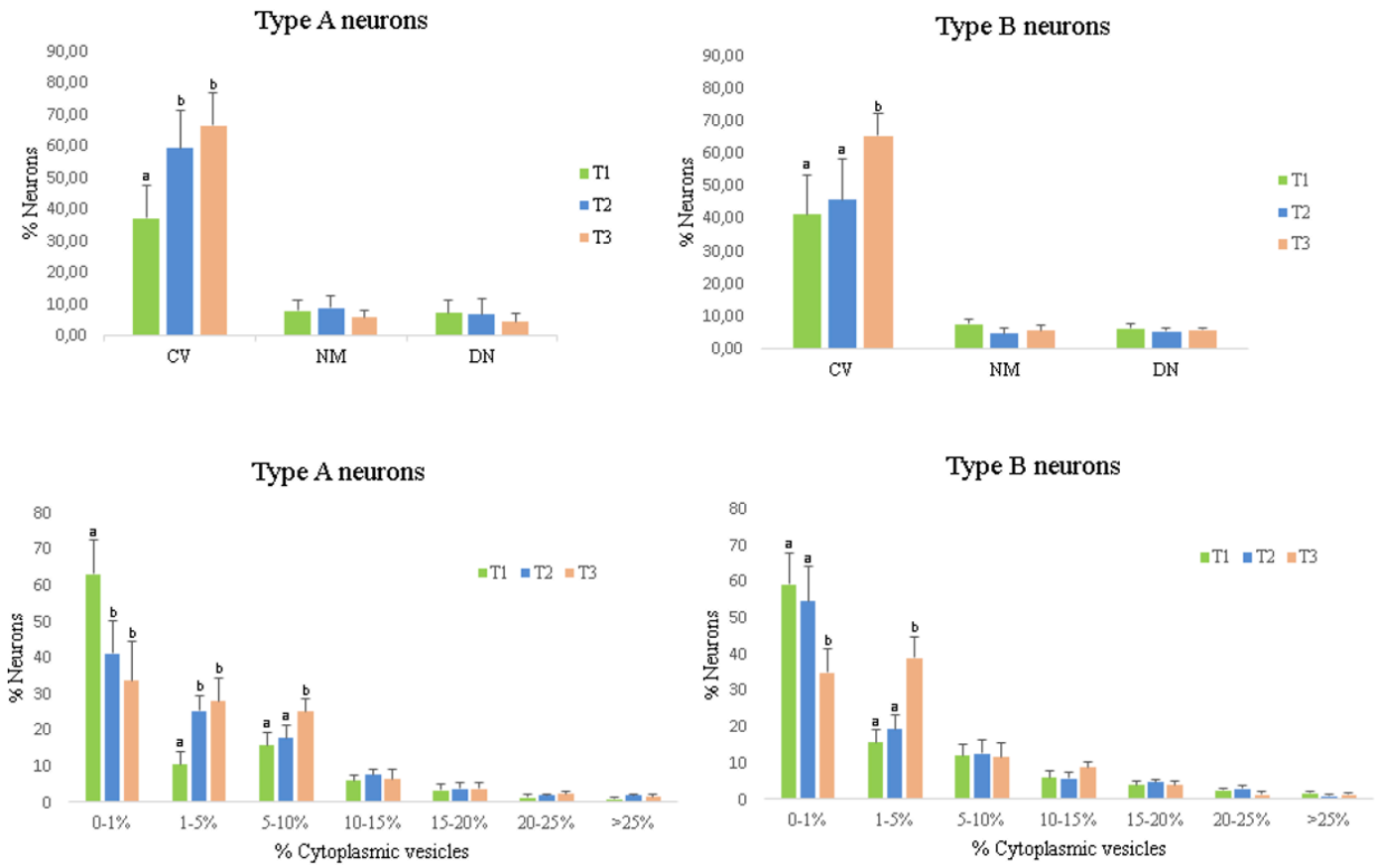


Figure 4

Percentage of A and B neurons with alterations: cytoplasmic vesicles (CV) migrated nuclei (MN) and double nucleoli (DN). Percentage of cytoplasmic vesicles in type A and B neurons. Each value corresponds to mean +/- standard deviation. Different letters indicate significant differences for each parameter ($p<0.05$).

Supplementary Files

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- FinalTable300DPI.jpg